

## **Original Research Article**

### **Bio-efficacy of certain chemicals against *Diaphorina citri* (Homoptera:Psyllidae)**

#### **ABSTRACT**

Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) is identified as a major pest of citrus in subtropical and tropical Asia. Citrus greening disease or Huanglongbing 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 ACP are generally ineffective for preventing the introduction and spread of HLB, 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 IPM 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, 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.

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Key-words: Asian Citrus Psyllid, Huanglongbing, neonicotinoids

#### **INTRODUCTION**

Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is identified as a major pest of citrus in subtropical and tropical Asia (Halbert and Manjunath, 2004). Citrus

**Comment [U1]:** This term has not been used for a long time, please consult nomenclatural data

**Comment [U2]:** Homoptera is no longer used, please replace with Hemiptera: Sternorrhyncha

**Comment [U3]:** Put the name described and not the acronym. So too for HLB and IPM. What does it mean?

greening disease or Huanglongbing is found to be the most serious disease –of –citrus in –the world, causing the highest economic loss (Aubert *et al.*,1996). Its status 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 (Wang *et al.*, 2002). 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 (Hall *et al.*, 2013). 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 (Tiwari *et al.* 2011). 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 (Grafton-Cardwell *et al.* 2013). 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 (de Graca 1991; Hall *et al.* 2013; Zhang *et al.* 2013). IPM actions depend on infestation levels of psyllids, and the percentage of infected trees (Bové 2006; Halbert and Manjunath 2004). 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 (McCoy, 1985). Some biological agents which control the *D. citri* such as lady beetles, lacewings and spiders are all well known as predators (Qureshi and Stansly, 2010 and Michaud, 2004). 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 (McFarland and Hoy, 2001). *Tamaraxia radiata* is now widely distributed in Florida citrus ecosystems (Hoy and Nguyen, 2001). Insecticides are presently a critical component of ACP management. The systemic neonicotinoid insecticides,

Comment [U4]: Whose status?

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 (Qureshi *et al.*, 2011). 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.

## MATERIALS AND METHOD

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. 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-A). All the prepared formulation of respective chemicals were used for the application on adults of *D,citri* under controlled conditions. The counted number of adults (10 individuals) were selected from stock culture maintained in 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.

**Comment [U5]:** Where were the bioassays carried out?

**Comment [U6]:** Were 10 individuals used for each concentration or for each insecticide? Please detail and clarify

**Table A: Types of chemicals used**

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Sl. No.	Formulations	Concentration used( $\mu$ l/100ml)	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
7.	Control	-	-	-	-

**Leaf dip method:**

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.

Comment [U7]: Please draw a scheme to make it clearer

## RESULTS AND DISCUSSION

The studies on bio-efficacy of chemicals (Bifenthrin, Neem oil, Lambda cyhalothrin, Cypermethrin + Profenophos, Deltamethrin + Triazophos and Quinalphos) against *D.citri* Kuwamaya were carried out under controlled conditions at Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh in 2019.

**Comment [U8]:** This is in the methodology and not here

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  $75.56 \pm 15.03\%$ . It was followed by Lambda cyhalothrin with  $74.45 \pm 13.47\%$  total mortality which was at par with Quinalphos. This was followed by Deltamethrin+Triazophos and Bifenthrin with  $71.11 \pm 11.70\%$  and  $70.00 \pm 18.56\%$  total per cent mortality respectively. Least per cent mortality was observed by Neem oil at the maximum concentration, amongst all, which was  $65.56 \pm 15.03\%$  (Table 2). It was also concluded by the probit analysis that the  $LC_{50}$  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 (Table 2,3,4). Out of 30 adults, mortality (in numbers) was shown for each chemical at 12, 24, 48 hours (Figure- 1 to 6). 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- cypermethrin+profenophos > lambda cyhalothrin=quinalphos > deltamethrin+triazophos > neem oil.

Chakravarthi *et al.*, 1998 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%). Chakravarthy, 1998 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. Rao and Shivankar, 2011 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.*, (2015) 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.

**Comment [U9]:** The work data remains to be discussed and compared with data from other authors. For example, discuss about ecological and behavioral aspects of the insect and environmental damage.

## CONCLUSION

With regard to mode of exposure, leaf dip method was preferred to be used over any other mode. 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 per cent 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- cypermethrin+profenophos> lambda cyhalothrin=quinalphos> deltamethrin+triazophos> neem oil.

**Comment [U10]:** What other mode was used?

**Comment [U11]:** This is a result and not a conclusion, please elaborate another conclusion.

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UNDER PEER REVIEW



Plate 1 A: Plastic container (100ml) with treated leaves in BOD



Plate 1 B: Insecticides used and micro pipette

Table-1: Bio-efficacy of different insecticides at different concentrations and time intervals:

Insecticides	Recommended concentration	Mortality (Mean $\pm$ SD)			Total Mortality (%)
		12 hours	24 hours	48 hours	
Bifenthrin	10 $\mu$ l/100ml	1.67 $\pm$ 0.58 <sup>d</sup>	3.00 $\pm$ 1.00 <sup>d</sup>	4.00 $\pm$ 1.00 <sup>f</sup>	28.89 $\pm$ 11.70 <sup>c</sup>
	15 $\mu$ l/100ml	2.33 $\pm$ 1.53 <sup>c</sup>	3.67 $\pm$ 1.53 <sup>c</sup>	5.00 $\pm$ 2.00 <sup>e</sup>	36.67 $\pm$ 13.34 <sup>c</sup>
	20 $\mu$ l/100ml	5.33 $\pm$ 1.53 <sup>a</sup>	6.67 $\pm$ 1.53 <sup>a</sup>	9.00 $\pm$ 1.00 <sup>a</sup>	70.00 $\pm$ 18.56 <sup>a</sup>
Cypermethrin + Profenophos	30 $\mu$ l/100ml	3.00 $\pm$ 2.00 <sup>c</sup>	5.33 $\pm$ 1.53 <sup>b</sup>	6.00 $\pm$ 1.73 <sup>d</sup>	47.78 $\pm$ 15.75 <sup>b</sup>
	40 $\mu$ l/100ml	3.33 $\pm$ 2.08 <sup>b</sup>	6.67 $\pm$ 2.52 <sup>a</sup>	8.00 $\pm$ 1.73 <sup>b</sup>	60.00 $\pm$ 24.04 <sup>a</sup>
	50 $\mu$ l/100ml	6.00 $\pm$ 1.00 <sup>a</sup>	7.67 $\pm$ 1.53 <sup>a</sup>	8.33 $\pm$ 1.00 <sup>a</sup>	75.56 $\pm$ 15.03 <sup>a</sup>
Lambda cyhalothrin	40 $\mu$ l/100ml	2.67 $\pm$ 0.58 <sup>c</sup>	4.00 $\pm$ 1.00 <sup>c</sup>	7.00 $\pm$ 1.00 <sup>c</sup>	45.56 $\pm$ 22.19 <sup>b</sup>
	70 $\mu$ l/100ml	3.33 $\pm$ 1.53 <sup>b</sup>	5.33 $\pm$ 0.58 <sup>b</sup>	7.67 $\pm$ 1.53 <sup>c</sup>	54.44 $\pm$ 21.69 <sup>b</sup>
	100 $\mu$ l/100ml	6.00 $\pm$ 1.00 <sup>a</sup>	7.67 $\pm$ 0.58 <sup>a</sup>	8.67 $\pm$ 0.58 <sup>a</sup>	74.45 $\pm$ 13.47 <sup>a</sup>
Deltamethrin +Triazophos	40 $\mu$ l/100ml	3.00 $\pm$ 1.00 <sup>c</sup>	4.67 $\pm$ 1.15 <sup>c</sup>	5.67 $\pm$ 1.53 <sup>d</sup>	44.45 $\pm$ 13.47 <sup>b</sup>
	70 $\mu$ l/100ml	4.00 $\pm$ 1.00 <sup>b</sup>	5.00 $\pm$ 1.00 <sup>b</sup>	7.33 $\pm$ 0.58 <sup>c</sup>	54.44 $\pm$ 17.10 <sup>b</sup>
	100 $\mu$ l/100ml	6.00 $\pm$ 1.00 <sup>a</sup>	7.00 $\pm$ 1.00 <sup>a</sup>	8.33 $\pm$ 1.15 <sup>a</sup>	71.11 $\pm$ 11.70 <sup>a</sup>
Quinalphos	100 $\mu$ l/100ml	3.33 $\pm$ 0.58 <sup>b</sup>	4.00 $\pm$ 1.00 <sup>c</sup>	6.33 $\pm$ 0.58 <sup>d</sup>	45.55 $\pm$ 15.75 <sup>b</sup>
	150 $\mu$ l/100ml	4.33 $\pm$ 1.53 <sup>b</sup>	6.00 $\pm$ 1.00 <sup>b</sup>	8.00 $\pm$ 1.00 <sup>b</sup>	61.11 $\pm$ 18.36 <sup>a</sup>
	200 $\mu$ l/100ml	6.00 $\pm$ 1.73 <sup>a</sup>	7.67 $\pm$ 1.53 <sup>a</sup>	8.67 $\pm$ 1.53 <sup>a</sup>	74.45 $\pm$ 13.47 <sup>a</sup>
Neem oil	300 $\mu$ l/100ml	1.67 $\pm$ 0.58 <sup>d</sup>	3.67 $\pm$ 0.58 <sup>c</sup>	5.00 $\pm$ 1.00 <sup>c</sup>	47.78 $\pm$ 30.06 <sup>b</sup>
	400 $\mu$ l/100ml	2.33 $\pm$ 0.58 <sup>c</sup>	4.67 $\pm$ 1.53 <sup>c</sup>	6.67 $\pm$ 0.58 <sup>c</sup>	45.56 $\pm$ 21.69 <sup>b</sup>
	500 $\mu$ l/100ml	5.00 $\pm$ 1.00 <sup>a</sup>	6.67 $\pm$ 1.53 <sup>a</sup>	8.00 $\pm$ 1.00 <sup>b</sup>	65.56 $\pm$ 15.03 <sup>a</sup>
<i>LSD</i>		1.221	1.277	1.184	17.522
<i>DF</i>		17.53	17.53	17.53	17.53
<i>F</i>		6.05	7.02	10.34	3.4
<i>P</i>		0	0	0	0.001

Table-2: Effect of insecticides on citrus psylla, *Diphorina citri* after 12 hours:

Insecticides	Chi-Square	LC <sub>50</sub> μl/100ml	Log(con.) Value	Correlation 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 - 11.0

Table-3: Effect of insecticides on citrus psylla, *Diphorina citri* after 24 hours:

Insecticides	Chi-Square	LC <sub>50</sub> μl/100ml	Log(con.) Value	Correlation coefficient	R <sup>2</sup>	Regression 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-4: Effect of insecticides on citrus psylla, *Diphorina citri* after 48 hours:

Insecticides	Chi-Square	LC <sub>50</sub> μl/100ml	Log(con.) Value	Correlation coefficient	R <sup>2</sup>	Regression 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
Lambda cyhalothrin	0.23	17.54	1.24	0.99	0.99	y = 0.09x + 17.5
Deltamethrin +Triazophos	0.01	33.20	1.52	0.99	0.98	y = 0.14x + 12.0
Quinalphos	0.02	73.35	1.87	0.97	0.94	y = 0.07x + 12.5
Neem oil	0.02	302.08	2.48	1.00	1.00	y = 0.05x + 1.67

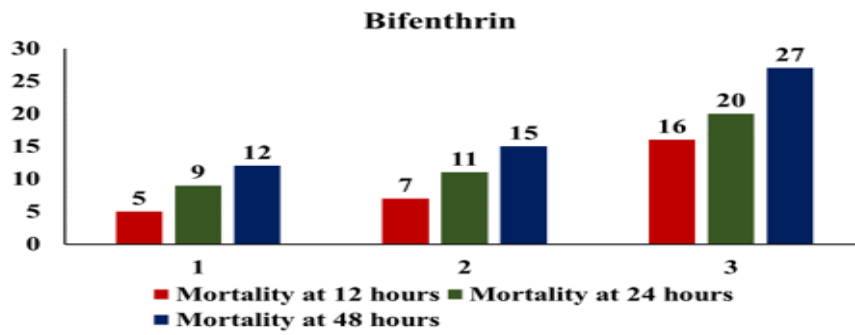


Fig 1: Efficacy of Bifenthrin

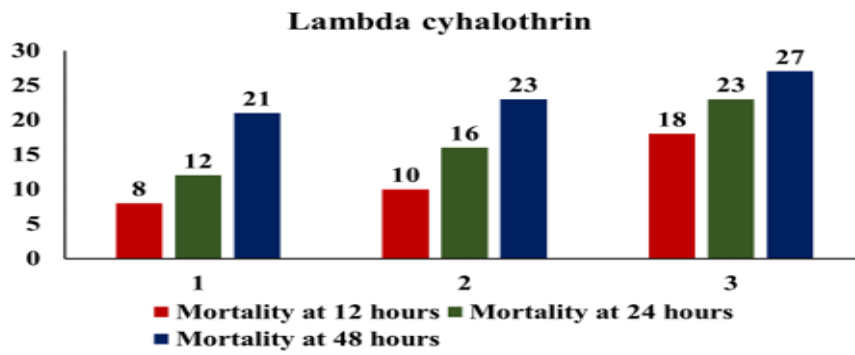


Fig 2: Efficacy of Lambda cyhalothrin

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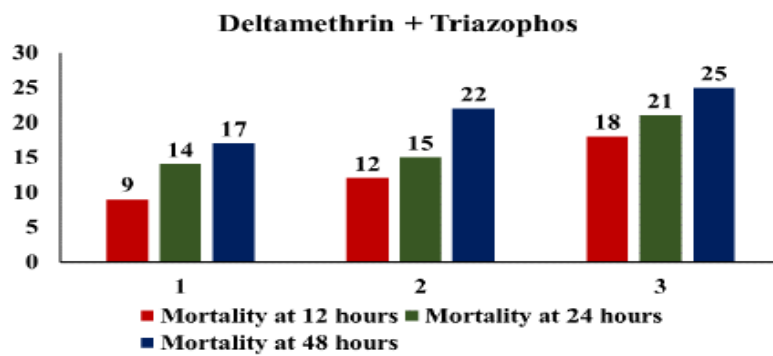


Fig 3: Efficacy of Deltamethrin+Triazophos

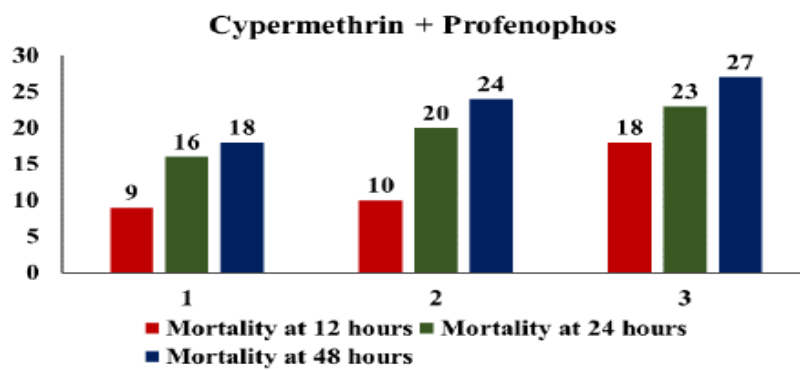


Fig 4: Efficacy of Cypermethrin+Profenophos



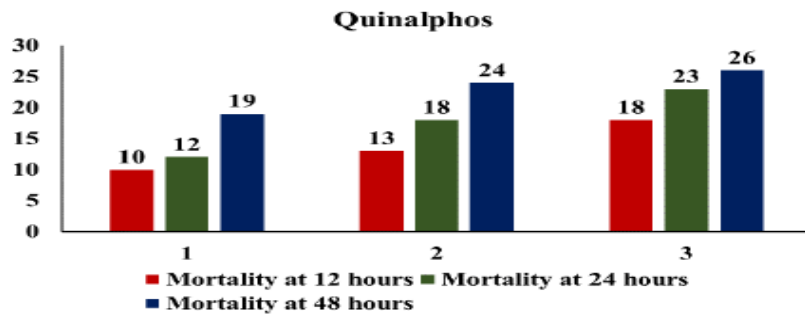


Fig 5: Efficacy of Quinalphos

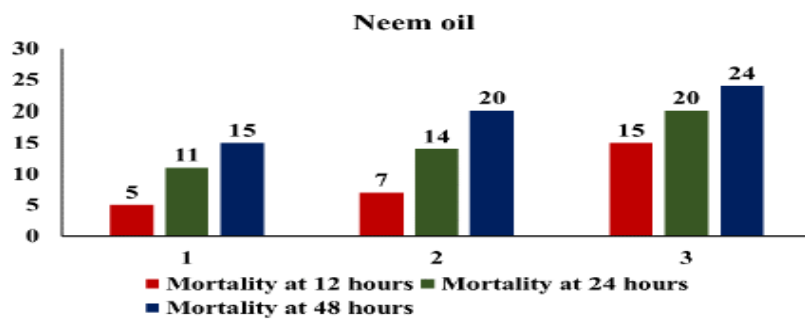


Fig 6: Efficacy of Neem oil

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