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# Toxicity of botanicals and selective insecticides to Asian citrus psylla, *Diaphorina citri* K. (Homoptera: Psyllidae) in laboratory conditions

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## ABSTRACT

Present study was designed to evaluate the effectiveness of toxicity of some botanicals and selective insecticides against Asian citrus psyllids *Diaphorina citri* Kuwayama which is a vector of huanglongbing or citrus greening and widely spread in citrus growing regions. In laboratory, cumulative mortality of adult *D. citri* with Confidor® (imidacloprid), Movento® (spirotetramat) and Radiant® (spinetoram) was more than 95% at commercially recommended doses. These three insecticides showed similar trend toward mortality of adult psyllids either applied through spray or leaf dip method. The cumulative mortality of adult psyllids was 77 % and 86% with spray and leaf dip method respectively. Datura showed 86% mortality of nymphs in leaf dip method. The mortality of adults after spray with Datura was 31% and after leaf dip treatment 60%. It is concluded that the products we used in our study are effective against *D. citri* and due to their bio-based chemistries these are supposed to be less or nontoxic to beneficial insects and environment.

**Key words:** Eco-friendly, Bioassay, Huanglongbing, Biopesticides, Citrus IPM.

## INTRODUCTION

Asian citrus psylla (ACP), *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) was first described in Tiawan Kuwayama (1908), afterward found in Indo-Pak region (Hussain & Nath, 1927) and now widely distributed in Asia and several countries of the world. It is a serious pest of citrus in Pakistan and other Asian countries (India & China). It is a vector of *Candidatus liberibacter asiaticus* which cause a fatal citrus disease called 'Huanglongbing' (HLB) or citrus greening (Halbert & Manjunath, 2004). Due citrus greening 20% of trees in poorly managed orchards were lost within few years of planting (Aubert, 1990). Due to severe and rapid spread of the disease orchards may lose their commercial value within 7-8 years of planting. In India a survey revealed that, citrus suffered 25 percent yield losses by insect pests, out of which *D. citri* contributed 83-95 percent (Shivankar & Singh, 2006).

In Pakistan the problem of ACP and greening disease of citrus has not been addressed as of much importance. In addition to *D. citri* some environmental factors and susceptible rootstock are also responsible for greening problem (Hoodle, 2012), but exact losses of citrus due to psyllids need to be estimated in Pakistan. In Florida the losses due to invasion of ACP and its vectored disease huanglongbing is about 10 percent resulting in about 8257 job losses, revenue reduction $ 2.7 billion (US) and $ 1.8 billion (US) economic activities related to citrus work force (Hodges & Spreen, 2012).

Application of insecticides is a major part for the management of *D. citri.* Some synthetic insecticides like neem oil, petroleum oils, insect growth regulators and organics, were tried against citrus ACP, which showed encouraging results in reducing its population

(Dahiya et al., 1994; Nakano et al., 1999; Shivankar et al., 2000; Qureshi & Stansly, 2007, 2008, 2010; Qureshi et al., 2009, 2010, 2011, 2012; Rogers, 2008; Rogers et al., 2012; Setamou et al., 2010; Tiwari et al., 2011). Traditionally synthetic insecticides e.g., imidacloprid, thioclam and mineral oil have been used for the control of citrus pests e.g. imidacloprid, thioclam, mineral oil (Boulahia et al., 1996) and combination of mineral oils and abamactin (Rezk et al., 1996) have been found to be effective in reduction of pest densities. Foliar application of systemic insecticides, imidacloprid, thiamethoxam, clothianidin (Neonicotinoids) reduced 50 to 70 percent *D. citri* in one month after treatment. More than 80 percent mortality of *D. citri* was attained within 10 days after treatment of neonicotinoids on seedling plants of citrus and this level was through 90 days in green house and 60 days in field conditions (Ichinose et al., 2010). Serikawa, et al., (2012) observed that when imidacloprid (Neonicotinoid) is applied systemically, the chances of *D. citri* feeding on citrus plants are reduced. Furthermore, the vector acquisition of pathogen is also minimized which protect the spread of HLB through psyllids.

Most of the synthetic insecticides have toxic effects and are dangerous to human health, these insecticides when applied to crops against some pests; also remain in shape of residues in fruit (Hussaina & Siddique, 2010). With the change of agriculture trends and organic forming and requirements of World Health Organization (WHO), the use of bio-pesticides against phytophagous insects/pests is increasing gradually, especially in the agro-ecosystem associated with natural enemies that are considered as important control agents in the IPM modules (Heinrichs, 1998; Tengerdy & Szakacs, 1998; Rausell et al., 2000). In addition to reduction in the environmental pollution, use of these natural chemicals in place of conventional pesticides also conserve non-target organisms, and reduce pest resurgence as compared to the conventional compounds.

Biodegradable and insecticidal liminoid azadirachtin formulated from neem tree is effective chemical against several pests (Isman, 1999). Effects of a neem (4.5% azadirachtin) based biopesticide, were assessed against the *D. citri* adults and nymphs, no adult mortality or oviposition preference against treated plants observed, but significant repellent effect was observed in choice experiment. Nymphs were found susceptible to azadirachtin at very low concentrations and at 22.5ppm azadirachtin, ecdysis observed past 4 days after treatment and all nymphs were dead within 7 days. The densities of psyllid nymphs on treated plants in greenhouse population was reduced significantly, by concentrations of 10 ppm azadirachtin (Weathersbee & McKenzie, 2005).

A longer and strong effect against *D. citri* was observed for the treatments of 435 Oil (horticultural spray oil) alone and spirotetramat (Movento® 240SC), tolfenpyrad, abamectin + thiamethoxam (Agriflex®), and diflubenzuron (Micromite® 80WGS) all applied with 435 Oil. Flubendiamide (Belt® 4SC) + Induce (surfactant), M-pede® (soap) + Addit® (vegetable oil) and fenpyroximate (Portal® 0.4EC). A significant psyllids population decrease was observed for all of these treatments even after $17^{th}$ day after treatment. During growing season these selective insecticides provide required level of ACP control with minimal impact on bio-control agents that would be at risk if broad spectrum insecticides are applied at that time (Qureshi et al., 2011).

The ACP and HLB pose a serious threat to citrus production in Pakistan. Therefore, development of sustainable pest and disease management systems that involve environment friendly approaches to control vector psyllids and conserve bio-control agents, such as lady beetles, lacewings, syrphid flies and spiders etc., is required. Therefore, we conducted this study to evaluate effectiveness of relatively safe synthetic and botanical insecticides against *D. citri*. The present study was involved, leaf dip and spray methods for bioassay of botanicals and selective insecticide in laboratory conditions.

## MATERIALS AND METHODS

### Psyllids colony

The psyllid colony was maintained on *Murraya paniculata* (Citrus Jasmine) plants in controlled temperature glass house at 25 ±2°C and 60±5RH at 16L: 8D at National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad, Pakistan. The detail of synthetic insecticides and botanicals along with their formulations, active ingredients used in the study are given in Table 1.

### Preparation of Botanicals Solution

Dry powers of *Azadirachta indica* A. juss (Neem) and *Datura alba* Nees (Datura) were obtained by grinding the dry leaves of these botanicals in blender (Anex-176GL). The dry powder (100gm) of each botanical was mixed in one liter of water to prepare 100 % solution. The solutions were kept in corked bottles (400ml) at 4°C to prevent contamination (Sridhar & Vijaya, 2002; Dawar et al., 2010).

**Spray method**

For spray experiment, we first checked revival activity of adult psyllids. The purpose was to inactivate the adult psyllids for proper application of test solutions on their body. For revival activity 30 psyllid adults were put in falcon tubes (15ml) and placed in freezer at -4$^{o}$C for 4, 6 and 8 minutes and after mentioned time tubes were taken and place at normal temperature and their reactivation time was noted. The adults kept in refrigerator for at least 8 minutes were observed to be reactivated in 3.28±0.13 minutes (Figure 1).

A total of 245 adults were distributed to 7 treatments (5+2) with 7 replicates. The adult psyllids were sprayed using mister sprayer (800ml hand operated). A separate sprayer was used for each of the solution. After spray the adult psyllids were released in 40 dram ventilated snap cap vials (17/8$^{//}$ X 35/16$^{//}$). A fresh washed shoot of *M. paniculata* as mentioned in leaf dip method was also provided in each vial as a food source for psyllids. Mist sprayers were calibrated prior to application and recommended doses of insecticides were used as mentioned in Table 1.

**Leaf dip application method**

The shoots M. *paniculata* were dipped in prepared solution for 2-3 seconds and immediately placed at paper towel to suck up the excess solution from leaves. To keep the shoots fresh the stalk of shoots were put into the eppendorf tubes (1.5ml micro centrifuge) filled with water and covered with parafilm to prevent water flow out of tube. Each of the treated shoot was provided with five psyllid nymphs and five adults. Each shoot was put in 100ml snap cap vial and covered with perforated parafilm for ventilation. The experiment was carried out in controlled conditions as mentioned in colony maintenance. The mortality data of psyllid nymphs and adults was recorded after 24h, 48h and 72h after the treatment. Adult psyllids were disturbed by soft hair brush and nymphs with needle to confirm whether they are alive or dead. The dead individuals were taken out of the vials on each observation time.

**Statistical Analyses**

Normality of the data was checked before statistical analysis by using Kolmogorov Simonov test. We used generalized linear models (GLM) procedure under one way Analysis of Variance (ANOVA). Multiple comparison for treatments were performed using Tukey's test at P=0.05 to discern differences in number of nymphs and adults of *D. citri* died in each method leaf dipped or sprayed. Data are reported as percent mortality (Mean ±SE). Analysis was done in SPSS® IBM® Version-20, 2011.

## RESULTS

**Mortality of *D. citri* Adults in Spray**

A significant difference in mortality of *D. citri* adults was recorded among groups at three different time intervals (DF = 6, 42; F = 7.555; P<0.001 for 24h, DF = 6, 42; F = 22.988; P<0.001 for 48h and DF = 6, 42; F = 39.48; P<0.001 for 72h). The mortality % against Radiant® was 77.14±10.17, 91.43±4.04 and 100 at 24h, 48h and 72h respectively (Table 2). We observed 77.14±11.07 percent mortality against Neem at 24h and it remained the same even at72h. The percent mortality against Confidor® and Movento® was <50% at 24h which reached to 97.14±2.86 percent at 72h. Although Datura caused 31.43±11.43

percent adults mortality but results of the Tukey's test indicated the mortality did not differ significantly from the control.

**Mortality of *D. citri* Adults in Leaf dip**

We also recorded a significant difference in mortality of *D. citri* adults in leaf dip method (DF = 6, 42; F = 33.13; P<0.001 for 24h, DF = 6, 42; F = 66.203; P<0.001 for 48h and DF = 6, 42; F = 72.333; P<0.001 for 72h). Confidor®, Movento® and Radiant® showed more than 80% psyllids adults mortality at 24h leading to 100% at 72h. The results of Tukey's test showed that the mortality due to Confidor®, Movento® and Radiant® was significantly higher compared to the control, Neem and Datura (Table 3). The percent mortalities due to Neem and Datura at 72h were 45.71±5.71 and 60±9.76 respectively.

**Mortality of *D. citri* Nymphs in Leaf dip**

Similar trend of mortality for psyllid nymphs as in adults was recorded in leaf dip method for adults (DF = 6, 42; F = 18.66; P<0.001 for 24h, DF = 6, 42; F = 82.44; P<0.001 for 48h and DF = 6, 42; F = 57.27; P<0.001 for 72h). Treated group showed significantly higher mortalities of psyllid nymphs as compared to untreated (Table 3). We also observed that significantly higher psyllid nymph's mortality after treatment with Datura and Neem (Table 4) compared to psyllid adults in same method (Table 3). Cumulative mortality (%) of adult and nymphs of *D. citri* assessed in different methods are given in the Figure 2.

## DISCUSSION

Present studies regardless of methods either sprayed or leaf dip showed significantly good percent mortality of *D. citri* nymphs and adults at final hours of observation. At 72h in spray method the adult mortality was almost 100% for Confidor®, Movento® and

Radiant®. Qureshi et al. (2010, 2011, 2012) studied that foliar application of bio-pesticides and relatively selective chemistries provide good suppression of *D. citri*. In our study psyllids adult mortality with Neem is 77.14% compared to 45.71% mortality in nymphs. Datura caused higher nymph mortality (60%) compared to the Neem (45.5%). Weathersbee & McKenzie (2005) assessed 4.5% azadirachtin against the *D. citri* adults and nymphs and reported similar results. Qureshi et al. (2013) has reported 87% cumulative mortality of adults *D. citri* with bacterial insecticidal Entrust® (spinosad) and 25% adult mortality against Aza-Direct® (Neem) provided up to 25% adult mortality. He also observed >90% mortality against *D. citri* nymphs.

Khan et al. (2012) found 30-80% psyllids mortality using different concentrations of neem oils and extracts and 100% psyllids mortality for imidacloprid. The findings are also authenticating the results of the present study. Immaraju et al., (1990) found two-fold more mortalities in leaf dip method, when evaluated different bioassay methods against citrus pests, also supporting our results for leaf dip method (for Neem & Datura). However, the selective insecticides of new chemistry like Confidor® (neonicotinoid) or bacterial insecticides Movento® (spirotetramat) and Radiant® (spinetoram) found to be excellent chemicals against both adults and nymphs of psyllids regardless of the application methods. The lesser mortality of *D. citri* adults when presented to Neem in leaf dip and to Datura in spray methods is evidenced of the reason that Datura act more aggressively when applied on plant and pest do feeding on it, as systemic insecticide but neem has no such properties.

Today it is a common practice to use insecticides for vector management of greening disease of citrus (Setamou, et al., 2010) but in response to similar mode of action of different insecticides ACP is developing varying levels of resistance to these chemicals and there are evidences of detoxifying enzymes in their field populations of ACP regarding insecticide resistance (Tiwari, et al., 2011) which also leads to environmental disruption and disturbance to natural enemies of psyllids in filed. Also the use of synthetic insecticides incurs high costs along with harmful effects to citrus ecosystems (Wang, 2002).

So it is need of the day to involve such classes of insecticides that would have less toxic to bio-control agents and no resistance evidenced in psyllids against these insecticides. The products we used in our present study are botanical, bacterial or new chemistry which has good mortality toward psyllids and due to their bio-based chemistry these are supposed to be less or nontoxic to beneficial insects and has no residual effects in fruits. Field trials for this eco-friendly insecticide are warranted to determine their efficacy against psyllids in citrus orchards.

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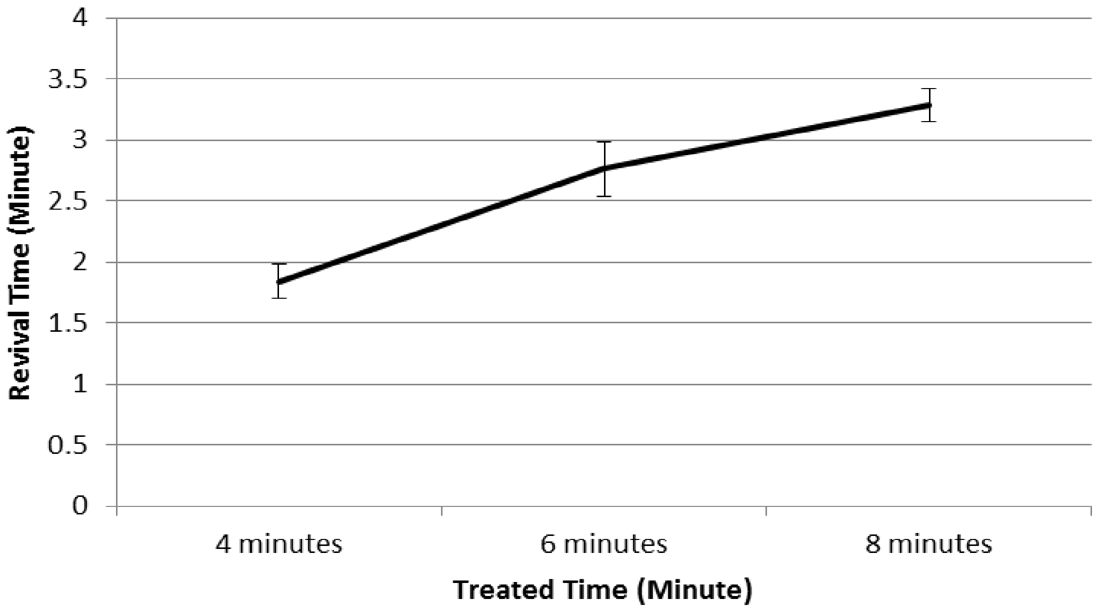


**Figure 1. Revival time of adult *D. citri* kept in freezer for different time periods. Falcon tubes containing *D. citri* were kept in freezer for 4, 6 and 8 minutes and their revival time was noted immediately after keeping them out at room temperature.**

**Table 1.** Detail of synthetic insecticides and botanicals along with their formulations used in the study.

| **Product** | **Active Ingredient** | **Formulation/ Lt. water** | **Company Name** |
|---|---|---|---|
| Confidor® | imidacloprid | 12.50ml | Dow Agro Science |
| Movento® | spirotetramat | 1.25ml | Bayer Crop Science |
| Radiant® | spinetoram | 0.40ml | Bayer Crop Science |
| Neem | azadirachtin | 20.00ml | Self-Made |
| Datura | tropane | 20.00ml | Self-Made |
| Control (Tape water) | --- | --- | --- |
| Control (Untreated) | --- | --- | --- |

**Note:** Recommended field doses of insecticides and botanicals @ 2% were used.

**Table 2. Percentage mortality (Mean±SE) of *D. citri* adults at the assessment times, 24h, 48h or 72h day after spraying.**

| Treatments | 24 hours | 48 hours | 72 hours |
|---|---|---|---|
| **Confidor®** | 40 ±15.11[c] | 91.43 ± 5.94[b] | 97.14 ± 2.86[b] |
| **Movento®** | 42.86 ±14.75[c] | 82.86 ± 9.18[b] | 97.14 ± 2.86[b] |
| **Radiant®** | 77.14 ±10.17[d] | 91.43 ± 4.04[b] | 100 b |
| **Neem** | 77.14 ±11.07[d] | 77.14 ± 11.07[b] | 77.14± 11.07[b] |
| **Datura** | 31.43 ±11.43[bc] | 31.43 ± 11.43[a] | 31.43 ± 11.43[a] |
| **Control (Tape water)** | 8.57 ± 5.95[b] | 14.29 ± 7.19[a] | 11.43 ± 5.95[a] |
| **Control (Untreated)** | 0.00 [a] | 2.86 ± 2.86[a] | 5.71± 3.69 [a] |

**Note:** Mean values in columns with the same letters are not significantly different.

**Table 3. Percentage mortality (Mean±SE) of *D. citri* adults at the assessment times, 24h, 48h or 72h after treating leaves (leaf dip) with insecticide solution.**

| Treatments | 24 hours | 48 hours | 72 hours |
|---|---|---|---|
| **Confidor®** | 80 ± 8.73$^{c}$ | 97.14± 2.86$^{c}$ | 100 $^{c}$ |
| **Movento®** | 88.57± 8.57$^{c}$ | 94.29 ±5.71$^{c}$ | 100 $^{c}$ |
| **Radiant®** | 94.29 ± 5.71$^{c}$ | 100 $^{c}$ | …. |
| **Neem** | 32.43±8.57 $^{b}$ | 40± 8.73$^{b}$ | 45.71± 5.71$^{b}$ |
| **Datura** | 25.71± 8.41$^{ab}$ | 34.29± 8.41$^{b}$ | 60 ±9.76$^{b}$ |
| **Control (Tape water)** | 5.71 ±3.69$^{ab}$ | 5.71 ±3.69$^{a}$ | 11.43 ±5.95$^{a}$ |
| **Control (Untreated)** | 0.00 $^{a}$ | 0.00 $^{a}$ | 2.86±2.86 $^{a}$ |

**Note:** Mean values in columns with the same letters are not significantly different.

**Table 4. Percentage mortality (Mean±SE) of *D. citri* nymphs at the assessment times, 24h, 48h or 72h after treating leaves (leaf dip) with insecticide solution.**

| Treatments | 24 hours | 48 hours | 72 hours |
|---|---|---|---|
| **Confidor®** | 57.14± 11.07[bc] | 100 [b] | ….. |
| **Movento®** | 94.29± 5.71[c] | 100 [b] | …. |
| **Radiant®** | 88.57±5.94 [c] | 91.43 ±5.95[b] | 91.43 ±5.95[b] |
| **Neem** | 37.14 ±13.4[ab] | 85.71 ±5.71[b] | 85.71± 5.71[b] |
| **Datura** | 31.43± 11.43[ab] | 85.71± 8.41[b] | 85.71± 8.41[b] |
| **Control (Tape water)** | 2.86 ±2.86[a] | 2.86 ±2.86[a] | 8.57 ±5.94[a] |
| **Control (Untreated)** | 2.86 ±2.86[a] | 5.71 ±3.69[a] | 8.57 ±5.94[a] |

**Note:** Mean values in columns with the same letters are not significantly different.

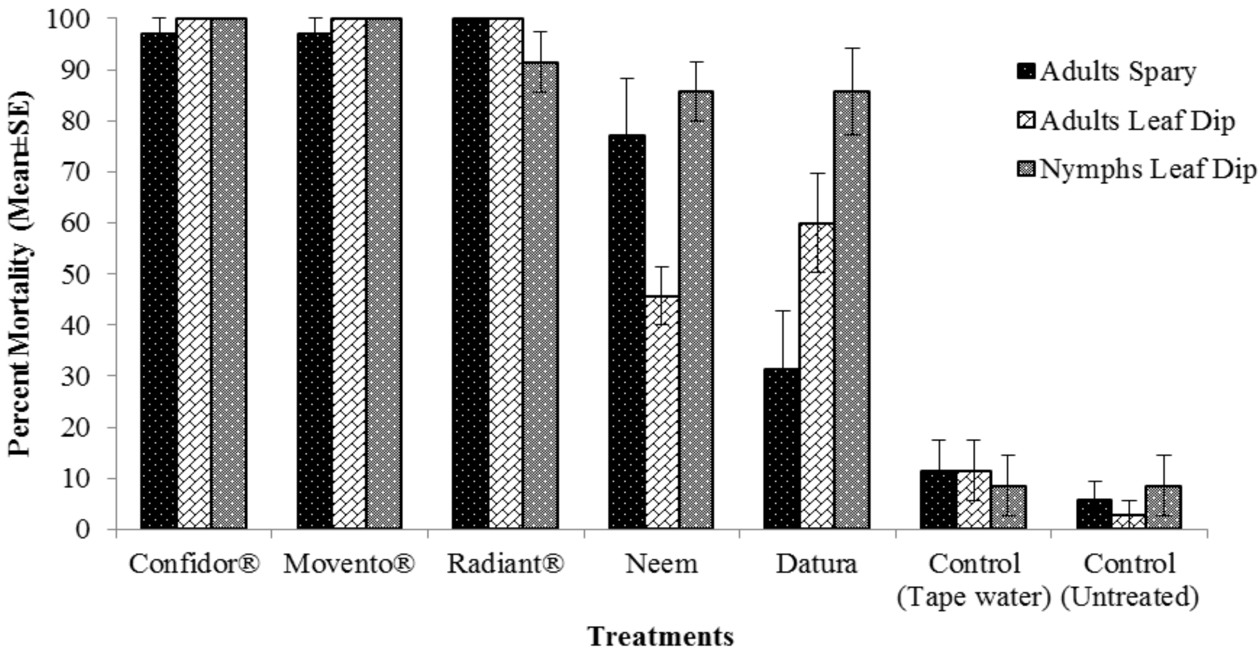


**Figure 2. Cumulative mortality (%) of adult and nymphs of *D. citri* at final assessment time of 72h for Confidor®, Movento®, Radiant®, Neem, Datura, Tape water and untreated, which were all applied at field rate using lead dip and spray methods.**