

PERCEPTION OF THE USE OF NEONICOTINOIDS, SPINOSAD, AND NEREISTOXIN IN INSECTS POLLINATING PAPAYA (*Carica papaya* L.), PERSIAN LEMON (*Citrus × latifolia* Tanaka), AND SOURSOP (*Annona muricata* L.) IN VERACRUZ, MEXICO

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ABSTRACT

Pollinating insects in tropical fruit trees are of utmost importance for flower pollination and ensuring fruit production. However, given the current climatic conditions and the chemical management practices, they are affected by the residual effects of neonicotinoids, spinosad, and nereistoxin on the flowering of papaya (*Carica papaya* L.), Persian lemon (*Citrus × latifolia* Tanaka), and soursop (*Annona muricata* L.). The overuse of these insecticides has led to the decline of pollinating insects such as honeybees. The objective of this study was to assess the use of neonicotinoid insecticides spinosad and nereistoxin during flowering of papaya, Persian lemon, and soursop, and evaluate stakeholders' perceptions of the impact of pollinator population density in central Veracruz, Mexico. A survey was carried out among papaya, Persian lemon, and soursop producers through a questionnaire with open and closed questions. The data were analyzed using frequency and multivariate analyses with the statistical package STATISTICA version 7.0. Results show that producers use imidacloprid, thiamethoxam, acetamiprid, spinosad, and the nereistoxin known as thiocyclam to control mites and insect pests. Producers observed damage to bees and beneficial insects after applying these chemicals. In conclusion, the management and application of toxic insecticides for pest control pose a significant risk to pollinating insects.

Keywords: Tropical fruit trees, insecticides, toxicity, residues, *Apis mellifera* L.

INTRODUCTION

Due to climate change, natural pollinator populations are declining, which has implications for ecosystems and agroecosystems. Mutualistic interactions, such as plant-pollinator associations, are critical for pollination. Plants benefit from the visit of organisms that act as pollinators by carrying pollen to other flowers (Maglianesi-Sandoz, 2016). According to estimates, 70 % of tropical crops produce significantly more when pollinators visit their flowers (Garibaldi *et al.*, 2012).

The decline of honey bee (*Apis mellifera* L.) colonies is attributed to multiple factors, such as the use of pesticides, genetically modified crops, habitat loss, and the presence

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of diseases or parasites. Among the pesticides, neonicotinoids stand out for their toxicity, as they are systemic neurotoxic insecticides that accumulate in pollen, nectar, and guttation fluids of plants, increasing their impact on pollinators (Fairbrother *et al.*, 2014). Their translocation to floral tissues causes residual effects that generate both lethal and sublethal mortality in different bee species.

Riano-Jiménez and Cure (2016) evaluated the toxicity of the insecticides imidacloprid, spinosad, and thiocyclam hydrogen oxalate (nereistoxin) in neotropical bees of the species *Bombus atratus*, determining their median lethal doses (LD50). Imidacloprid was the most toxic, with a topical and oral LD50 of 0.000048 and 0.00001 mg L⁻¹, equivalent to 0.048 and 0.01 mg per bee, respectively. For thiocyclam hydrogen oxalate, the topical and oral LD50 were 0.000244 and 0.000056 mg L⁻¹ (0.244 and 0.056 µg per bee). In the case of spinosad, the oral LD50 reached 0.0002 mg L⁻¹ (0.2 µg per bee).

Neonicotinoids present in pollen and nectar can cause mortality in crop-pollinating insects (Fairbrother *et al.*, 2014). Nereistoxin, a neurotoxic substance isolated from the marine annelid *Lumbriconereis heteropoda*, acts as a poison for weevils and caterpillars and may pose a risk to pollinators and humans (Kumar *et al.*, 2011). Since the mid-20th century, honeybee populations maintained in artificial hives have declined. In contrast, managed colonies in Asia, Africa, and Australia have increased since 1991 (Fairbrother *et al.*, 2014). In Mexico, studies on papaya (*Carica papaya* L.) from Cotaxtla, Veracruz, showed residues of thiamethoxam in water, plant, and fruit matrices, which poses an additional risk to pollinators associated with flowering (Megchún-García *et al.*, 2019, 2024).

Chemical insecticides affect pollinators through different mechanisms of action, either through direct toxicity that causes mortality or sublethal effects that alter their behavior, reproduction, navigational ability, and immune function (Maggi and Chreil, 2023). During flowering in tropical fruit trees, the movement of pollinators favors the transfer of male gametes and ensures the sexual reproduction of plants. If pollinators are scarce or inefficient, pollination can fail due to poor quality and quantity of the pollen transported, as well as inadequate depositing on the stigma that prevents germination (Garibaldi *et al.*, 2012).

In Mexico, papaya and Persian lemon (*Citrus × latifolia* Tanaka) are crops of national and international economic importance, while emerging fruit trees such as soursop (*Annona muricata* L.) have significant market potential (Megchún-García *et al.*, 2023). In papaya, physiological triggers regulate primordia formation and flower opening in the androecious, andromonoecious, and gynoecious sexual forms. Stigma receptivity begins on the same day as flower opening, at which time flowers can be visited by pest insects or beneficial pollinator-associated insects, such as stingless bees (Pares *et al.*, 2002; Aspeitia-Echegaray *et al.*, 2014; Hoyos and Hurtado-Salazar, 2017; Megchún-García *et al.*, 2018).

In the case of lemon, there are numerous floral flows that are continuously visited by pollinators throughout the year (Almaguer-Vargas *et al.*, 2011). Lemon tree pollination is carried out by entomophilous insects, primarily bees and flies. Citrus fruits vary

greatly in their dependence on pollinators, with cases such as tangerines that are completely dependent on them (Moreno *et al.*, 2024).

In soursop, natural pollination is limited by dichogamy or protogyny, resulting in poor and erratic fruit production. During this stage, pollinating insects such as beetles and ants play a key role during anthesis (Rodríguez *et al.*, 2010), reducing dependence on manual pollination to ensure fruit production. However, the use of insecticides such as neonicotinoids, spinosad, and nereistoxin during flowering has led to a decline in pollinator populations. Therefore, the objective of this study was to assess the use of these insecticides during flowering of these fruit trees and analyze local stakeholders' perceptions of their impact on pollinator population density in central Veracruz, Mexico.

MATERIALS AND METHODS

The study was carried out in the central region of the state of Veracruz, Mexico, in the municipalities of Cotaxtla, Tlalixcoyan, and Tlapacoyan (Figure 1). A technical survey was conducted in the towns of Avi6n, Colonia Ejidal, Mata Tambor, Los Bajos de Tlachiconal, Los Bajos Platanal, Cotaxtla, ejido Anexo Mundo Nuevo, and La Palmilla to identify the study crops. The municipality of Cotaxtla produces 1643.5 ha of lemon, 737.5 ha of papaya, and 7 ha of soursop; the municipality of Tlalixcoyan has 543.71 ha of papaya and 423 ha of lemon; and the municipality of Tlapacoyan produces 3758 ha of lemon and 8 ha of soursop (SIAP, 2024).

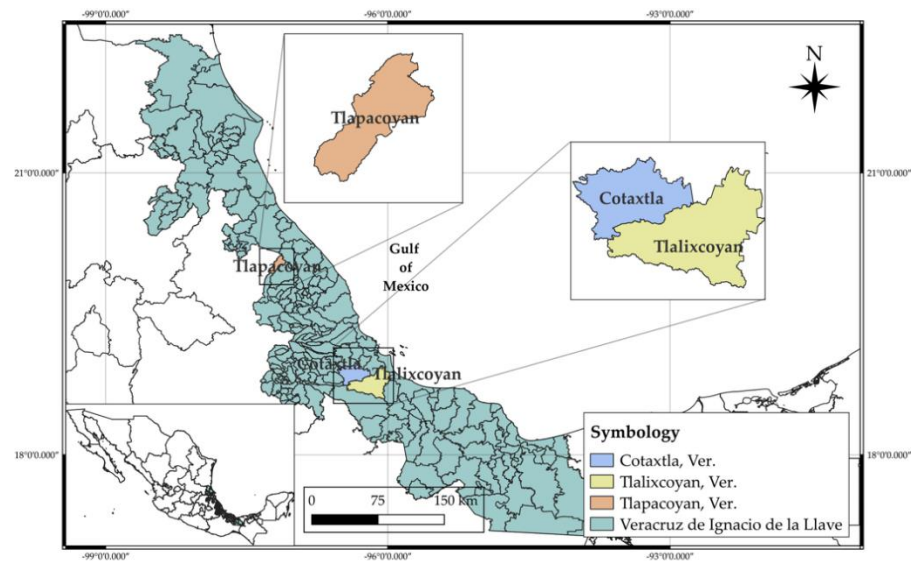


Figure 1. Location of the study area in municipalities of Veracruz, Mexico.

A survey was conducted to gather information on the technical management of neonicotinoid insecticides, spinosad, and nereistoxin, considered the main compounds that affect natural pollinators, particularly bees. Non-probability sampling was used, concentrating on the identification of key informants, including prominent producers and agricultural stakeholders. The sample interviewed consisted of 66 producers dedicated to the cultivation of papaya, Persian lemon, and soursop, with crop areas ranging from 1 to 10 ha. A semi-structured questionnaire with open and closed questions was used, and an infographic was created to classify the insecticides based on the most common brand names for these fruit trees.

The questionnaire was validated using SPSS version 25 software (IBM SPSS Statistics), with the collaboration of five judges who assessed the clarity and comprehension of the questions on a scale of 1 to 5, considering their correct wording. A pilot test was also conducted to rule out poorly reasoned questions. A technical survey was carried out in the field, starting in the municipalities of Cotaxtla, Tlalixcoyan, and Tlapacoyan. The activity began in April and ended in September 2024.

The study variables evaluated were: production system; type of fruit tree (papaya, Persian lemon, soursop); cultivated area (ha); distance between plants and rows (m); planting date; flowering and harvest periods; presence of pests; management and application of insecticides, including the type of insecticide that affects natural pollinators, time of application, product mix, frequency and effectiveness; insecticides that damage crops or cause mortality in pollinating bees; phenology of fruit trees and application of insecticides according to phenological stage; application and protection equipment; unused insecticides and reasons for not using them; and flower drop on fruit trees, as well as problems present in the ecosystem. The data were coded in Microsoft Excel 2009 and analyzed in Statistica version 7. Frequency and multivariate principal component analyses were carried out.

RESULTS AND DISCUSSION

According to the survey, 84.8 % of producers are dedicated to growing Persian lemons. Of the total producers, 69.6 % operate an intensive monoculture production system, while the rest establish crops in association with grasses and vegetables, such as corn, watermelon, coconut, and banana (Table 1). The growing demand and consumption

Table 1. Producer distribution by crop and production system in the central area of Veracruz, Mexico.

Tropical fruit tree	Frequency	Producers (%)	Production system	Frequency	Producers (%)
Papaya (<i>Carica papaya</i> L.)	9	13.6	Associated cropping	19	28.8
Persian Lemon (<i>Citrus × latifolia</i> Tanaka)	56	84.8	Monoculture	46	69.7
Soursop (<i>Annona muricata</i> L.)	1	1.6	In rotation	1	1.5
Total	66	100	Total	66	100

of tropical fruits like lemons is increasing worldwide, particularly in Europe, at an average rate of 6 % per year. Pollinating insects are essential for fruit production as they ensure pollination rates and fruit set (de la Peña-Alonso *et al.*, 2018). However, the main cause of pollinator decline is habitat loss, as more than 40 % of the ice-free land surface has been modified by humans for agricultural purposes. This alteration limits the availability of food and nesting sites, hindering pollinator adaptation (Bartomeus and Bosch, 2018).

It was observed that producers use a specific group of chemical insecticides as part of integrated pest management in lemon, papaya, and soursop crops. Among the insecticides that affect pollinators, 3 % of producers use imidacloprid and abamectin, while 2 % use only imidacloprid. Most producers use neonicotinoids such as acetamiprid, imidacloprid, and thiamethoxam.

Imidacloprid is primarily used to control leaf miner (*Phyllocnistis citrella*), aphids, mites, and red spider mites (*Tetranychus urticae*). In the case of spinosad, it is often combined with other active ingredients, such as imidacloprid, dimethoate, and cypermethrin. Spinetoram, a fermentation product derived from the actinobacterium *Saccharopolyspora spinosa*, was also used. Its active component, spinosyn, exhibits effects similar to those of spinosad. Overall, 48.9 % of producers use imidacloprid for agronomic pest management (Table 2).

The chemical structure of neonicotinoids gives them systemic properties, allowing for complete plant protection by distribution throughout the vascular system, reaching the pollen and nectar. This characteristic can lead to the presence of residues of imidacloprid, clothianidin, and thiamethoxam in wildflowers, which pose a risk to pollinators such as domestic, commercial, and wild bees, hoverflies, wasps, beetles, and ants present in tropical fruit trees (Botías and Sánchez-Bayo, 2018). Pollinators play an important role in floral evolution, particularly in angiosperms, by promoting mutualistic interactions in pollination processes. However, external factors such as exposure to neonicotinoids negatively affect these processes (Simón-Porcar *et al.*, 2018; Megchún-García *et al.*, 2019).

In studies on honeybees (*Apis cerana* Fabricius), exposure to more than two pesticide combinations (imidacloprid, chlorobenzamide, and glyphosate) reduced bee flight time and distance. Furthermore, transcriptomic results indicated that concentrations of 0.001 mg L⁻¹ of imidacloprid could affect the survival rate of queen bees (Guo *et al.*, 2025). Other studies indicated that the neonicotinoid acetamiprid significantly altered the gut microbial community in adult and larval bees, suggesting that it directly affects bee health (Su *et al.*, 2024).

In the interview, 56 % of producers reported not perceiving any harm to pollinating insects due to insecticides (Figure 2). Of those who apply neonicotinoid insecticides, 16.6 % identified imidacloprid as the main cause of harm to natural pollinators. Meanwhile, 9 % associated spinosad with the damage. In this case, 1.5 % of producers reported using nereistoxin (thiocyclam), usually in a mixture with other active ingredients to control whiteflies (*Bemisia tabaci*), red spider mites (*Tetranychus urticae*), *Diaphorina citri*, thrips (Thysanoptera), and ants (Formicidae).

Table 2. Insecticides used by producers for pest control in papaya (*Carica papaya* L.), Persian lemon (*Citrus × latifolia* Tanaka), and soursop (*Annona muricata* L.) and their potential impact on pollinators.

Groups of insecticides used	Frequency (number of interviewees)	Fruit tree producers (%)
Cypermethrin, chlorpyrifos ethyl	5	7.6
No insecticides used	5	7.6
Cypermethrin	3	4.5
Imidacloprid, abamectin	3	4.5
Abamectin, chlorpyrifos ethyl	2	3
Abamectin, imidacloprid, spinetoram	2	3
Abamectin, imidacloprid, tolfenpyrad	2	3
Acetamiprid, spinetoram etaxazole	2	3
Imidacloprid	2	3
Abamectin	1	1.5
Abamectin, chlorpyrifos ethyl, carbofuran	1	1.5
Abamectin, chlorpyrifos ethyl, diazinon	1	1.5
Abamectin, dimethoate, diazinon	1	1.5
Abamectin, pinol, soap	1	1.5
Abamectin, spinetoram, spirotetramat	1	1.5
Bifenazate, organic insecticide	1	1.5
Cypermethrin, pyraclostrobin	1	1.5
Chlorpyrifos ethyl, carbaryl, cypermethrin	1	1.5
Diazinon, malathion, dimethoate	1	1.5
Botanical extracts	1	1.5
Imidacloprid, abamectin, elemental sulfur	1	1.5
Imidacloprid, abamectin, bifenazate	1	1.5
Imidacloprid, abamectin, cypermethrin	1	1.5
Imidacloprid, abamectin, chlorpyrifos ethyl	1	1.5
Imidacloprid, abamectin, diazinon	1	1.5
Imidacloprid, abamectin, diazinon, chlorpyrifos ethyl, spinetoram	1	1.5
Imidacloprid, abamectin, dimethoate	1	1.5
Imidacloprid, abamectin, soap	1	1.5
Imidacloprid, abamectin, malathion	1	1.5
Imidacloprid, abamectin, sulfoxaflor, chlorpyrifos ethyl	1	1.5
Imidacloprid, elemental sulfur, malathion	1	1.5
Imidacloprid, cypermethrin	1	1.5
Imidacloprid, chlorpyrifos ethyl, paraffinic oil	1	1.5
Imidacloprid, chlorpyrifos ethyl, diazinon	1	1.5
Imidacloprid, chlorpyrifos ethyl, organic insecticide	1	1.5
Imidacloprid, dimethoate, cypermethrin	1	1.5
Imidacloprid, neem extract	1	1.5
Imidacloprid, organic insecticide	1	1.5
Imidacloprid, malathion, chlorpyrifos ethyl	1	1.5
Imidacloprid, spinetoram, carbofuran	1	1.5
Imidacloprid, spinosad	1	1.5
Imidacloprid, spinosad, abamectin	1	1.5
Organic insecticide	1	1.5
Methamidophos, spinetoram, cypermethrin, abamectin, chlorpyrifos ethyl	1	1.5
Spinetoram	1	1.5
Spinosad	1	1.5
Spinosad, cypermethrin, permethrin	1	1.5
Spinosad, dimethoate	1	1.5
Thiamethoxam, organic insecticide	1	1.5
Total	66	100

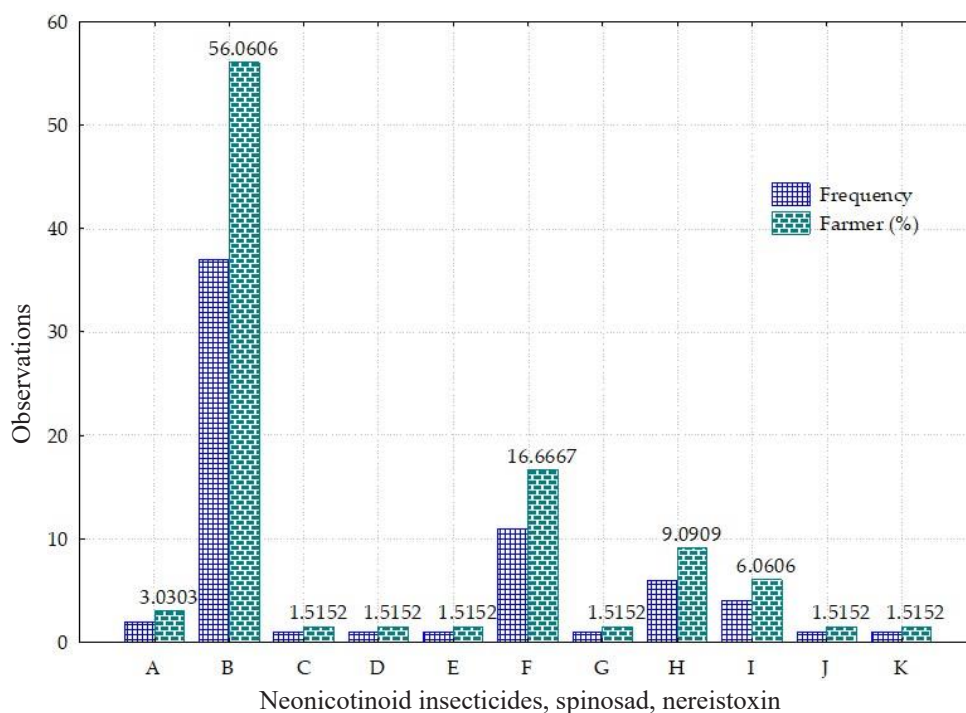


Figure 2. Frequency and percentage of producers who identified neonicotinoid insecticides, spinosad, and nereistoxin as causing mortality in natural pollinators in tropical fruit crops. A: dinotefuran; B: no perception of effects; C: thiamethoxam, lambda-cyhalothrin; D: thiamethoxam, thiocyclam; E: thiamethoxam; F: imidacloprid; G: clothianidin, thiamethoxam, thiocyclam, tolfenpyrad; H: spinosad; I: tolfenpyrad; J: diazinon; K: imidacloprid, tolfenpyrad.

Pollinators can be exposed to insecticides through direct contact with airborne particles or treated plant surfaces, through the ingestion of pollen, nectar, and contaminated water, or through inhalation of volatile pesticides (Botías and Sánchez-Bayo, 2018). One of the risks of applying neonicotinoids such as imidacloprid is their negative effect on natural enemies, such as the predator *Arma chinensis*. This shows that although imidacloprid is effective in pest control, it can also harm beneficial insects (Cheng *et al.*, 2025).

Of the insecticides that kill bees during the flowering stage of tropical fruit trees, producers mentioned spinosad and clothianidin in order of importance with 3 %, followed by imidacloprid and other active ingredients with 1.5 % (Figure 3). Spinosad has demonstrated low toxicity to bees when allowed to dry on plant foliage for 3 h (Mayes *et al.*, 2003). Bumblebees have been reported to be more vulnerable than honeybees to the presence of imidacloprid in pollen and nectar (Martin-Culma and Arenas-Suárez, 2018). The phenomenon of bee poisoning, known as Colony Collapse Disorder, is caused by exposure to neonicotinoids and pyrethroids such as cypermethrin, which have long-term impacts on honeybee populations and

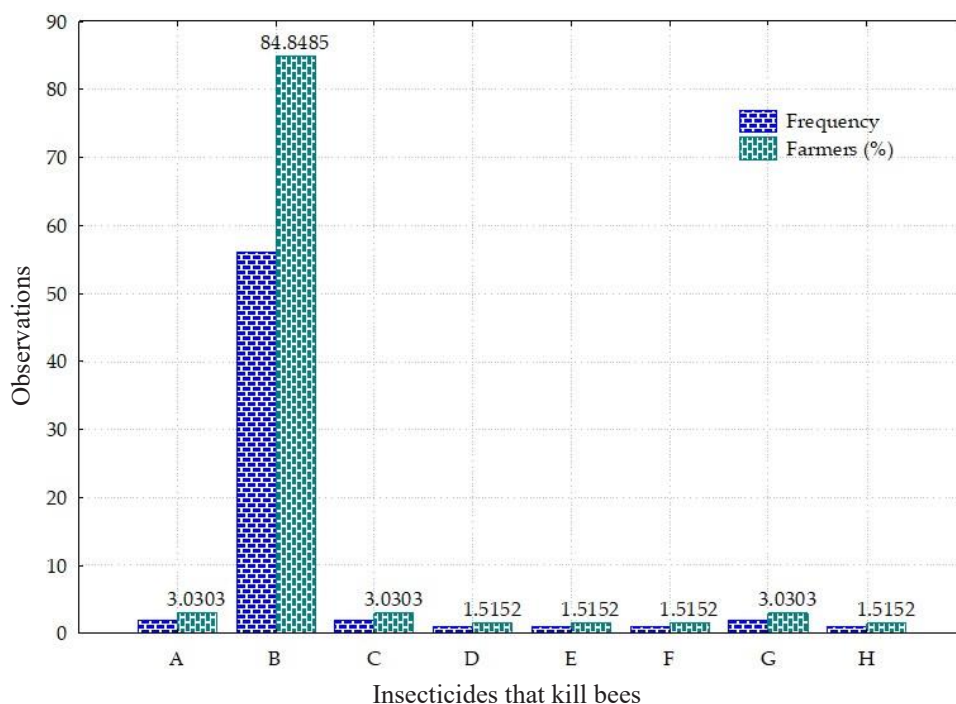


Figure 3. Frequency and percentage of producers who identified insecticides causing mortality in honeybees (*Apis mellifera* L.) during flowering of tropical fruit trees. A: spinosad, clothianidin; B: no effects perceived; C: cypermethrin; D: lambda-cyhalothrin; E: imidacloprid, abamectin, spinetoram; F: abamectin; G: all insecticides; H: tolfenpyrad.

communities and wild pollinators. Therefore, in countries like Italy, permethrin and cypermethrin are banned in agriculture (Gasparini *et al.*, 2025).

From the total of producers, 75.7 % reported no damage from the application of chemically synthesized insecticides, but 4.5 % argued that bees are killed when insecticides are applied at different phenological stages. Around 1.5 % identified the death of natural predators, and 3 % observed the death of beneficial insects and fish. Similarly, 4.5 % of producers reported flower drop in all three crops (Figure 4).

Pollinator research indicates the existence of secondary pollinators, or anthophilous insects, including beetles, lepidopterans (butterflies), flies, wasps, and ants. Although they are less efficient pollinators, their flower visitation intensity contributes in a complementary way to bees (Stefanescu *et al.*, 2018). Factors such as land-use change, habitat loss, pollution, and climate change have accelerated the decline of pollinators. In tropical regions, the most important pollinating insects include bees, wasps, and ants (Hymenoptera), flies (Diptera), butterflies and moths (Lepidoptera), and beetles (Coleoptera). In addition, there are vertebrate pollinators such as nectarivorous birds (Trochilidae), nectarines (Nectariniidae), honey eaters (Meliphagidae), and parrots (Psittacidae), as well as bats that pollinate agaves in deserts and tropical rainforests (Meléndez-Ramírez *et al.*, 2020).

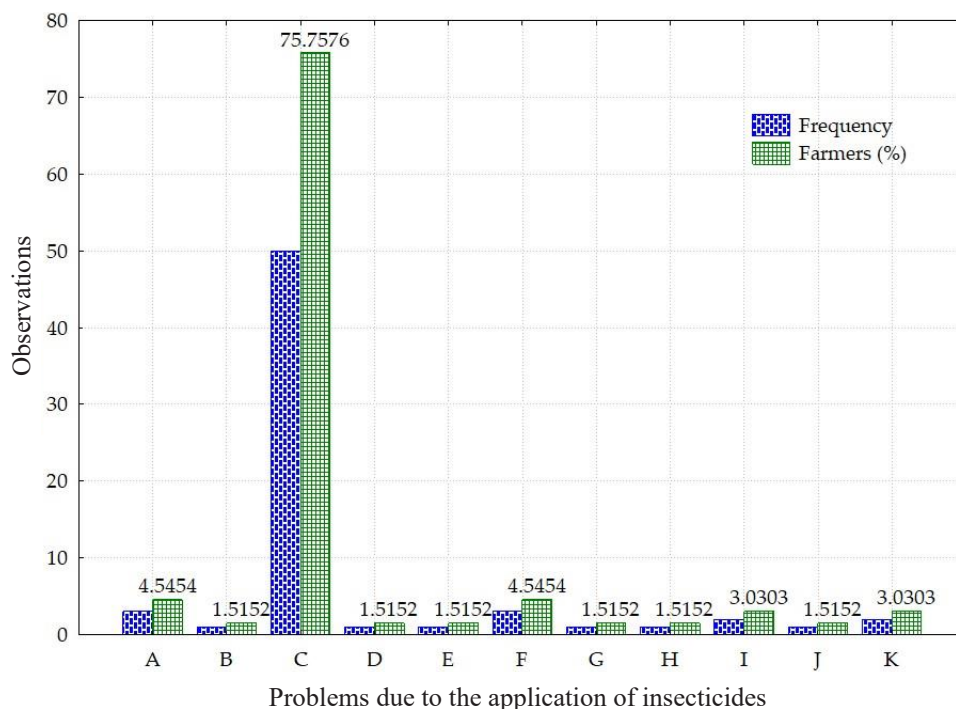


Figure 4. Frequency and percentage of observations reported by producers regarding problems caused by the application of synthetic chemical insecticides on tropical fruit trees. A: flower drop; B: plant burning; C: no damage perceived; D: flower drop and death of butterflies; E: death of bees and butterflies; F: death of bees; G: flower and fruit drop; H: death of natural predators; I: insecticide resistance; J: attraction of more insects; K: death of beneficial insects and fish.

In the multivariate principal components analysis, a positive relationship was observed between insecticide application equipment and method according to the phenological stage (Figure 5), which directly influences insecticide effectiveness within integrated pest management. On the other hand, a negative relationship was observed with insecticide management, pest presence, active ingredient mix, and planting date. These results suggest that the level of damage caused by systemic insecticides such as neonicotinoids depends on the method and management of application in the field. Previous studies have shown that the inappropriate use of neonicotinoids affects bees and other beneficial insects, reducing their survival and causing mortality due to contact or ingestion, mainly through consumption of contaminated nectar, pollen, resins, or water (Cajamarca *et al.*, 2020). This remarks the need to implement local bee poisoning surveillance and prevention programs (Gasparini *et al.*, 2025).

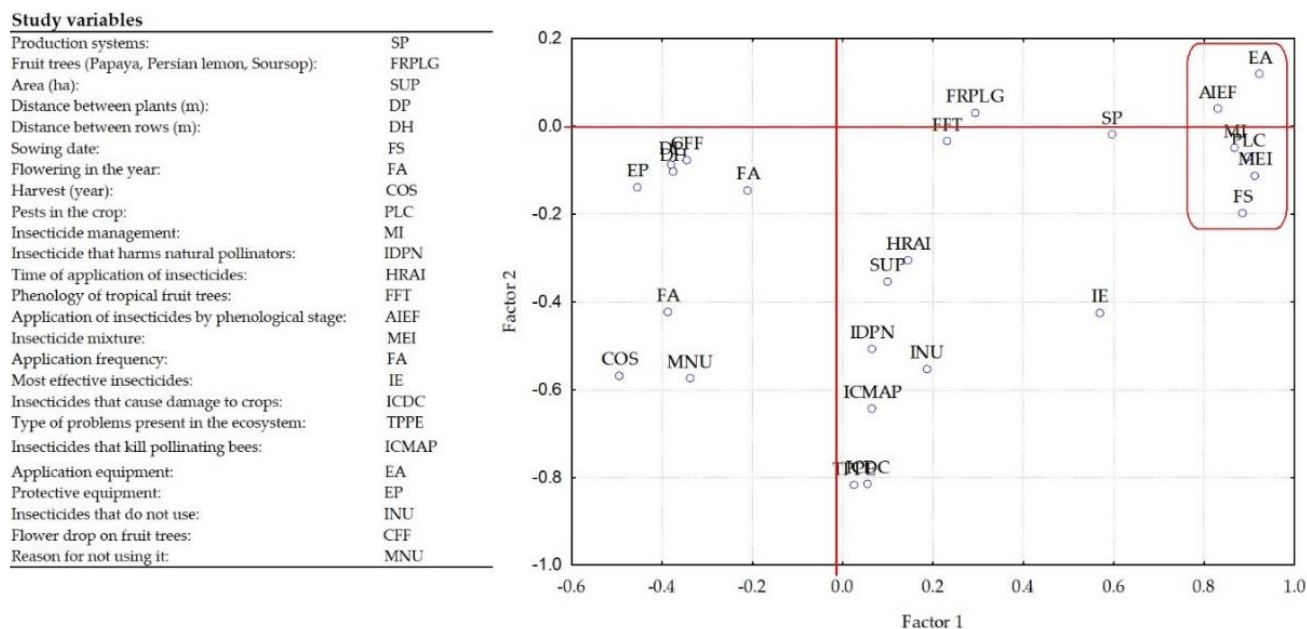


Figure 5. Multivariate principal components analysis by two factors for the management of neonicotinoid insecticides, spinosad, and nereistoxin in papaya (*Carica papaya* L.), Persian lemon (*Citrus × latifolia* Tanaka), and soursop (*Annona muricata* L.) cultivation.

CONCLUSIONS

Producers use insecticides that affect pollinating insect populations, such as neonicotinoids and spinosad, although few realize the impact these chemical compounds have on beneficial insects associated with flowering and the ecosystem. Imidacloprid is the most widely used, generally in combination with other active ingredients, due to its high effectiveness in controlling pests in the crops analyzed. Other emerging insecticides, such as nereistoxin, are beginning to gain relevance in integrated pest management, making it important to conduct residual studies on flowers to assess their impact on natural pollinators. Therefore, there is a potential risk of frequent damage to pollinating insects if management and use of toxic insecticides in fruit crops are not properly regulated.

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