

Relative toxicity of different insecticides to Asian citrus psylla (*Diaphorinacitri* Kuwayama)

ABSTRACT

Asian citrus psyllids *Diaphorinacitri* (Kuwayama) act as vector of the devastating citrus disease, Huanglongbing (HLB) or citrus greening which is widely spread in citrus growing regions. The use of insecticide for control of citrus psylla is one of the prominent and effective method to overcome their menace. However, recently very few insecticide have been found under label claim for this pest. The present investigation was conducted in order to know the toxicity of some new chemistry insecticide against *D. citri*. The bioassay experiment was conducted during the year 2022-23 ~~at Toxicology Laboratory, Department of Entomology, Dr. PDKV., Akola (M.S.)~~. The toxicity was assessed through uptake bioassay technique for systemic insecticide by using fresh citrus twig. The results demonstrated that, amongst the five tested insecticide imidacloprid 17.8% SL was the most the effective insecticide with lowest LC₅₀ values. However, except spirotetramat 15.3 % OD, the LC₅₀ values of thiamethoxam 25% WG, abamectin 1.9% EC, fenpropathrin 30% EC were ~~lying-placed~~ within the fiducial limit of most toxic insecticide i.e. imidacloprid 17.8% SL. They are considered as at par and equally toxic to *D. citri*. The relative toxicity trend against adult psylla was imidacloprid > abamectin > thiamethoxam > fenpropathrin > spirotetramat and for nymph imidacloprid > thiamethoxam > abamectin > fenpropathrin > spirotetramat against psylla. Abamectin showed least toxic effect against nymph than adult psylla.

Keywords: ~~Huanglongbing disease, Bioassay, Abamectin, Bioassay, Huanglongbing, Imidacloprid, Psylla, Thiamethoxam, relative toxicity.~~

Comment [s1]: Please mention means of mortality for treatments while comparing.

Introduction:

The Asian citrus psyllid, (*Diaphorinacitri* Kuwayama) (Homoptera: Psyllidae), is widely distributed in southern Asia. It is an important pest of citrus in several countries as it acts as a vector of "*Candidatus Liberibacter asiaticus*" a most devastating disease called greening disease or Huanglongbing (HLB), caused by "*Candidatus Liberibacter asiaticus*". The heavy citrus crop losses to citrus orchards imposed by the disease emanates has resulted in the destruction of several citrus industries in Asia and Africa (Manjunath *et al.*, 2008). The fifth instar nymphs of the pest, acting as a main vector of greening bacterium, which accelerates are accounted for the decline about 83 to 95 percent crop losses (Dawane *et al.* 2013). Both nymphs and adults of citrus psylla suck the cell sap with the help of their sharp piercing mouth parts, resulted curling, leaves and flowers defoliation and die back of branches from tip to downward and premature dropping of fruits (Shah and Saleem, 2000). HLB acts to disrupt the phloem of the tree by limiting its ability to uptake nutrients. Initially this leads to yellowing of leaves, infested trees showing stunted growth, sparsely foliated branches, unseasonal bloom, promotion of premature leaf and fruit drop, twig dieback, production of small, misshapen fruit that contain bitter juice with no economic value (Salifu *et al.* 2012). Young leaves are chlorotic with green banding along the major veins. Mature leaves have yellowish-green patches between veins, fruits with small size, generally lopsided, underdeveloped, unevenly colored, hard, and poor in juice (Capoore *et al.*, 1974). The population of citrus psylla has two peaks in a year during spring and summer (Belot *et al.*, 2013), these peaks obtained coincided with the availability of new flush. Insecticides are best strategic measure to control psyllid populations. Imidacloprid and aldicarb are suggested effectively in the period of November and April (Qureshi and Stansly, 2007; 2008; Rogers *et al.*, 2008). Similarly, broad spectrum insecticides are used in winter, spring and summer season as foliar sprays (Rogers *et al.*, 2008; Rogers, 2008; Stansly *et al.*, 2009), but they give short term protection against immature psyllids (Qureshi and Stansly, 2007 and 2009). Even if, cultural, mechanical and biological control practices are not easily applicable, labour demanding and not greatly effective in heavy attack due to different reasons. The use of chemical for control of citrus psylla is one of the prominent, effective and rapid method to overcome their menace. However, recently a very few insecticide have been found under label claim for this pest. Therefore, it's a need to test some novel chemical insecticides against *D. citri*, as alternate

option to label claim insecticide in view to reduce the development of resistances and residual effect. So, keeping these facts in mind, the present investigation was aimed to determine the toxicity of some new chemistry insecticide against *D. citri*.

Methodology:

The bioassay experiment was conducted during the year 2022-23 in the Toxicology Laboratory, Department of Entomology, Dr. PDKV., Akola (M.S.). The toxicity was assessed through uptake bioassay technique for systemic insecticides (Molina *et al.*, 2022) using five insecticides *viz.* Thiamethoxam 25 % WG, Abamectin 1.9 % EC, Imidacloprid 17.8 % SL, Spirotetramat 15.3% OD, Fenprothrin 30% EC etc. Bioassays were conducted against nymph and adult stages in laboratory with four replicates per insecticide. The nymphal population of psylla was collected in the zipped plastic bags and brought to the laboratory. The adult population were carefully captured in glass vial or polythene bag and tied it with rubber band to avoid the escape. They were provided them with fresh and moisten leaves in the laboratory till the start of the bioassay. Citrus shoots (about 15 cm in length and with at least two-three terminal leaves) infested with psyllid nymphs of 15 cm (at least two three terminal leaves) was were placed in a glass tube (50 ml) with one containing an insecticide solution for 24 h. Distilled water was used as a control treatment. Each stem was protected with a paper cone with glycerin in the corners to prevent nymphs from escaping. After that, shoots was were transferred to a glass tube with water, where nymph mortality was checked after 24, 48, 72 and 96 hours.

For the adult experiment, citrus shoots were placed in each insecticide solution for 24 h. After that, shoots were transferred to a clean 50 ml tube with water. Each tube was placed inside a plastic container (1 L) with a filter paper disc on at the bottom, a 2 ml water vial as a humidity source, ten adults were released in container and covered with muslin cloth. The numbers of dead and live psyllids adults were counted observations were recorded at 6, 12, 24 and 48 hr after treatment. The corrected per cent mortalities were determined as per Abbott's formula (Abbott, 1925) and the LC_{50} values were determined by running probit analysis (Finney, 1952).

Results and Discussion:

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Comment [s2]: As far as the bioassay is concerned, I am afraid to mention that **not all the prerequisites of the tests are held properly:**

1. A very important part is the number of individuals exposed to the treatments and then counts of dead / alive numbers at proper intervals.
2. Calculation of mortality in each replication and the mean of each treatment.
3. Correction by Abbott's (if the number subjected to treatments is equal in all replications) or Henderson Tilton's if the primary subjects are unequal.
4. And the rest of the procedure....

Comment [s3]: If the Material and Methods is not clear, then the Results and subsequently Discussion cannot be evaluated.

The nymph and adult mortality of *D. citri* were exposed to different concentrations of insecticides. LC₅₀ and LC₉₀ value calculated to compare the toxicity of various insecticides (Pandi *et al.*, 2013). The bioassay results demonstrated that, amongst the five tested insecticide imidacloprid was the most effective insecticide with lowest LC₅₀ values. In case of nymphal bioassay, at the LC₅₀ levels the imidacloprid was 3.61 time more toxic than spirotetramat (Unity). Abamectin, thiamethoxam and fenpropathrin 30% EC recorded 2.97, 3.48 and 2.73 fold higher toxic than spirotetramat (Table 1). In adult, the relative toxicity of imidacloprid was found 3.13 fold more toxic than the unity i.e. spirotetramat, The next better insecticide were abamectin, thiamethoxam and fenpropathrin recording relative toxicity values, respectively of 2.94, 2.70 and 2.44 fold more toxic than spirotetramat (Unity), which was found least toxic to *D. citri* (Table 2).

A similar trend of toxicity at LC₉₀ level was observed against nymph and adult for the all the tested insecticides. However, except spirotetramat, the LC₅₀ values of thiamethoxam, abamectin, fenpropathrin were lying within the fiducial limit of most toxic insecticide i.e. imidacloprid. They are considered as at par and equally toxic to *D. citri*.

The above results, regarding the relative toxicity of different insecticides are in agreement with the findings of previous workers; Chauhan and Srivastava (2018) who tested the field efficacy of different insecticide against *D. citri* and revealed that, imidacloprid in most effective in reduction of nymph and adult population of psylla, followed by thiamethoxam and novaluron. The present study is also supported by Powell *et al.* (2007) who reported that, the biannual or more frequent applications of Admire (imidacloprid) significantly reduced psyllid population, percentage of trees infestations and percentage of flushes infestations.

Similar results were reported by Childers and Rogers (2005) reported that, foliar applications with thiamethoxam and imidacloprid was found to reduce psylla population of 86.9-96.4 % and 91.5-98.0 % over control up to 12 DAT (Sharma 2008). Foliar application with abamectin @ 0.3ml/l resulted 90.2-92.5% reduction in psylla population (Rao and Shivankar 2011). Sarada *et al.* (2014) also reported that, foliar application of abamectin @ 0.0007 % reduced psylla population more than 80 % after 3 and 7 DAT. The present findings are in line with the findings of Boina *et al.* (2009) who reported that feeding by psylla adults on daily treated plants with a sublethal concentration (0.1µ/L) of imidacloprid significantly reduced the population of citrus psylla. Khan *et al.* (2012) reported that, bioassay with methomyl and imidacloprid inflicted 97-100% mortality to *D. citri*. They also observed gradually decline in

mortality in case of methomyl but it was very quick in imidachloprid and lambda cyhalothrin.

According to Wankhade *et al.*(2015) the treatment with abamectin 1.9 EC @ 0.4 ml/l (0.0007%) was found most effective in recording the least nymphal population under field conditions followed by imidacloprid 17.8 SL which proved effective against citrus psylla. The present studies are in line with Molina *et al.*(2022) who reported that thiamethoxam and imidacloprid were equally effective on African citrus psylla (*Trioza erytreae*) nymphs and were persistent over time. Spirotetramat and abamectin were the least effective on *T. erytreae* nymph control, which collaborates with the results in the present studies. Khan *et al.* (2013), reported a cumulative mortality of adult *D. citri* with Confidor® (imidacloprid), Movento® (spirotetramat) and Radiant® (spinetoram) were more than 95% at commercially recommended doses. Qasim and Hussain (2015) reported that, imidacloprid was better than Bifenthrin against *D. citri*. The results are in congruence with Dalvaniya *et al.* (2015) who reported that foliar applications of imidacloprid and thiamethoxam significantly reduced *D. citri* population in lime plants. In accordance with the present finding of Rao *et al.*(2011) revealed that, module with foliar application of thiamethoxam, abamectin and imidacloprid at 20 days interval before, during and after flushing periods was found to be superior in protecting new flush against *D. citri*.

Conclusion:

Based on the relative toxicity values obtained in the present studies the various insecticide tested can be arranged in sequence of better toxicity as imidacloprid > abamectin > thiamethoxam > fenprothrin > spirotetramat against adult psylla and imidacloprid > thiamethoxam > abamectin > fenprothrin > spirotetramat against nymph.

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Fig. 1 Adult psylla bioassay



Fig. 2 Nymph psylla bioassay

UNDER PEER REVIEW

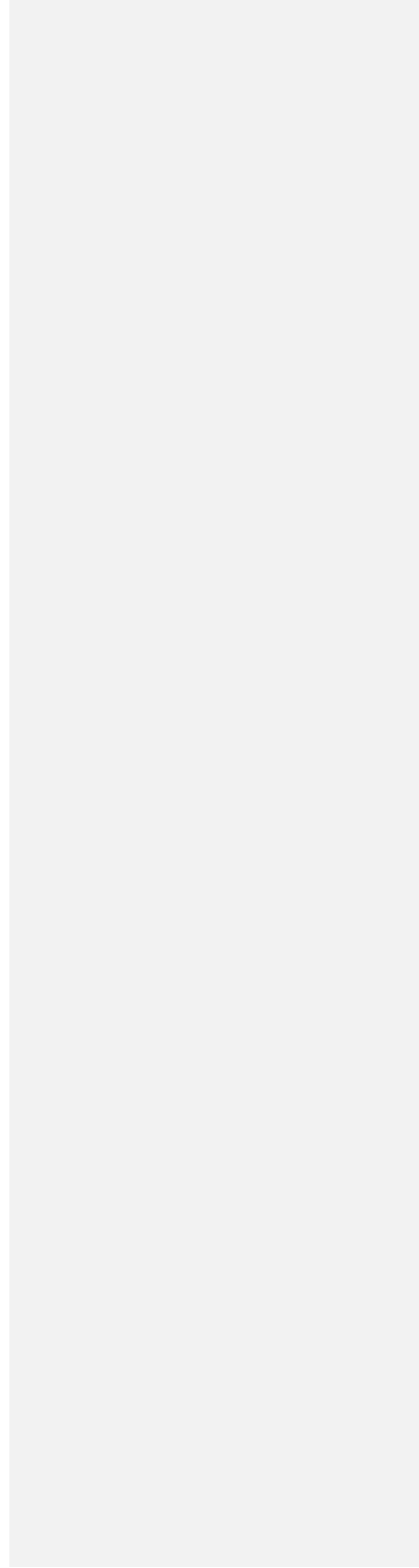


Table 1. Median lethal concentration (LC 50) of different insecticides on citrus psylla (nymph) under laboratory conditions.

Sr. no.	Insecticide	Slope	Regression equation (y)	Chi-Square	LC50 %	Fiducial Limit		LC90 %	Fiducial Limit		Relative Toxicity atLC50	Relative Toxicity Rank
						Lower	Upper		Lower	Upper		
1	Thiamethoxam 25 % WG	2.72	$Y=4.19+2.72x$	0.566	0.029	0.018	0.045	0.086	0.055	0.134	3.48	2
2	Abamectin 1.9 % EC	17.28	$Y=25.35+17.28x$	0.622	0.034	0.032	0.037	0.040	0.038	0.044	2.97	3
3	Imidacloprid 17.8 % SL	4.33	$Y=6.70+4.33x$	0.754	0.028	0.021	0.038	0.056	0.041	0.076	3.61	1
4	Spirotetramat 15.3 % OD	4.46	$Y=4.44+4.46x$	0.737	0.101	0.077	0.133	0.196	0.149	0.257	1.00	5
5	Fenproprathrin 30 % EC	20.04	$Y=28.73+20.04x$	0.755	0.037	0.035	0.039	0.043	0.040	0.045	2.73	4

Table 2. Median lethal concentration (LC 50) of different insecticides on citrus psylla (Adult) under laboratory conditions.

Sr. no.	Insecticide	Slope	Regression equation (y)	Chi-Square	LC ₅₀ (%)	Fiducial Limit		LC ₉₀ (%)	Fiducial Limit		Relative Toxicity at LC ₅₀	Relative Toxicity Rank
						Lower	Upper		Lower	Upper		
1	Thiamethoxam 25 % WG	2.56	Y=3.67+2.56x	0.020	0.037	0.023	0.060	0.117	0.072	0.191	2.70	3
2	Abamectin 1.9 % EC	16.94	Y=24.97+16.94x	0.952	0.034	0.031	0.036	0.040	0.037	0.043	2.94	2
3	Imidacloprid 17.8 % SL	3.30	Y=4.92+3.30x	0.283	0.032	0.022	0.047	0.079	0.054	0.114	3.13	1
4	Spirotetramat 15.3 % OD	4.54	Y=4.54+4.54x	0.811	0.100	0.076	0.131	0.192	0.146	0.251	1.00	5
5	Fenproprithrin 30 % EC	16.35	Y=22.73+16.35x	0.837	0.041	0.037	0.044	0.049	0.045	0.053	2.44	4