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Alternative insecticides for controlling *Diaphorina citri* Kuwayama (Hemiptera: Lividae) in lemon

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Abstract

In Mexico, the management of *Diaphorina citri* in Mexican lime is mainly based on scheduled applications of synthetic pesticides, without prior sampling, or rotation of the different toxicological groups, which leads to an excessive growth of the populations of this insect due to the development of resistance because of the excessive application of these products. This study aimed to evaluate the efficacy of organosynthetic insecticides for the control of *D. citri* in Mexican lime and the effect of these new molecules on its main natural enemies. Sixteen new molecules were evaluated with ten replications and a completely randomized experimental design, and the variables studied were number of psyllids and natural enemies. Sampling was carried out on 2, 8, 15, 20 and 29 days after application. The following was performed using the SAS statistical program: normality and homoscedasticity test on the data of variances, Anova, and Duncan's test ($p< 0.05$). Compared to the previous sampling, Tolfenpyrad® had a mortality rate of 97.5, 92.6, 95.1 and 100% at 8, 15, 20 and 29 days after application, respectively. In contrast, Cyantraniliprole® showed 93.9, 69.7, 81.81 and 100% at 8, 15, 20 and 29 days after application. However, some other products, such as Chlorantraniliprole® and Neem plus oil can be an alternative at a low pest density.

Keywords:

Asian psyllids, control, natural enemies.

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Introduction

Mexico is the leading producer of Mexican limes in the world, with an area of 120 000 ha, of which Michoacán has 75 000 ha and produces 900 000 t, with an economic spillover of $2 595 billion pesos (SIAP, 2022). Citrus are attacked by a large number of pests and diseases that affect their vigor, reduce production and fruit quality, and sometimes cause the loss of trees. These organisms attack various parts of the tree; the Asian citrus psyllid, scales, thrips and red spider mites stand out for their importance (Miranda-Salcedo *et al*., 2020a).

Currently, the Asian citrus psyllid *Diaphorina citri* (Kuwayama) 1908 (Hemiptera: Liviidae) is the most important pest that attacks citrus in Mexico. The insect is distributed throughout the Mexican Republic (López-Arroyo *et al*., 2008) and is the vector of Huanglongbing (HLB), the most important citrus disease in the world (Bové, 2006; Stansly, 2012).

Its management has been based mainly on the use of different chemical ingredients (organophosphorus insecticides, pyrethroids, neonicotinoids, entomopathogenic fungi, and plant-based products), which has caused the resurgence of new pests in the country, such as thrips, scales, and red spider mites, as the number of pesticide applications has increased; for example, in Michoacán, around 40 applications per year, which affects natural enemies and pollinators (Miranda-Salcedo *et al*., 2020a).

In Michoacán, SENASICA implements a Phytosanitary Epidemiological Management Areas (AMEFIS, for its acronym in Spanish) program each year in approximately 30 000 ha of citrus (SENASICA, 2019). Nonetheless, there is a wide range of natural enemies (Miranda-Salcedo and López-Arroyo, 2010) and predator releases are not made under an inundative biological control approach. An alternative to mitigate this problem is to evaluate new molecules with low environmental impact that affect beneficial insects (predators, parasitoids and pollinators) less.

The set of these strategies will reduce the number of sprays and the level of economic damage. The present study aimed to evaluate relatively recent generation insecticides for the control of the Asian citrus psyllid in Mexican limes. The hypothesis is that synthetic and biorational insecticides-acaricides for use in citrus cause mortalities greater than 90% of *Diaphorina citri* and do not impact on non-target organisms.

Materials and methods

The biological effectiveness test was established in a three-year-old thorny Mexican lime grove located in the Valle de Apatzingán Experimental Field (19° 0’ 44.10” north latitude, 102° 13’ 38.57” west longitude and 346 masl). The following molecules and doses were evaluated in this study: 1) Flupyradifurone 1.56 ml L^{-1} water; 2) Pyriproxyfen 1 ml L^{-1}; 3) Pymetrozine 1.5 ml L^{-1}; 4) Flonicamid 0.6 ml L^{-1}; 5) Buprofezin 4 ml L^{-1}; 6) Tolfenpyrad 2 ml L^{-1}; 7) Fenpyroximate 3 ml L^{-1}; 8) Spirotetramat 1 ml L^{-1}; 9) Cyantraniliprole 0.8 ml L^{-1}; 10) Chlorantraniliprole 0.2 ml L^{-1}; 11) Neem plus oil 4 + 6 ml L^{-1}; 12) Neem plus diatoms 4 ml L^{-1} + 6 g L^{-1}; 13) Silicon 6 g L^{-1}; 14) Kaolin 20 g L^{-1}; 15) *Bacillus amyloliquefaciens* 5 g L^{-1}; 16) potassium salts 5 g L^{-1} and 17) water control.

Each treatment consisted of ten replications (each tree was a replication); a completely randomized design was used, where in each treatment the trees were distributed in a row independent of another. A Swissmex manual backpack sprayer was used to apply each treatment, and one liter of insecticide solution per tree and one mL of adherent per liter of water were applied.

The specimens were quantified using the tapping technique, which consisted of selecting a branch at a height of 1.5 m, three blows were given with a stick, and the specimens were quantified on a purple board measuring 38 x 21 cm (Miranda-Salcedo, 2019). Likewise, another sampling method was used, which consisted of checking a new shoot from each tree. The response variable was the number of adults of *D. citri*. In the case of natural enemies (lacewings, coccinellids and red spiders mites), their presence was only recorded per sampling unit.

Subsequently, the samples were collected and stored in containers with 70% alcohol for identification with taxonomic keys. Sampling was performed before the application and at 2, 8, 15,

Revista Mexicana de Ciencias Agrícolas
REMEXCA
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20 and 29 days after application. The data were tested for normality and homoscedasticity, then the Anova was run with the SAS statistical program (2010); the comparison of means test used was Duncan's ($p < 0.05$).

To convert the number of insects of the variables into percentage, equation 1 was used.

$$\text{Population of arthropods in } \%=\left[\frac{\text{Number or arthropods in the previous sampling}}{\text{Number of arthropods in the subsequent samplings(daa)}}\right]*100\%$$

1). Arthropod population expressed in %.

Results and discussion

In the Apatzingán Valley, Michoacán, *D. citri* is present throughout the year due to environmental conditions and management practices promoted by producers (Miranda-Salcedo and López-Arroyo 2009, 2010). These factors, such as crop irrigation, promote a frequent emission of new vegetative shoots, which ensure food availability and oviposition sites for the insect. In the case of Mexican limes, there are four population peaks per year (September, December, April and July).

In this study, it was observed that after eight days the treatments were applied. The significantly different treatments were Tolfenpyrad®, Cyantraniliprole®, and Spirotetramat ($p < 0.0001$); 15 days after applying the product, the best treatment was Tolfenpyrad®; at 20 days, Buprofezin® and Tolfenpyrad; finally, at 29 days, Tolfenpyrad®, Cyantraniliprole®, and Spirotetramat (Table 1). Regarding the mortality exerted, compared to the previous sampling, Tolfenpyrad® presented a mortality of 97.5% at eight days, 92.6% at 15 days, 95.1% at 20 days and 100% at 29 days after application.

Table 1. Number of adults of *D. citri* controlled with products with low environmental impact in Mexican lime (Duncan 0.05).

Treatment	Previous $F_{16,153}$; $p < 0.0001$	8 days $F_{16,153}$; $p < 0.0001$	15 days $F_{16,153}$; $p < 0.0001$	20 days $F_{16,153}$; $p < 0.0001$	29 days $F_{16,153}$; $p < 0.0001$
1) Flupyradifurone	4.1 bcd	6.5 abc	11.5 a	3.5 bcde	0.9 bc
2) Pyriproxyfen	3.1 cde	3.9 bc	11.3 ab	3.1 cde	1.5 abc
3) Pymetrozine	4.3 bcd	6.7 ab	7.0 bcd	4.2 bc	2.4 ab
4) Flonicamid	0.3 e	5.8 abc	4.9 cdef	0.5 ed	0.3 c
5) Buprofezin	4.1 bcd	11.4 a	1.5 efg	0.2 e	0.4 c
6) Tolfenpyrad	4.1 bcd	0.1 c	0.3 g	0.2 e	0 c
7) Fenpyroximate	5.4 bc	4.2 bc	5.7 cde	3.2 cde	0.7 c
8) Spirotetramat	0.1 e	0.2 c	1.2 fg	0.6 ed	0.1 c
9) Cyantraniliprole	3.3 cde	0.2 c	1 fg	0.6 ed	0 c
10) Chlorantraniliprole	7.6 b	2.1 bc	4.9 cdef	1.9 cde	0.5 c
11) Neem - oil	2.2 cde	1.8 bc	4.4 efg	3.4 bcde	0.5 c
12) Neem- Diatoms	5.2 bc	2.8 bc	8.9 abc	3.9 bcd	0.6 c
13) Silicon	5.2 bc	5 bc	12.2 a	6.7 ab	0.8 c
14)) Kaolin	5.1 bc	3.4 bc	10.1 ab	6.7 ab	1.4 abc
15) *B. amyloliquefaciens*	7.9 b	2.4 bc	8.3 abcd	9.3 a	2.6 a
16) Potassium salts	12.3 a	7.2 ab	8.5 abcd	8.8 a	2.7 a
17) Control	4.7 b	5.7 abc	11 ab	8.6 a	2.4 ab

Data corresponds to the number of adults per sampling unit; letters indicate significant differences between treatments.

Revista Mexicana de Ciencias Agrícolas
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In contrast, Cyantraniliprole® showed 93.9% at eight days, 69.7% at 15 days, 81.81% at 20 days and 100% at 29 days post-application. The treatments of Tolfenpyrad® and Cyantraniliprole® were the ones that best controlled *D. citri* during the biological effectiveness test. For its part, Spirotetramat® was also significantly different from the rest of the treatments at 8, 15, 20 and 29 days after application; however, in the previous sampling, it presented the lowest density, 0.1 psyllids per sampling unit.

The best products after two days of application were Flupyradifurone® (95% mortality), Tolfenpyrad® (93% mortality), and Cyantraniliprole® (100% mortality). Nevertheless, after 20 days of applying the products, the best treatments were Tolfenpyrad® (95% mortality), Cyantraniliprole® (82% mortality), Buprofezin® (75% mortality), and Chlorantraniliprole® (75% mortality). In contrast, Pyriproxyfen®, Flonicamid®, Spirotetramat®, Neem plus oil, Silicon, Kaolin, *Bacillus amyloliquefaciens*, and the control presented zero percent mortality (Figures 1 and 2).

Figure 1. Number of *D. citri* adults controlled with biorational products in Mexican lime (July 2021).

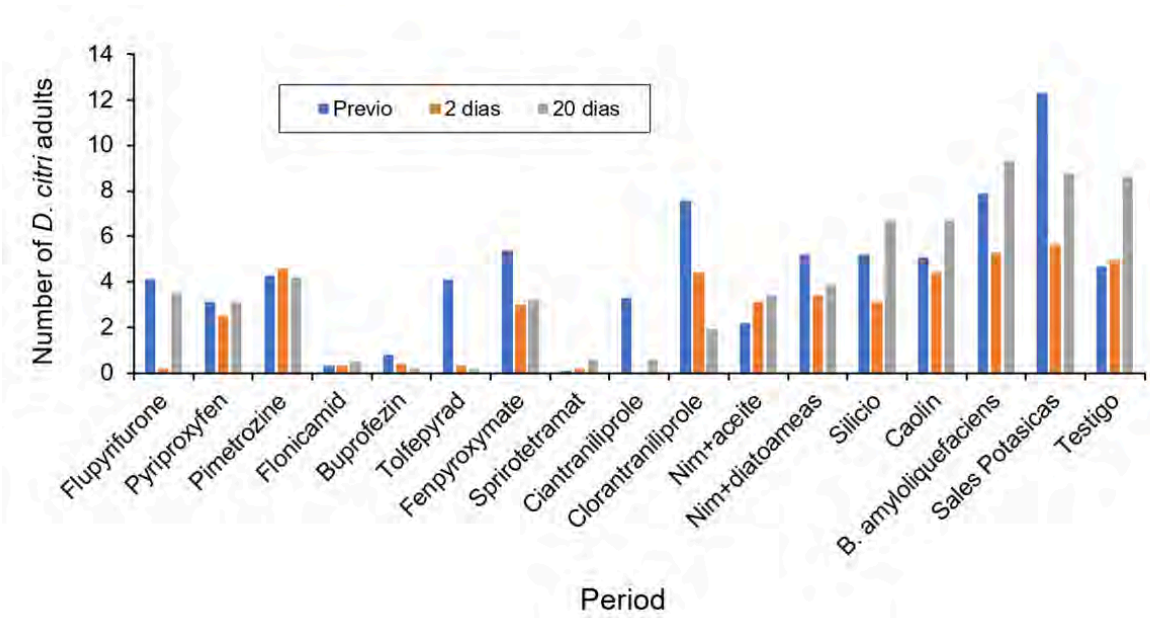

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Figure 2. Number of adults *D. citri* killed with biorational products in Mexican lime (July 2021).

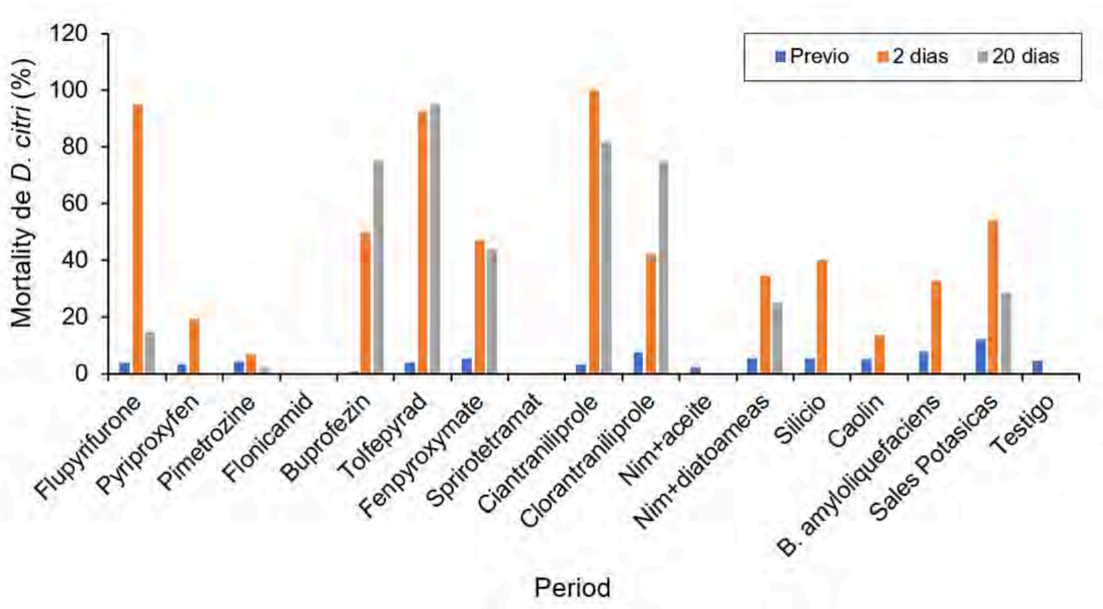
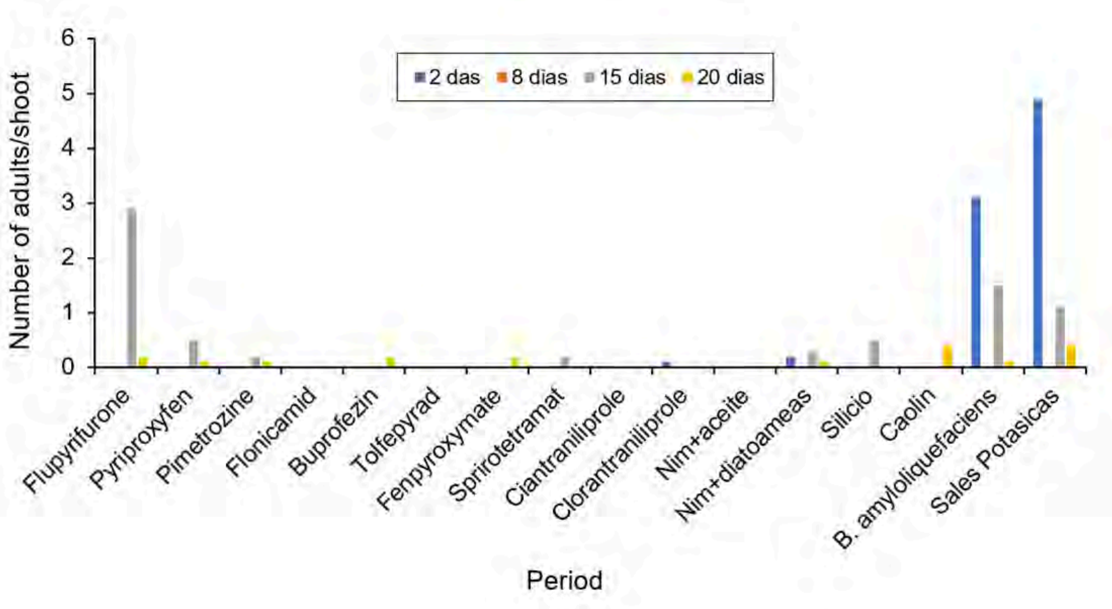


Figure 3 shows the effect of the different treatments on the presence of *D. citri* adults per shoot during the four post-application samplings (2, 8, 15 and 20 days). With this type of sampling, the presence of adults was low. Nonetheless, the treatments of Flonicamid®, Tolfenpyrad®, Cyantraniliprole®, and Neem plus oil presented zero individuals per shoot in all samplings.

Figure 3. Number of *D. citri* adults per shoot controlled with biorational products in Mexican lime (July 2021).



Regarding the presence of natural enemies of *D. citri* in the Apatzingán Valley, a large group was found and the following stand out: *Chrysoperla rufilabris* Burmeister 1839, *Ceraeochrysa* sp. nr. *cincta* (Mexico) (Neuroptera: Chrysopidae); *Cycloneda sanguinea* (L., 1763), *Hippodamia convergens* Guerin-Meneville 1842, *Olla v-nigrum* (Mulsant, 1866) (Coleoptera: Coccinellidae),

Revista Mexicana de Ciencias Agrícolas
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Zelus renardii (Kolenati, 1857) (Hemiptera: Reduviidae), and different species of red spider mites. The most important for their abundance were *C. rufilabris* and *C. cincta*.

These natural enemies are important regulators of the complex of pests that attack lemons. In the case of lacewings, two of the most promising products, Tolfenpyrad® and Cyantraniliprole®, did not show the presence of lacewings or coccinellids. In a previous study, where Spirotetramat® and other products not included in this bioassay were evaluated, the presence of lacewings was affected for 28 days after application (Miranda-Salcedo *et al*., 2020a). Therefore, the high susceptibility of lacewings to products such as Tolfenpyrad®, Ciantraniliprole®, and Spirotetramat® was observed (Figures 4 and 5).

Figure 4. Number of lacewings present with the control of biorational products against *Diaphorina citri* in Mexican lime (July 2021).

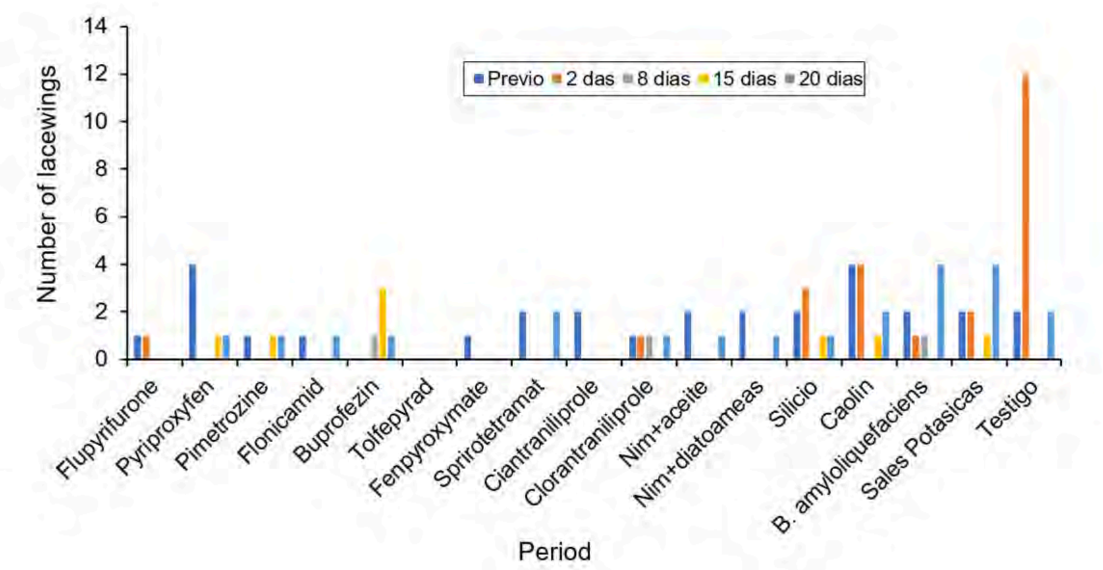

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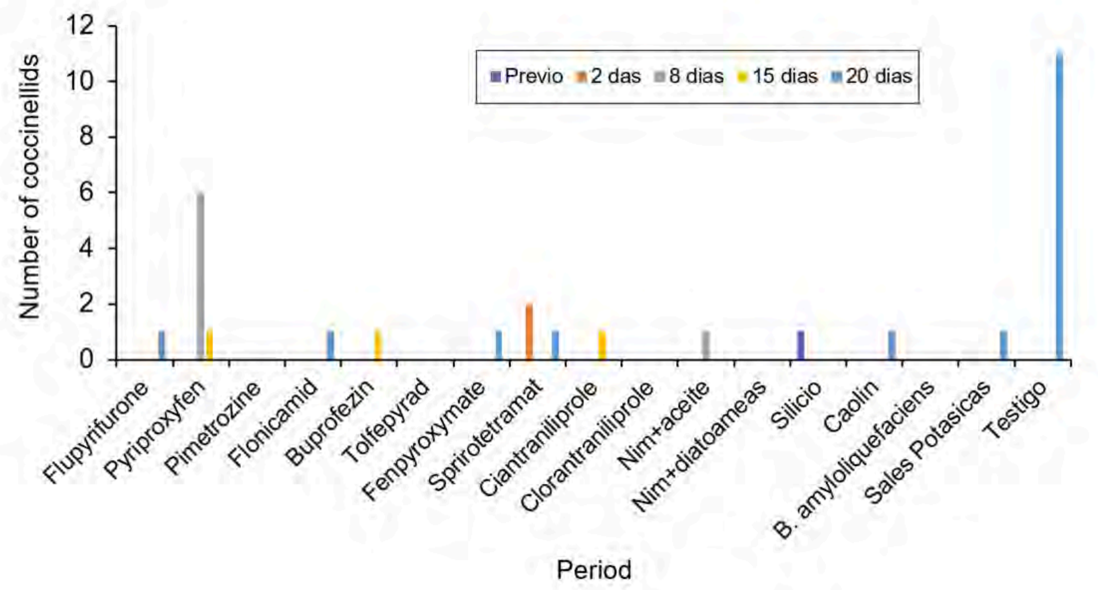
Figure 5. Number of coccinellids present with the control of biorational products against *Diaphorina citri* in Mexican lime (July 2021).



It can be concluded that Tolfenpyrad®, Cyantraniliprole®, and Spirotetramat® are an alternative for the control of *D. citri* and can replace highly toxic insecticides, such as organophosphorus insecticides, pyrethroids and neonicotinoids, which have been shown to be resistant to the Asian citrus psyllid in different citrus-growing areas of the country (Osorio *et al*., 2019).

It is important to emphasize that several bioresidual products act synergically and increase bioresiduality (total mortality that includes the chemical plus mortality produced by biological factors). For example, the use of lacewings within a biological control program by augmentation or conservation (López and Villanueva, 2019). Red spider mites were observed to be scarce in most treatments, except for T15 *Bacillus amyloliquefaciens* (Figure 6). Finally, several of the products assessed can be part of a portfolio of friendly alternatives in the control of this pest.

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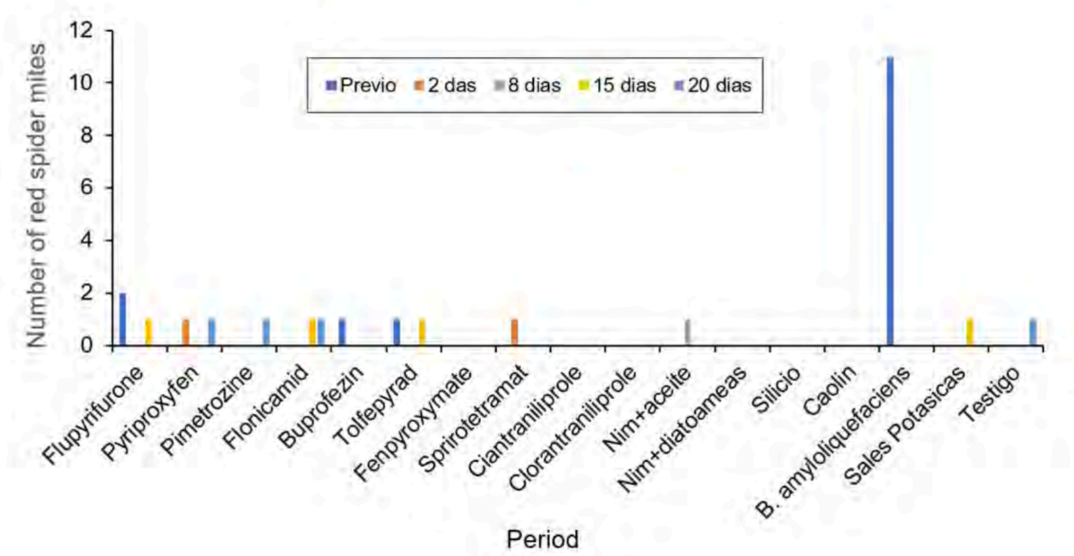
Figure 6. Number of red spider mites present with the control of biorational products against *Diaphorina citri* in Mexican lime (July 2021).



Finally, the interaction of natural enemies, the environment, the producer's management, and sampling affect decision-making. A point to consider is that most producers apply by calendar and do not have sampling data, which led to an excess of chemical applications (Cortés-Moncada *et al*., 2010b; Villanueva-Jiménez *et al*., 2019; Miranda-Salcedo *et al*., 2020b). Given this, products with low environmental impact and the use of natural enemies are an option for integrated pest management (Ables and Ridgway, 1981).

Many biological effectiveness tests have been conducted on this pest; however, there are some insecticides that are an alternative for its management (Table 2). Reducing a chemical application in the 75 000 ha of citrus in the Apatzingán Valley represents a saving of 35 million pesos and if extrapolated to the 40 applications ml L^{-1} that are carried out on average per year, the cost would be 1 400 million pesos.

Table 2. Insecticides recommended for the control of *D. citri* in Mexican lime.

Product	Dose	Author
Berni Labs-garlic + chamomile	2 ml L^{-1}	Miranda-Salcedo *et al.* (2020a)
Berni Labs- citrus seed extract	4 ml L^{-1}	
Yellow weed extract preparation, *Reseda luteola* (Resedaceae) at 5%	4 ml L^{-1}	
Paraffinic petroleum oil	10 ml L^{-1}	Ruíz-Galván *et al.* (2015)
Spirotetramat®	0.5 ml L^{-1}	
Biotech-garlic oil	2 ml L^{-1}	Miranda-Ramírez *et al.* (2021)
Berni Labs- citrus seed extract	2 ml L^{-1}	
Nichino-Fenpyroximate®	1.25 ml L^{-1}	

To obtain good management of *D. citri*, it is necessary to carry out field monitoring of the psyllid population before making any application to determine its density and thus make a well-informed decision.

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It is also important to include, within a sustainable management of *D. citri*, the use of the parasitoid *Tamarixia radiata* at certain times of the year, which has been reported with a parasitism of 59.6% in Sinaloa (Cortez-Mondaca *et al*., 2010a) and 27% in groves with agroecological management in the Apatzingán Valley (Miranda-Salcedo, 2019). Other studies that report different species of predators in the control of the Asian psyllid are Cortés-Mondaca *et al*. (2010b), Lozano-Contreras and Jasso-Argumedo (2012), and Kondo *et al*. (2017).

Conclusions

The products that controlled *D. citri* significantly better were Tolfenpyrad®, Cyantraniliprole®, and Spirotetramat®. Nevertheless, some other products such as Chlorantraniliprole and Neem plus oil can be an alternative at a low pest density. The products with the best performance in the control of *D. citri* (Tolfenpyrad®, Cyantraniliprole®, and Spirotetramat®) affected the presence of non-target organisms, such as lacewings and coccinellids. The results obtained generate new research opportunities with molecules with low environmental impact and alternatives for biological control by conservation.

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