



## **Bio-efficacy of Certain Chemicals against *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)**

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### **Authors' contributions**

This work was carried out in collaboration between both authors. Author AR designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Author PQR managed the literature searches. Both authors read and approved the final manuscript.

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### **ABSTRACT**

Asiatic citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is identified as a major pest of citrus in subtropical and tropical Asia. Citrus greening disease or Huanglongbing (HLB) is found to be the most serious disease of citrus in the world, causing the highest economic loss. *Diaphorina citri* Kuwayama is the only known vector of Huanglongbing. Intensive insecticide programs against Asian Citrus Psyllid are generally ineffective for preventing the introduction and spread of Huanglongbing, especially in new plantings. Extensive pesticide applications are causing psyllid resistance and probably damage to bees and beneficial insects. So, it is important to devise Integrated Pest Management schemes that minimize the amount of pesticides applied, especially neonicotinoids and other broad-spectrum materials. An integrated strategy involving both biological and chemical control tactics is required for sustainable management of the pest to reduce disease spread. Some biological agents which control the *D. citri* such as lady beetles, lacewings and spiders are all well known as predators. Therefore, the present investigation had been aimed to study the biology and efficacy of various chemicals, both, solely and in combination, viz., Bifenthrin, Lambda cyhalothrin, Azadirachtin (neem oil), Cypermethrin + Profenofos,

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Deltamethrin + Triazophos and Quinalphos under laboratory conditions against *D. citri* as management strategies of an insect pest must be based on thorough ecological studies of the concerned insect pest.

**Keywords:** Asian Citrus Psyllid (ACP); Huanglongbing (HLB); neonicotinoids.

## 1. INTRODUCTION

Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is identified as a major pest of citrus in subtropical and tropical Asia [1]. Citrus greening disease or Huanglongbing is found to be the most serious disease of citrus in the world, causing the highest economic loss [2]. The status of ACP derives, not from the damage it causes, but from its functioning as the only known vector of Huanglongbing, a phloem-limited bacterial disease of international importance. Sap is sucked from the phloem of tender shoots and buds by both adults and nymphs, while adults also feed on the phloem of mature leaves. Severe infestations in orchards and gardens may cause withering, distortion, and loss of immature leaves with irregular-shaped canopies. Growth of sooty mould fungi on honeydew excreted by nymphs leads to blemishing of foliage and fruit, and can reduce photosynthesis [3]. Some synthetic conventional insecticides like neem oil, petroleum oils and organics have been successfully used in reducing its population (Setamou, et al. 2010). Foliar application of systemic insecticides viz., imidacloprid, thaimethoxam reduces 50 to 70 percent *D. citri* population. Even intensive insecticide programs against ACP are generally ineffective for preventing the introduction and spread of HLB, especially in new plantings [4]. Extensive pesticide applications are causing psyllid resistance and probable damage to bees and beneficial insects. So, it is important to devise IPM schemes that minimize the amount of pesticides applied, especially neonicotinoids and other broad-spectrum materials. Possible strategies include trapping, biological control, repellents, attractants, and application of pesticides such as horticultural oils with low impact on beneficial insects and biological controls [5]. Infected trees may respond to heat and nutrients. Antibiotics such as ampicillin will kill the pathogen. Long range hopes are resistant species for replants, cross protection with non-pathogenic microbes, or treatment with bacterial antagonists [6,4,7]. IPM actions depend on infestation levels of psyllids, and the percentage of infected trees [8,1]. An integrated strategy involving both biological and chemical control

tactics is required for sustainable management of the pest to reduce disease spread. Biological control has always been an important component of citrus insect pest management [9]. Some biological agents which control the *D. citri* such as lady beetles, lacewings and spiders are all well known as predators [10,11]. These and other predators were observed to inflict 80–100% mortality to *D. citri*. Two exotic hymenopteran parasitoids of ACP, *Diaphorencyrtis aligarhensis* (Encyrtidae) and *Tamaraxia radiata* (Eulophidae) were introduced in Florida [12]. *Tamaraxia radiata* is now widely distributed in Florida citrus ecosystems [13]. Insecticides are presently a critical component of ACP management. The systemic neonicotinoid insecticides, thiamethoxam, imidacloprid and clothianidin and a new insecticide cyantraniliprole are allowed in Florida citrus but their use as soil applications is limited by rate restrictions to young trees [14]. Therefore, the present investigation has been aimed to study the biology and efficacy of some chemicals against *D. citri*, as management strategies of an insect pest must be based on thorough ecological studies of the concerned insect pest. The study is supposed to provide essential bridging information for devising effective management strategies against *D. citri* by providing information in areas concerning with citrus production.

## 2. MATERIALS AND METHODS

To assess the efficacy of various chemicals, singly and in combination, viz., Bifenthrin, Lambda cyhalothrin, Azadirachtin (neem oil), Cypermethrin + Profenofos, Deltamethrin + Triazophos and Quinalphos, the experiments were conducted on adults of *D. citri* under laboratory conditions at Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh in 2019. There were six treatments. A control experiment (spray with distilled water) was run parallel with the main experiment and each was replicated thrice. The recommended doses of respective chemicals were prepared by adding distilled water in a conical flask (Table 1). All the prepared formulation of respective chemicals used for the application on adults of *D. citri* under

controlled conditions. The counted number of adults (10 individuals for each concentration and each insecticide) were selected from stock culture maintained in Biochemical Oxygen Demand (BOD) incubator and were exposed to above prepared formulations of respective chemicals by “Leaf dip bioassay” and mortality was observed after 12, 24 and 48 hours (Plate 1 & Plate 2).

**Leaf dip method dipping technique:** The citrus leaves were plucked from the tree and cleaned with moist cotton to remove the dirt particles, if any. After cleaning, the leaves were dipped in respective chemicals for one minute and then allowed to dry at room temperature for 15-20 minutes. Adults were released for feeding on separate leaves, after wrapping its tip with a moist cotton swab to prevent it from drying.

**Table 1. Types of chemicals used**

| Sl. no. | Formulations                     | Concentration used( $\mu$ l/100 ml) | Trade name  | Source                     | Type |
|---------|----------------------------------|-------------------------------------|-------------|----------------------------|------|
| 1.      | Bifenthrin 10%                   | 10, 15, 20                          | Markar      | Dhanuka Agritech Limited   | EC   |
| 2.      | Azadirachtin 0.15%, 1500ppm      | 300, 400, 500                       | Aastha Neem | Aastha Organics            | EC   |
| 3.      | Lambda cyhalothrin 5%            | 40, 70, 100                         | Baghban     | Biomass Lab.               | EC   |
| 4.      | Cypermethrin 4% + Profenofos 40% | 30, 40, 50                          | Hitcel      | Excel Crop Care Ltd.       | EC   |
| 5.      | Deltamethrin 1% + Triazophos 35% | 40, 70, 100                         | Deltex      | Excel Crop Care Ltd.       | EC   |
| 6.      | Quinalphos 25%                   | 100, 150, 200                       | Flash       | Indofil Industries Limited | EC   |



**Plate 1. Plastic container with treated leaves in incubator**



**Plate 2. Chemicals used and micro pipette**

### 3. RESULTS AND DISCUSSION

The studies on bio-efficacy of chemicals (Bifenthrin, Neem oil, Lambda cyhalothrin, Cypermethrin + Profenophos, Deltamethrin + Triazophos and Quinalphos) against *D. citri* Kuwayama were carried out under controlled conditions. With regard to efficacy of chemicals, the total percent mortality and concentration (dose) of the chemicals was found to be positively correlated. Highest mortality was found after 48 hours of the treatment at the maximum concentration of each chemical. Amongst the three concentrations undertaken for each chemical, maximum per cent mortality occurred at the maximum concentration in each of them. Cypermethrin + Profenophos was overall found to be most effective at all the three concentrations at 12, 24, 48 hours with a total mortality of 75.56±15.03%. It was followed by

Lambda cyhalothrin with 74.45±13.47% total mortality which was at par with Quinalphos. This was followed by Deltamethrin + Triazophos and Bifenthrin with 71.11±11.70% and 70.00±18.56% total per cent mortality respectively. Least per cent mortality was observed by Neem oil at the maximum concentration, amongst all, which was 65.56±15.03% (Table 2). It was also concluded by the probit analysis that the LC<sub>50</sub> value of Bifenthrin was found to be the least, hence the most toxic and that of neem oil was found to be maximum, hence the least toxic, after 12 hours of time interval. Similar results were obtained after 24 hours and 48 hours of time interval (Tables 3,4,5).

Out of 30 adults, mortality (in numbers) was shown for each chemical at 12, 24, 48 hours (Figs. 1 to 6).

**Table 2. Bio-efficacy of different insecticides at different concentrations and time intervals**

| Insecticides               | Recommended concentration | Mortality (Mean ± SD)  |                        |                        | Total mortality (%)      |
|----------------------------|---------------------------|------------------------|------------------------|------------------------|--------------------------|
|                            |                           | 12 hours               | 24 hours               | 48 hours               |                          |
| Bifenthrin                 | 10 µl/100 ml              | 1.67±0.58 <sup>d</sup> | 3.00±1.00 <sup>d</sup> | 4.00±1.00 <sup>f</sup> | 28.89±11.70 <sup>c</sup> |
|                            | 15 µl/100 ml              | 2.33±1.53 <sup>c</sup> | 3.67±1.53 <sup>c</sup> | 5.00±2.00 <sup>e</sup> | 36.67±13.34 <sup>c</sup> |
|                            | 20 µl/100 ml              | 5.33±1.53 <sup>a</sup> | 6.67±1.53 <sup>a</sup> | 9.00±1.00 <sup>a</sup> | 70.00±18.56 <sup>a</sup> |
| Cypermethrin + Profenophos | 30 µl/100 ml              | 3.00±2.00 <sup>c</sup> | 5.33±1.53 <sup>b</sup> | 6.00±1.73 <sup>d</sup> | 47.78±15.75 <sup>b</sup> |
|                            | 40 µl/100 ml              | 3.33±2.08 <sup>b</sup> | 6.67±2.52 <sup>a</sup> | 8.00±1.73 <sup>b</sup> | 60.00±24.04 <sup>a</sup> |
| Lambda cyhalothrin         | 50 µl/100 ml              | 6.00±1.00 <sup>a</sup> | 7.67±1.53 <sup>a</sup> | 8.33±1.00 <sup>a</sup> | 75.56±15.03 <sup>a</sup> |
|                            | 40 µL/100 ml              | 2.67±0.58 <sup>c</sup> | 4.00±1.00 <sup>c</sup> | 7.00±1.00 <sup>c</sup> | 45.56±22.19 <sup>b</sup> |
|                            | 70 µl/100 ml              | 3.33±1.53 <sup>b</sup> | 5.33±0.58 <sup>b</sup> | 7.67±1.53 <sup>c</sup> | 54.44±21.69 <sup>b</sup> |
| Deltamethrin +Triazophos   | 100 µl/100 ml             | 6.00±1.00 <sup>a</sup> | 7.67±0.58 <sup>a</sup> | 8.67±0.58 <sup>a</sup> | 74.45±13.47 <sup>a</sup> |
|                            | 40 µl/100 ml              | 3.00±1.00 <sup>c</sup> | 4.67±1.15 <sup>c</sup> | 5.67±1.53 <sup>d</sup> | 44.45±13.47 <sup>b</sup> |
|                            | 70 µl/100 ml              | 4.00±1.00 <sup>b</sup> | 5.00±1.00 <sup>b</sup> | 7.33±0.58 <sup>c</sup> | 54.44±17.10 <sup>b</sup> |
| Quinalphos                 | 100 µl/100 ml             | 6.00±1.00 <sup>a</sup> | 7.00±1.00 <sup>a</sup> | 8.33±1.15 <sup>a</sup> | 71.11±11.70 <sup>a</sup> |
|                            | 100 µl/100 ml             | 3.33±0.58 <sup>b</sup> | 4.00±1.00 <sup>c</sup> | 6.33±0.58 <sup>d</sup> | 45.55±15.75 <sup>b</sup> |
|                            | 150 µl/100 ml             | 4.33±1.53 <sup>b</sup> | 6.00±1.00 <sup>b</sup> | 8.00±1.00 <sup>b</sup> | 61.11±18.36 <sup>a</sup> |
| Neem oil                   | 200 µl/100 ml             | 6.00±1.73 <sup>a</sup> | 7.67±1.53 <sup>a</sup> | 8.67±1.53 <sup>a</sup> | 74.45±13.47 <sup>a</sup> |
|                            | 300 µl/100 ml             | 1.67±0.58 <sup>d</sup> | 3.67±0.58 <sup>c</sup> | 5.00±1.00 <sup>e</sup> | 47.78±30.06 <sup>b</sup> |
|                            | 400 µl/100 ml             | 2.33±0.58 <sup>c</sup> | 4.67±1.53 <sup>c</sup> | 6.67±0.58 <sup>c</sup> | 45.56±21.69 <sup>b</sup> |
|                            | 500 µl/100 ml             | 5.00±1.00 <sup>a</sup> | 6.67±1.53 <sup>a</sup> | 8.00±1.00 <sup>b</sup> | 65.56±15.03 <sup>a</sup> |
| LSD                        |                           | 1.221                  | 1.277                  | 1.184                  | 17.522                   |
| DF                         |                           | 17,53                  | 17,53                  | 17,53                  | 17,53                    |
| F                          |                           | 6.05                   | 7.02                   | 10.34                  | 3.4                      |
| P                          |                           | 0.000                  | 0.000                  | 0.000                  | 0.001                    |

**Table 3. Effect of insecticides on citrus psylla, *Diphorina citri* after 12 hours**

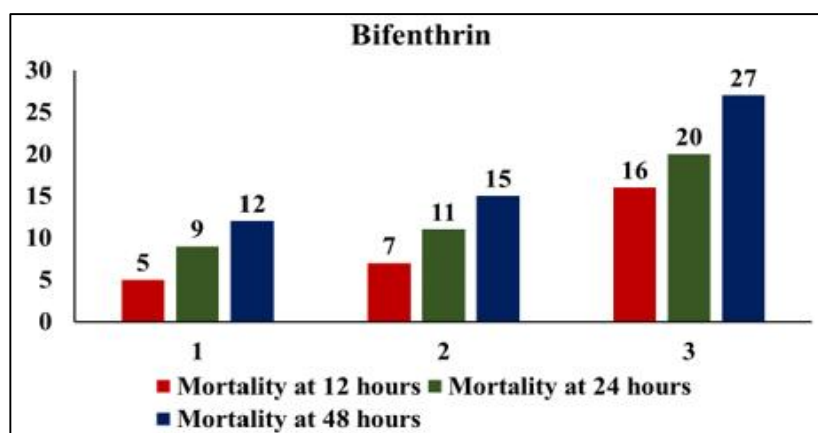
| Insecticides              | Chi-Square | LC <sub>50</sub> (µl/100 ml) | Log(con.) value | Co-relation coefficient | R <sup>2</sup> | Regression equation |
|---------------------------|------------|------------------------------|-----------------|-------------------------|----------------|---------------------|
| Bifenthrin                | 1.48       | 20.55                        | 1.31            | 0.94                    | 0.88           | y = 1.10x - 7.17    |
| Cypermethrin+ Profenophos | 1.40       | 45.85                        | 1.66            | 0.91                    | 0.83           | y = 0.45x - 5.67    |
| Lambda cyhalothrin        | 1.37       | 87.86                        | 1.94            | 0.95                    | 0.89           | y = 0.17x + 0.33    |
| Deltamethrin+ Triazophos  | 0.50       | 81.09                        | 1.91            | 0.98                    | 0.96           | y = 0.15x + 2.50    |
| Quinalphos                | 0.23       | 162.62                       | 2.21            | 0.99                    | 0.98           | y = 0.08x + 1.67    |
| Neem oil                  | 0.98       | 525.99                       | 2.72            | 0.95                    | 0.89           | y = 0.05x + 11.00   |

**Table 4. Effect of insecticides on citrus psylla, *Diphorina citri* after 24 hours**

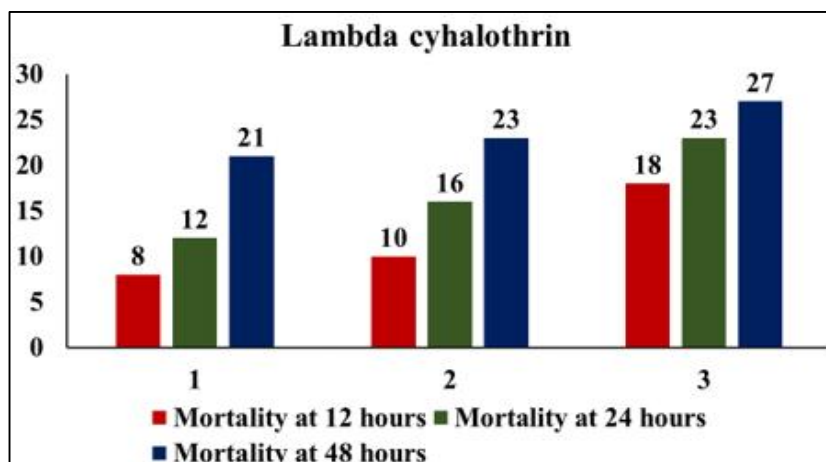
| Insecticides              | Chi-Square | LC <sub>50</sub><br>(µl/100 ml) | Log(con.)<br>value | Co-relation<br>coefficient | R <sup>2</sup> | Regression<br>equation |
|---------------------------|------------|---------------------------------|--------------------|----------------------------|----------------|------------------------|
| Bifenthrin                | 1.67       | 16.14                           | 1.21               | 0.94                       | 0.88           | y = 1.10x - 3.17       |
| Cypermethrin+ Profenophos | 0.00       | 28.16                           | 1.45               | 1.00                       | 0.99           | y = 0.35x + 5.67       |
| Lambda cyhalothrin        | 0.82       | 54.79                           | 1.74               | 0.99                       | 0.98           | y = 0.19x + 4.17       |
| Deltamethrin+ Triazophos  | 1.02       | 51.67                           | 1.71               | 0.92                       | 0.86           | y = 0.12x + 8.50       |
| Quinalphos                | 0.05       | 121.38                          | 2.08               | 1.00                       | 1.00           | y = 0.12x + 1.17       |
| Neem oil                  | 0.39       | 391.49                          | 2.59               | 0.98                       | 0.96           | y = 0.045x - 3.00      |

**Table 5. Effect of insecticides on citrus psylla, *Diphorina citri* after 48 hours**

| Insecticides              | Chi-square | LC <sub>50</sub><br>(µl/100 ml) | Log(con.)<br>value | Co-relation<br>coefficient | R <sup>2</sup> | Regression<br>equation |
|---------------------------|------------|---------------------------------|--------------------|----------------------------|----------------|------------------------|
| Bifenthrin                | 4.44       | 12.38                           | 1.09               | 0.95                       | 0.89           | y = 1.50x - 4.50       |
| Cypermethrin+ Profenophos | 0.00       | 26.44                           | 1.42               | 0.98                       | 0.96           | y = 0.45x + 5.00       |
| Quinalphos                | 0.23       | 17.54                           | 1.24               | 0.99                       | 0.99           | y = 0.09x + 17.50      |
| Deltamethrin+ Triazophos  | 0.01       | 33.20                           | 1.52               | 0.99                       | 0.98           | y = 0.14x + 12.00      |
| Quinalphos                | 0.02       | 73.35                           | 1.87               | 0.97                       | 0.94           | y = 0.07x + 12.50      |
| Neem oil                  | 0.02       | 302.08                          | 2.48               | 1.00                       | 1.00           | y = 0.05x + 1.67       |



**Fig. 1. Efficacy of bifenthrin**



**Fig. 2. Efficacy of lambda cyhalothrin**

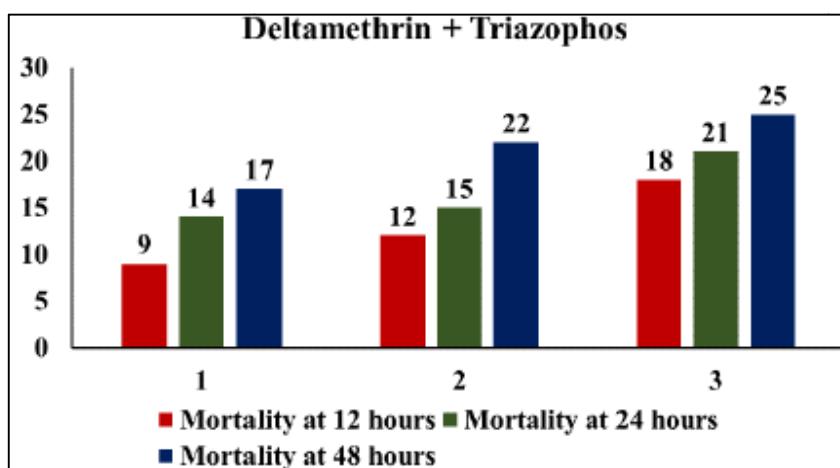


Fig. 3. Efficacy of Deltamethrin + Triazophos

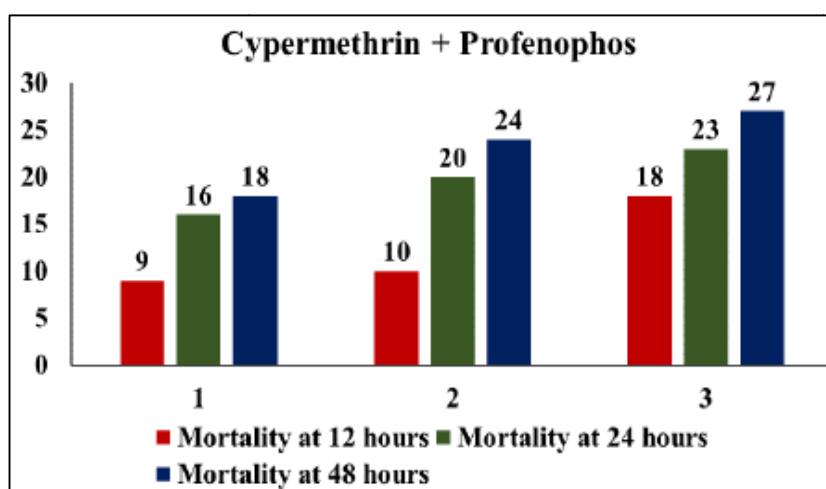


Fig. 4. Efficacy of Cypermethrin + Profenophos

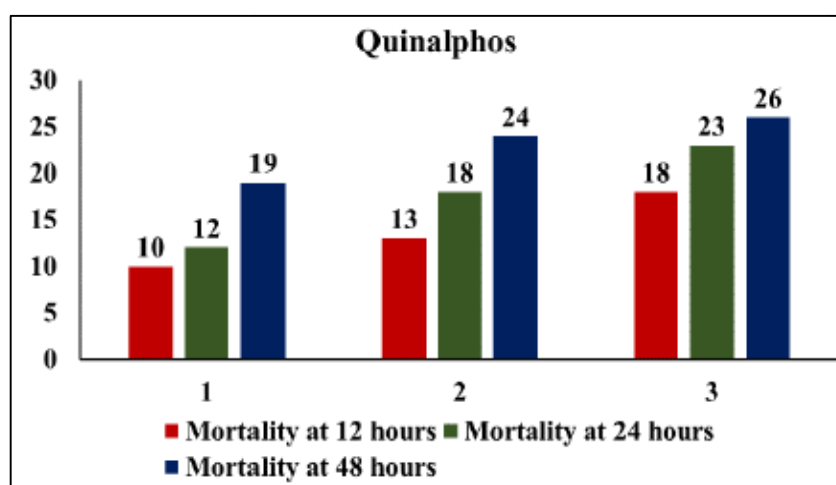


Fig. 5. Efficacy of quinalphos

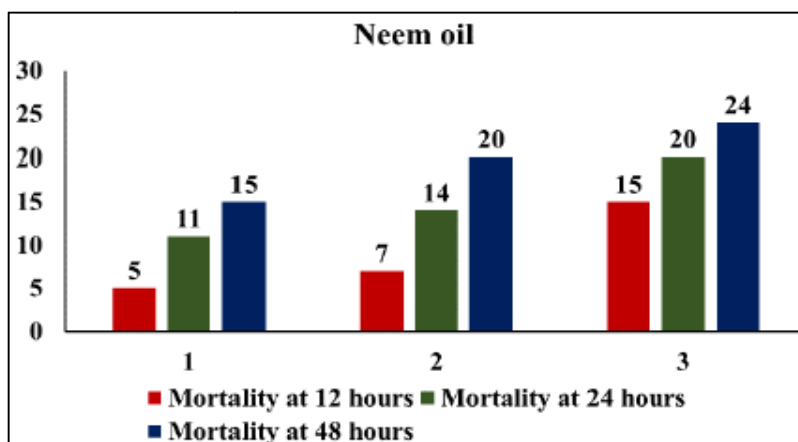


Fig. 6. Efficacy of neem oil

Finally, it was inferred that insecticides proved to be more effective than botanical (neem oil). Effectiveness against *D. citri* adults was rated in the following order- Bifenthrin > Lambda Cyhalothrin > Cypermethrin + Profenophos > Deltamethrin + Triazophos > Quinalphos > Neem Oil.

Chakravarthi, et al. [15] tested nine insecticides and observed that profenofos, triazophos and imidacloprid gave complete control after 7 days of spraying, whereas, azadirachtin (0.03%) and neem (*Azadirachta indica*) oil also achieved higher level of control (around 90%). Chakravarthi, et al. [15] also observed that triazophos @ 0.025% and profenophos @ 0.025% were effective and persistent in action as compared to botanicals like neem oil (1.0%), pongamia oil (1.0%) and animal origin insecticide, fish oil rosin soap (0.2%) which were only effective for a short period after their application. Sharma, 2008 tested the bio-efficacy of different insecticides viz, dimethoate (0.075%), oxydemeton methyl (0.075%), imidacloprid (0.008%), chlorpyrifos (0.1%), triazophos (0.1%), thiamethoxam (0.008%) and quinalphos (0.075%) showed significantly high (91-100%) nymphal reduction of citrus psylla. It was reported that neem soap @ 5 g/litre, pongamia soap @ 5 g/litre, neem oil @ 6.76 ml/litre and azadirachtin (10000 ppm) @ 3.65 ml/litre were found most effective than *Bacillus thuringiensis*, *Verticillium lecanii* and sweet flag against second instar nymphs of *D. citri* at 15 days after application. Dalvaniya, et al. [16] reported that among the treatments, imidacloprid 17.8 SL @ 3.0 ml/10 litres water was most effective with minimum population of *D. citri* at one, three, seven and 10 days after spray, followed by

diafenthiuron 50 EC @ 4.0 ml/10 litres water and thiamethoxam 25 WDG @ 3.20 g/10L of water.

#### 4. CONCLUSION

With regard to mode of exposure, leaf dip method was preferred to be used. It was found that insecticides proved to be more effective than botanical (neem oil). Amongst insecticides, Cypermethrin + Profenophos was overall found the most effective at all the three concentrations at 12, 24, 48 hours with a total percent mortality of  $75.56 \pm 15.03\%$ . Out of 12, 24 and 48 hours after treatment, highest mortality was found after 48 hours at all the concentrations taken. Amongst the three concentrations undertaken for each chemical, maximum mortality occurred at the maximum concentration in each of them. Least per cent mortality was observed by Neem oil at the maximum concentration, amongst all, which was  $65.56 \pm 15.03\%$ . Out of 30 adults, mortality (in numbers) was shown for each chemical at 12, 24, 48 hours.  $LC_{50}$  value of Bifenthrin was found to be least, hence the most toxic and that of neem oil was found to be maximum, hence the least toxic, after 12 hours of time interval. Similar results were obtained after 24 hours and 48 hours of time interval. Effectiveness against *D. citri* adults was finally rated in the following order- Bifenthrin > Lambda Cyhalothrin > Cypermethrin + Profenophos > Deltamethrin + Triazophos > Quinalphos > Neem Oil.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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