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Assessment of residual effects of three neonicotinoids commonly used to control Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)

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Abstract

The Asian citrus psyllid, *Diaphorina citri*, Kuwayama (ACP), is widely distributed in southern Asia. It was first reported in USA during summer 1998 in Florida and spring 2001 in Texas. The damage of *D. citri* results mainly in *Candidatus liberibacter* spp. transmission and consequent greening disease induction. Moreover, plant-sap deprivation and honeydew excretion push the growth of abundant sooty mould. The initial and residual efficacy of three neonicotinoids was evaluated by laboratory and greenhouse trial at 3 and 32 days, respectively. Imidacloprid, Thiamethoxam and Dinotefuran were used as a foliar application at four rates (25%, 50%, 75% and 100% of recommended rate) in comparison with distilled water control. The greenhouse trial (semi-field test) was designed as a complete randomized bloc with individual cages as unit. The ACP adults (n=10) were introduced into cages and counted at 4, 18 and 29 days after treatment with and the residual effect was monitored up to 32 days. Regarding to laboratory bioassays, the treatments were very significant (P<0.0001) and showed an immediate (24 and 48h after treatment) effects of Dinotefuran on ACP adults at recommended rate with 88% and 92%, respectively. The mortality rates related to other neonicotinoids (Thiamethoxam and Imidacloprid) were statistically equal 72h after treatment and ranged from 74% to 100%. The results obtained in greenhouse trials showed that the 3 neonicotinoids provide a perfect control of ACP adults in a 18 days interval from distribution with mortality ranging from 93% to 100%. Interestingly, Thiamethoxam was still active 21 DAT. The corresponding LD_{50}'s obtained were 0.038, 0.041 and 0.250 ppm for Imidacloprid, Dinotefuran and Thiamethoxam, respectively.

Keywords: residue, yellow dragon disease, citrus, DL_{50}

Evaluation des effets résiduels de trois néonicotinoïdes couramment utilisés pour lutter contre le psylle Asiatique des agrumes *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)

Résumé

Le psylle asiatique des agrumes, *Diaphorina citri*, Kuwayama, est largement distribué en Asie du Sud. Il a été signalé pour la première fois aux États-Unis au cours de l'été 1998 en Floride et au printemps 2001 au Texas. Les dégâts de *D. citri* se manifestent principalement par transmission de la maladie du dragon jaune des agrumes causée par *Candidatus liberibacter* spp. De plus, la succion de la sève des feuilles et l'excrétion du miellat favorisent la croissance de la fumagine. L'efficacité initiale et résiduelle de trois néonicotinoïdes a été évaluée au laboratoire et par essai en serre pendant 3 et 32 jours, respectivement. L'imidaclopride, le thiaméthoxame et le dinotefuran ont été utilisés comme application foliaire à quatre doses (25%, 50%, 75% et 100% de la dose recommandée) et de l'eau distillée comme témoin. L'essai en serre (essai semi-terrain) a été conçu en bloc aléatoire complet avec des cages individuelles. Les adultes de *D. citri* (n=10) ont été introduits dans des cages initialement, 4, 18 et 29 jours après le traitement et l'effet résiduel a été suivi pendant 32 jours. En ce qui concerne les essais biologiques au laboratoire, les traitements étaient très hautement significatifs ($P < 0,0001$) et ont montré des effets immédiats (24 et 48 h après le traitement) du dinotefuran sur les adultes *D. citri* aux doses recommandées avec 88% et 92%, respectivement. Les taux de mortalité liés aux autres néonicotinoïdes (thiaméthoxame et imidaclopride) étaient statistiquement égaux 72 heures après le traitement et variaient de 74% à 100%. Les résultats obtenus dans les essais en serre ont montré que les 3 néonicotinoïdes fournissent un contrôle parfait des adultes *D. citri* pendant 18 jours et les taux de mortalité variaient de 93% à 100%. De façon intéressante, le thiaméthoxame a persisté pendant 21 jours après le traitement. Les DL_{50}s correspondantes obtenues étaient de 0,038, 0,041 et 0,250 ppm pour l'imidaclopride, le dinotéfurane et le thiaméthoxam, respectivement.

Mots-clés : résidus, maladie du dragon jaune, agrumes, DL_{50}

تقييم التأثيرات المتبقية لثلاثة نيونيكوتينويد شائعة الاستخدام للسيطرة على (Diaphorina citri Kuwayama (Hemiptera: Psyllidae

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ملخص

انتشار Diaphorina citri، Kuwayama على نطاق واسع في جنوب آسيا. تم الإبلاغ عنه لأول مرة في الولايات المتحدة الأمريكية خلال صيف 1998 في فلوريدا وربيع 2001 في تكساس. ينتج عن ضرر *D. citri* تشوه في أوراق الشجر مما يتسبب في نمو للعفن ونقل مرض التخضير الناجم عن *Candidatus Liberibacter*.

تم تقييم الفعالية الأولية والباقية لثلاثة نيونيكوتينويد في المختبر ومن خلال اختبار شبه ميداني خلال الفترة ما بين 3 و 32 يومًا على التوالي. تم استخدام إيميداكلوبريد و الثيامثوكسام و دينوتيفوران كرش ورقي بأربعة معدلات (25٪ و 50٪ و 75٪ و 100٪ من المعدل الموصى به) و الماء المقطر كشاهد. تم تصميم الاختبار شبه ميداني ككتلة عشوائية تمامًا مع أقفاص فردية. تم إدخال 10 من البالغين *D. citri*في أقفاص في البداية، ثم 4 و 18 و 29 يومًا بعد الرش وتم رصد التأثير المتبقي خلال 32 يومًا. فيما يتعلق بالمقاييس الحيوية المختبرية، كانت المعالجات ذات أهمية عالية للغاية (P <0.0001) وأظهرت آثار فورية (24 و 48 ساعة بعد الرش) من دينوتيفوران على *D. citri* بمعدل موصى به بنسبة 88٪ و 92٪ على التوالي. كانت معدلات الوفيات المتعلقة بالمبيدات الأخرى (الثيامثوكسام و إيميداكلوبريد) متساوية إحصائياً بعد 72 ساعة وتراوحت من 74٪ إلى 100٪. أظهرت النتائج التي تم الحصول عليها في تجارب اختبار شبه ميداني أن المبيدات الثلاث توفر تحكمًا مثاليًا على *D. citri* خلال 18 يومًا وتراوحت معدلات الوفيات من 93٪ إلى 100٪. ومن المثير للاهتمام أن الثيامثوكسام ظل متبقيا لمدة 21 يومًا بعد العلاج.

كانت قيم LD_{50} المقابلة التي تم الحصول عليها هي 0.038 و 0.041 و 0.250 جزء في المليون لكل من إيميداكلوبريد ودينوتيفوران وثياميثوكسام ، على التوالي.

الكلمات المفتاحية: بقايا، مرض التنين الأصفر، الحمضيات، جرعة مميتة 50%

Introduction

Citrus greening (or HLB: Huang Long Bing) has caused considerable losses in citrus production in some Asian countries, Africa, the Indian subcontinent and the Arabian Peninsula. Since 2004, the disease has been reported in Brazil and then in the United States of America in 2005. To date, the disease has not been detected in Australia and in the Mediterranean basin (CABI, 2020). Wherever the disease has appeared, citrus production is compromised by the loss of millions of trees. In 2006, production in the United States of America registered the lowest production (10.5 million tons) of its history after the introduction of this pathogen. The production was dropped from 15.7 million tons in 1997 to 7 million tons in 2017 (FAOSTAT, 2019). Economically, Citrus Greening directly affects the orchards killing infected trees, reducing yields (quantity and quality) and ultimately increasing production costs related to vector control and fertilization. In Florida, studies estimated that HLB reduced the value of production of $ 4.51 billions between the 2006-07 and 2010-11 seasons. Furthermore, chemical control actions to manage *Diaphorina citri* populations vectors can cost up to 1,000 US $ / Acre (21,000,00 Dhs / Ha) and foliar fertilizers applications to mitigate the HLB-induced greening disease push the costs of 200-600 US $ / Acre (ie 4,200.00 to 12,600.00 Dhs / Ha).

Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), was described from Taiwan and is native to Asia. It has been known in the Western Hemisphere for several decades in Brazil (Hodkinson & White 1981). During the past decade, its range has expanded into northern South America and the Caribbean. Females can lay up to 800 eggs and the following 5 larval stages life-cycle lasts from 14 to 48 days depending on the temperature. The Psyllid can give up to 10 generations per year. The presence of *D. citri* nymphs in a citrus orchard is very characteristic by the secretion of waxy exudates visible at a distance. Adults can overwinter for up to 6 months. The adult is winged and 2.5 mm long, standing with its brown spotted wings at 45° angle respect the plant support. Fluctuations in the population of *D. citri* are closely related to the phenology of the tree since spawning occurs only on young shoots. The 0.01 to 0.15 mm almond-shaped eggs are light yellow to orange, depending on the age. The lower T°C thresholds is -1° for nymphs and -10 ° for adults. ACP is better detected using sticky traps in citrus groves than by direct observations (Miranda et al., 2017). *D. citri* can transmit both African and Asian strains of Greening bacteria (*Candidatus liberibacter asiaticus* and *Candidatus liberibacter africanus*). To control this vector, application of chemical insecticide is the common measure, which increase consumer exposure to pesticide residues.

The amount of insecticide residues in plants can be estimated adopting many analytic methods, but pure analytical chemistry is not enough to evaluate the real toxic effect over the time on the target pests. For this reason, we structured the experiments to estimate the residual effect of 3 neonicotinoids (Imidacloprid, Thiamethoxam and Dinotefuran) in laboratory and greenhouse experiments in the interval between 3 and 32 DAT (Days After Treatment) respectively. In fact, the results could be a significant support to IPM program aiming to prevent Asian citrus psyllids damage and reducing the health and environment risks due to unnecessary distributions.

Material and Methods

Insects: The ACP adults used in both trials (Lab and semi-field) were collected from a rearing greenhouse at Weslaco Citrus Center (Texas A&M Kingsville University). *Diaphorina* reared on orange jasmine, *Murraya paniculata*. The collected population was maintained with any chemical distributions for more than 30 generations.

Laboratory bioassay:

Five newly emerged adults (24 to 48h old) of *D. citri* were introduced in one plastic tubes containing fresh citrus flushes. Each replicates were just sprayed (Table 1) with 2 ml of one of the formulate based on Imidacloprid (PROVADO, Bayer), Dinotefuron (VENOM, Valent) and Thiamethoxam (ACTARA, Syngenta).

Ten replicates (tubes) of 5 ACP adults and 4 concentrations of pesticides (25, 50, 75 and 100% of recommended dose) were used to complete these bioassays. The control flushes were sprayed with distillate water. The acute mortality rate was assessed 2, 24, 48 and 72 hours after treatments.

Semi-field test:

In each entomological cage (Figure 1, 80x50x50 cm) we have placed a grape fruit susceptible variety (*Citrus paradisi*) plant sprayed with one of three neonicotinoids (Imidacloprid, Dinotefuron and Thiamethoxam). Each pesticide was assessed at 4 concentration levels 25, 50, 75 and 100% of recommended dose. Distillate water was considered as control in this trial. Ten newly emerged *D. citri* adults (24 to 48h old) were introduced in each cage just after, 4, 11, 18 and 29 DAT. The mortality rate was assessed 2, 24, 48 and 72 hours after each ACP introduction to cages. Randomized block was adopted with 3 blocks (3 replicates) in the same ventilated greenhouse.

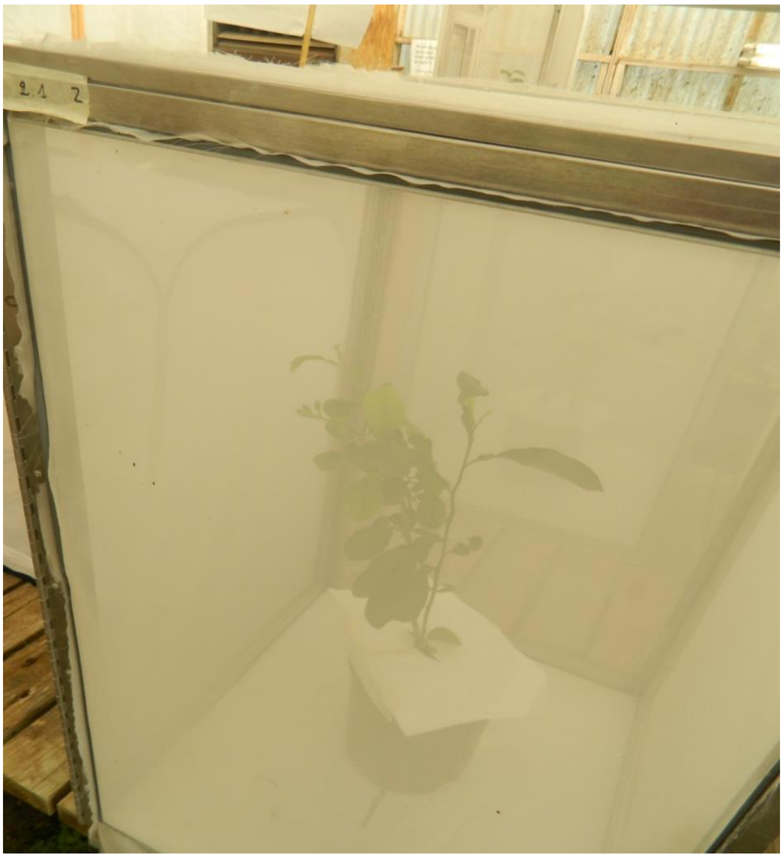

Figure 1: Experimental unit used in semi-field trial

Table 1: List of chemicals sprayed in both tests (Lab and semi-field)

Pesticide Brand Name	Active Ingredient	Rate/ Acre	PPM of A.I. in 20 ml	PPM of A.I. in 2 ml (Flush)
			Semi-field Trial	*Bioassay Trial*
ACTARA 25 WG	Thiamethoxam	5,5 oz	1.03	0.103
VENOM INSECTICIDE	Dinotefuran	4 oz	2.07	0.207
PROVADO 1.6 Flowable	Imidacloprid	20 fl oz	2.76	0.276

oz: ounce and fl oz: fuild ounce

Data analysis:

Data analysis was performed using the SAS general linear model (GLM) procedure for ANOVA and Student-Newman-Keuls for mean comparisons (SAS Institute, 2005). In order to rank and compare treatments to controls we did not use Abbott formula to correct mortality rates. The probit parameters of the concentration-mortality responses were performed using the POLO-PC statistical software (Leora Software, 1987). The LD_{50}'s and their corresponding 95% CL were calculated.

Results

Laboratory bioassay

At recommended rate, the Dinotefuran caused 70% of mortality 2h after ACP infestation (Table 2). This immediate toxic effect was not observed for Imidacloprid and Thiamethoxam which caused 28% and 38%, respectively. The Dinotefuran toxicity was maintained during the 1st, 2nd and 3rd DAT and caused 88%, 92% and 96% of mortality, respectively. Imidacloprid at recommended rate caused the same mortality 3 DAT as Dinotefuran while Thiamethoxam caused 82% of mortality.

Two hours after ACP infestation, the 75% of recommended rate of Dinotefuran and Thiamethoxam caused 52% and 48%, respectively which were statistically equal according to Student and Newman-Keuls. However, the same recommended rate of Imidacloprid has a toxic effect statistically similar to control (14%) 2h after ACP infestation but caused a complete mortality 3 DAT and then Imidacloprid was the most toxic compared to both other neonicotinoids. At the 1st and 2nd DAT, the effect of the 3 neonicotinoids was statistically similar and caused a mortality rates ranged from 64% to 78%. Three DAT, Dinotefuran and Thiamethoxam caused 82% of mortality.

At 50% of recommended rate, a low toxic effect (16% to 26%) was observed 2h after ACP infestation for 3 neonicotinoids. During the 1st and 2nd DAT, the mortality rates were moderate for Dinotefuran and Thiamethoxam and ranged from 44% to 56%. Two DAT, the Imidacloprid showed an important toxicity effect with 80% of mortality rate compared to Dinotefuran and Thiamethoxam with 54% and 56%, respectively. During the 3rd DAT, Imidacloprid caused 88% of mortality.

At 25% of recommended rate, the toxicity of 3 neonicotinoids was statistically similar immediately after treatment (2h after ACP infestation) then the mortality rates were low and ranged from 26% to 32%. The mortality rates increased moderately for 3 neonicotinoids during the 1st, 2nd and 3rd DAT and ranged from 52% and 54%, 56% and 60% and 66% and 74%, respectively.

The mortality rates of ACP adults showed an immediate (2 hours after pesticides spray) but moderate toxic effect for all doses (from full to quarter) compared to control which is statistically very highly significant (P<0,001). The Dinotefuran (70% at 2 hours after treatment) seems to have an immediate effect than two other neonicotinoids. The flushes sprayed at 75 and 100% of recommended dose showed the same effect of ACP adults (up to 80%). The doses of 75 and 100% of recommended rate gives the same control efficacy compared to control, 25 and 50.

Table 2: Mortality rates of Asian citrus psyllid adults (N=50 for each dose/Active Ingredient) caused by Dinotefuran, Imidacloprid and Thiamethoxam spray on citrus flushes in laboratory experiment.

	TREATEMENT	Dose (ppm)	Days After Treatment			
			D0SP+INF	D1	D2	D3
100% of R.R	Control	0,000	1,0±1,0a	2,0±1,4a	2,0±1,4a	6,0±2,6a
	Dinotefuran	0,207	70,0±6,1c	88,0±5,3d	92,0±5,3c	96,0±2,7c
	Imidacloprid	0,276	28,0±6,1b	60,0±3,0b	78,0±6,3b	96,0±2,7c
	Thiamethoxam	0,103	38,0±9,2b	74,0±7,9c	74,0±7,9b	82,0±6,3b
	Significance (P<5%)		***	***	***	***
75% of R.R	Control	0,000	1,0±1,0a	2,0±1,4a	2,0±1,4a	6,0±2,6a
	Dinotefuran	0,155	52,0±7,4b	66,0±6,0b	74,0±7,3b	82,0±7,0b
	Imidacloprid	0,207	14,0±5,2a	64,0±6,5b	76,0±7,2b	100,0±0,0c
	Thiamethoxam	0,077	48,0±8,0b	72,0±8b	78,0±6,3b	82,0±5,5b
	Significance (P<5%)		***	***	***	***
50% of R.R	Control	0,000	1,0±1 ,0a	2,0±1,4a	2,0±1,4a	6,0±2,6a
	Dinotefuran	0,104	26,0±7,3b	44,0±9,8b	54,0±10,3b	66,0±12,3b
	Imidacloprid	0,138	16,0±5,0b	60,0±10,8b	80,0±5,2c	88,0±3,3b
	Thiamethoxam	0,052	20,0±6,0b	52,0±8,0b	56,0±8,3b	70,0±8,0b
	Significance (P<5%)		***	***	***	***
25% of R.R	Control	0,000	1,0±1,0a	2,0±1,4a	2,0±1,4a	6,0±2,6a
	Dinotefuran	0,052	32,0±7,4b	54,0±11,2b	58,0±12,5b	66,0±10,8b
	Imidacloprid	0,069	26,0±10,8b	54,0±7,9b	60,0±6,0b	74,0±6,7b
	Thiamethoxam	0,026	26,0±7,9b	52,0±6,8b	56,0±8,3b	68,0±7,4b
	Significance (P<5%)		**	***	***	***

*D0, D1, D2 and D3: 2 hours, 1, 2 and 3 days after pesticide spray. SP: Spray, INF: ACP infestation, R.R: Recommended Rate. ** highly significant and *** very highly significant at P<5%. The means mortality within the same column for each dose followed by the same letter are not statistically different at P<5% according to the Student Newman-Keuls test.*

The LD_{50}'s analysis showed that the most toxic neonicotinoids were Imidacloprid followed by Dinotefuran (0,038 and 0,041 ppm, respectively). However, Thiamethoxam caused a moderate lower toxicity compared to both other neonicotinoids and recorded 6,6 and 6,1 times of LD_{50}'s more than Imidacloprid and Dinotefuran, respectively (Table 3).

Table 3: Susceptibility of *D. citri* to Dinotefuran, Imidacloprid and Thiamethoxam 72 hours after spray and equivalent LD_{50}

Active ingredient	LD_{50} (ppm)	95% CL	Slope±SE	g	Log (L)
Dinotefuran	0,041	0,006 to 0,066	1,948±0,504	0,443	-95,74
Imidacloprid	0,038	0,012 to 0,058	2,336±0,579	0,236	-62,95
Thiamethoxam	0,250	0,040 to 0,385	1,703±0,528	0,460	-107,2

Semi-field experiment

***At recommended rate* (Table 4)**

No significant effect was observed 2h after ACP infestation. During the 1st day after treatment (DAT, Dinotefuran and Imidaacloprid were the most toxic and showed 63% and 57% of mortality, respectively. However, the 2nd and 3rd DAT the 3 neonicotinoids caused 100% of mortality (Figure 2). During the 4th DAT and 2h after the 2nd ACP infestation no significant effect was observed. Five DAT (1 day after the 2nd ACP infestation), the mortality rates were moderate for both neonicotinoids Dinotefuran and Imidacloprid and caused 43% and 37%, respectively. At 6 and 7 DAT (2 and 3 days after the 2nd ACP infestation), the mortality rates caused by these 2 neonicotinoids were 80 and 100% for Dinotefuran and 50 and 97% for Imidacloprid. While, Thiamethoxam has no toxic effect since it caused 10% and 17% of mortality 5 and 6 DAT, respectively. However, it was very toxic 7 DAT and caused 93% of mortality which is statistically equal to both other neonicotinoids.

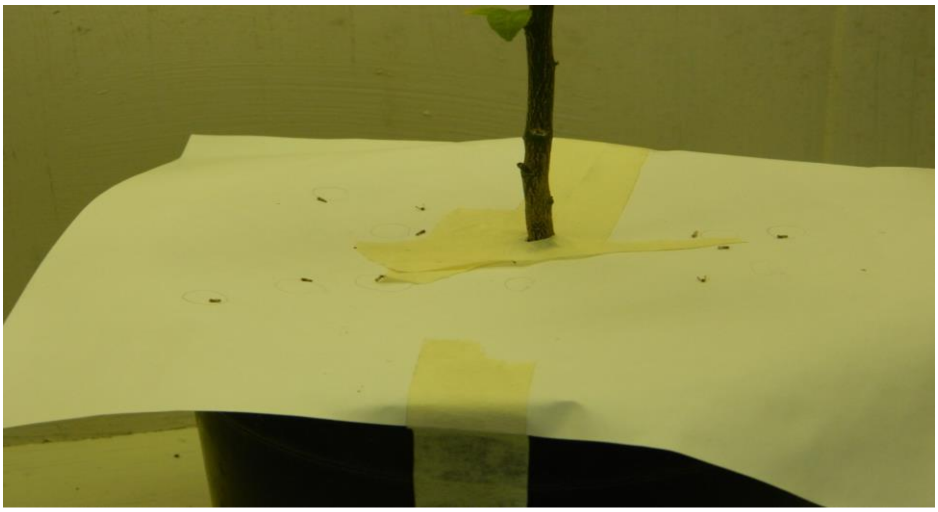

Figure 2: Total mortality of ACP adults observed 3 days after treatment of recommended rate of Imidacloprid.

During the 18 DAT (2h after 3rd ACP infestation), no immediate effect was observed. The 3 neonicotinoids caused a low toxicity 19 and 20 DAT (1 and 2 days after 3rd ACP infestation) and didn't exceed 43%. Dinotefuran showed a moderate toxic effect 21 DAT (3 days after 3rd ACP infestation) and caused 63% of mortality. Thiamethoxam was the most toxic at this time with 93% compared to Imidacloprid (80%).

During the 4th ACP infestation, no significant effect was observed during 29, 30 and 31 DAT. However, Dinotefuran, Imidacloprid and Thiamethoxam showed a low to moderate toxicity 32 DAT and the mortality rates recorded were 37%, 57% and 43%, respectively.

Table 4: Mortality rate (% ±SE) of ACP adults (N=30) after treatment of potted plants by 3 neonicotinoids at recommended rate

Days After Treatment	Control	Dinotefuran (2,07 ppm)	Imidacloprid (2,76 ppm)	Thiamethoxam (1,03 ppm)	Significance (P<5%)
D0SPINF1	-	-	-	-	
D1	0,0±0,0a	63,3±12,0b	56,7±23,3b	43,3±6,7 ab	*
D2	0,0±0,0a	73,3±17,6b	73,3±14,5b	56,7±8,8b	**
D3	13,3±6,7a	100,0±0,0b	100,0±0,0b	100,0±0,0b	***
D4INF2					
D5	3,3±3,3a	43,3±8,8b	36,7±12,0b	10,0±5,8a	*
D6	6,7±6,7a	80,0±0,0c	50,0±10,0b	16,7±6,7a	***
D7	20,0±0,0a	100,0±0,0b	96,7±3,3b	93,3±6,7b	***
D18INF4					
D19	0,0±0,0a	10,0±5,8ab	23,3±3,3b	13,3±3,3ab	*
D20	0,0±0,0a	26,7±3,3b	43,3±8,8b	26,7±3,3b	**
D21	16,7±3,3a	63,3±8,8b	80,0±5,7bc	93,3±3,3c	***
D29INF5					
D30	3,3±3,3	6,7±3,3	13,3±3,3	6,7±3,3	x
D31	3,3±3,3	16,7±6 ,7	16,7±6,7	13,3±8,8	x
D32	20,0±0,0a	36,7±3,3b	56,7±3,3b	43,3±8,8b	**

*D0, D1, D2 ………..D32: 2 hours, 1, 2 …….. 32 days after pesticide spray. SP: Spray, INF: ACP infestation, R.R: Recommended Rate. ^x No significant, * Significant, ** highly significant and *** very highly significant at P<5%. The mean mortality within the same line followed by the same letter are not statistically different at P<5% according to the Student Newman-Keuls test.*

***At 75% of recommended rate* (Table 5):**

A moderate toxicity was observed in cages treated with Imidacloprid immediately (2h after 1st ACP infestation) and showed 53% of mortality while Dinotefuran and Thiamethoxam caused low mortality rates with 33% and 37%, respectively. During the 3 following days after the 1st ACP infestation, all 3 neonicotinoids has statistically the same toxic effect increasingly from moderate to high with 47% and 100% of mortality.

At the 4th DAT (2h after 2nd ACP infestation), no significant effect was observed for 3 neonicotinoids. Five and 6 DAT (1 and 2 days after 2nd ACP infestation), the 3 neonicotinoids showed low toxic effect and the mortality rates didn't exceed 47%. The maximum toxic effect (97% to 100%) was observed 7 DAT (3 days after 2nd ACP infestation).

The 3rd ACP infestation (18, 19 and 20 DAT) showed no significant effect for 3 neonicotinoids. However, during 21 DAT (3 days after 3rd ACP infestation), a significant toxic effect was observed but didn't exceed 77% for 3 neonicotinoids. During the 4th ACP infestation (29, 30, 31 and 32 DAT), no significant effect was observed for the 3 neonicotinoids.

Table 5: Mortality rate (% ±SE) of ACP adults (N=30) after treatment of potted plants by 3 neonicotinoids at 75% of recommended rate

Days After Treatment	Control	Dinotefuran (1,55 ppm)	Imidacloprid (2,07 ppm)	Thiamethoxam (0,77 ppm)	Significance (P<5%)
D0SPINF1					
D1	0,0±0,0a	46,7±13,3b	60,0±15,3b	50,0±5,8b	*
D2	0,0±0,0a	53,3±16,7b	66,7±12,0b	56,7±8,8b	*
D3	13,3±6,7a	96,7±3,3b	100,0±0,0b	100,0±0,0b	***
D4INF2					
D5	3,3±3,3a	40,0±5,8b	23,3±3,3ab	26,7±12,0ab	*
D6	6,7±6,7a	43,3±3,3b	46,7±3,3b	36,7±8,8b	**
D7	20,0±0,0a	96,7±3,3b	100,0±0,0b	100,0±0,0b	***
D18INF4					
D19	0,0±0,0	16,7±6,7	23,3±8,8	10,0±0,0	x
D20	0,0±0,0	20,0±5,8	26,7±12,0	23,3±6,7	x
D21	16,7±3,3a	66,7±8,8b	73,3±6,7b	76,7±8,8b	*
D29INF5					
D30	3,3±3,3	13,3±6,7	3,3±3,3	0,0±0,0	x
D31	3,3±3,3	16,7±8,8	13,3±3,3	0,0±0,0	x
D32	20,0±0,0	50,0±0,0	40,0±10,0	50,0±10,0	x

*D0, D1, D2 ………..D32: 2 hours, 1, 2 …….. 32 days after pesticide spray. SP: Spray, INF: ACP infestation, R.R: Recommended Rate. [x] No significant, * Significant, ** highly significant and *** very highly significant at P<5%. The mean mortality within the same line followed by the same letter are not statistically different at P<5% according to the Student Newman-Keuls test.*

***At 50% of recommended rate* (Table 6):**

No significant effect was observed 2h after 1st ACP infestation for 3 neonicotinoids. However, the mortality rates recorded was low to moderate 1 and 2 DAT and ranged from 40% to 60% for all tested neonicotinoids. Three DAT, the high toxic effect was observed for 3 neonicotinoids (97% to 100%).

During the 2nd ACP infestation (4, 5 and 6 DAT), no significant effect was observed for 3 neonicotinoids. Seven DAT, the mortality rates were high and ranged from 83% to 93%.

At the 3rd ACP infestation (18 DAT), no significant effect was observed during the 1st and 2nd following DAT (18, 19 and 20) for 3 neonicotinoids. However, at 21 DAT, the mortality rates observed were moderate and caused 47%, 60% and 77% for Imidacloprid, thiamethoxam and Dinotefuran, respectively. No toxic effect was observed during the 4th ACP infestation (29 DAT) and 2 following days (30 and 31 DAT) for 3 neonicotinoids. These tested neonicotinoids has statistically the same toxic effect 32 DAT and the mortality rates ranged from 37% to 50%.

Table 6: Mortality rate (% ±SE) of ACP adults (N=30) after treatment of potted plants by 3 neonicotinoids at 50% of recommended rate

Days After Treatment	Control	Dinotefuran (1,04 ppm)	Imidacloprid (1,38 ppm)	Thiamethoxam (0,52 ppm)	Significance (P<5%)
D0SPINF1					
D1	0,0±0,0a	50,0±15,3b	46,7±12,0b	40,0±0,0b	*
D2	0,0±0,0a	56,7±18,6b	60,0±11,5b	60,0±5,8b	*
D3	13,3±6,7a	96,7±3,3b	96,7±3,3b	100,0±0,0b	***
D4INF2					
D5	3,3±3,3	16,7±3,3	23,3±6,7	16,7±3,3	x
D6	6,7±6,7	33,3±12,0	43,3±8,8	20,0±5,8	x
D7	20,0±0,0a	93,3±3,3b	86,7±8,8b	83,3±12,0b	***
D18INF4					
D19	0,0±0,0	16,7±8,8	3,3±3,3	3,3±3,3	x
D20	0,0±0,0	23,3±12,0	10,0±0,0	6,7±3,3	x
D21	16,7±3,3a	76,7±6,7b	46,7±6,7b	60,0±15,3b	**
D29INF5					
D30	3,3±3,3	0,0±0,0	3,3±3,3	0,0±0,0	x
D31	3,3±3,3	6,7±6,7	10,0±0,0	0,0±0,0	x
D32	20,0±0,0a	50,0±5,8b	36,7±6,7b	43,3±3,3b	*

*D0, D1, D2 ………..D32: 2 hours, 1, 2 …….. 32 days after pesticide spray. SP: Spray, INF: ACP infestation, R.R: Recommended Rate. [x] No significant, * Significant, ** highly significant and *** very highly significant at P<5%. The mean mortality within the same line followed by the same letter are not statistically different at P<5% according to the Student Newman-Keuls test.*

At 25% of recommended rate **(Table 7):**

Immediately after spray and 2h after the 1st ACP infestation, no significant effect was observed for 3 neonicotinoids. Low toxic effect was observed 1 DAT with 30%, 33% and 40% for Thiamethoxam, Imidacloprid and Dinotefuran, respectively. During the 2nd DAT, the mortality caused by 3 neonicotinoids was moderate and showed 37%, 40% and 63% for Imidacloprid, Thiamethoxam and Dinotefuran, respectively. High toxic

effect was observed 3 DAT with 100% for 3 neonicotinoids. No significant effect was observed during the 2nd ACP infestation (4 DAT) and the 2 following days (5 and 6 DAT) for 3 neonicotinoids. The 3rd day after the 2nd ACP infestation (7 DAT) showed a high toxic effect for 3 neonicotinoids with 83%, 87% and 100% for Dinotefuran, Imidacloprid and Thiamethoxam, respectively.

During the 3rd (18, 19, 20 and 21 DAT) and 4th (29, 30, 31 and 32 DAT) ACP infestation, no significant effect was observed for 3 neonicotinoids tested.

Table 7: Mortality rate (% ±SE) of ACP adults (N=30) after treatment of potted plants by 3 neonicotinoids at 25% of recommended rate

Days After Treatment	Control	Dinotefuran (0,52 ppm)	Imidacloprid (0,69 ppm)	Thiamethoxam (0,26 ppm)	Significance (P<5%)
D0SPINF1					
D1	0,0±0,0a	40,0±5,8b	33,3±12,0ab	30,0±10,0ab	*
D2	0,0±0,0a	63,3±3,3b	36,7±14,5b	40,0±10,0b	**
D3	13,3±6,7a	100,0±0,0b	100,0±0,0b	100,0±0,0b	***
D4INF2					
D5	3,3±3,3	20,0±10;0	16,7±8,8	3,3±3,3	x
D6	6,7±6,7	36,7±3,3	26,7±8,8	26,7±12,0	x
D7	20,0±0,0a	83,3±12,0b	86,7±3,3b	100,00±0,0b	***
D18INF4					
D19	0,0±0,0	6,7±6,7	6,7±3,3	6,7±3,3	x
D20	0,0±0,0	20,0±10,0	20,0±10,0	13,3±8,8	x
D21	16,7±3,3	43,3±3,3	36,7±3,3	36,7±12,0	x
D29INF5					
D30	3,3±3,3	3,3±3,3	3,3±3,3	10,0±10,0	x
D31	3,3±3,3	3,3±3,3	20,0±10,0	10,0±10,0	x
D32	20,0±0,0	20,0±0,0	26,7±6,7	33,3±3,3	x

*D0, D1, D2 ………..D32: 2 hours, 1, 2 …….. 32 days after pesticide spray. SP: Spray, INF: ACP infestation, R.R: Recommended Rate. ^{x} No significant, * Significant, ** highly significant and *** very highly significant at P<5%. The mean mortality within the same line followed by the same letter are not statistically different at P<5% according to the Student Newman-Keuls test.*

Discussion

The neonicotinoids are systemic insecticides affecting acetylcholine receptors in the nervous system of pests. They are classified by the Insecticide Resistance Action Committee Mode of Action (IRAC MoA) classification as nicotinic acetylcholine receptor (nAChR) competitive modulators which cause post-synaptic receptor blockage and belong to group 4A (IRAC, 2018). The mean active ingredients of this class of insecticides are acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam. The first one registered was imidacloprid (Palumbo et al., 2001). The foliar spray was reported to be effective to control many pests (Walgenbach & Scoof 2011). In fact, the soil application and trunk injection were more efficient than foliar spray regarding the residual efficacy on target pests and allow to reduce side effects on non-target species (Byrne et al., 2012 and Wallingford et al., 2012). Degradation of pesticides is governed by several factors such as evaporation that is dependent on temperature and biological dilution, which is dependent on the growth of plant, biochemical decomposition and photolysis (Walgenbach et al., 1991). However, recently Wu et al., 2020 reported that the accumulation of neonicotinoids residues has an environmental negative risks due to their relatively long half-life on soil. Among all neonicotinoids, acetamiprid represents the most toxic risks in soil according to the same study.

The study of Ugine et al., 2012 showed that imidacloprid was detected 21 days after treatment in Norway maple to control *Anoplophora glabripennis* (LC_{50}=1,3 ppm) and moreover this neonicotinoid was reported to have an effect on *D. citri* 95 days after treatment (Sétamou et al., 2010). George et al., (2007) reported that the residual effect of Imidacloprid still effective until 30 days after treatment of Japaneese beetle *Popillia japonica*. More recent study (Byrne et al., 2016), reported that the peak concentrations Imidacloprid and Dinetuferan was detected 1 week and 2 weeks for Thiamethoxam in citrus leaves which prevent the infestations by ACP eggs and nymphs. Also, the highest mortality of ACP was obtained by Thiamethoxam and the lowest by dinotefuran.

The current study could be a support to integrated pest management of Asian citrus psyllid especially as a preventive measure helping to face eventual introduced of this pest to Morocco. However, further studies regarding chemical and biological control should be undertaken under Moroccan environmental conditions.

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