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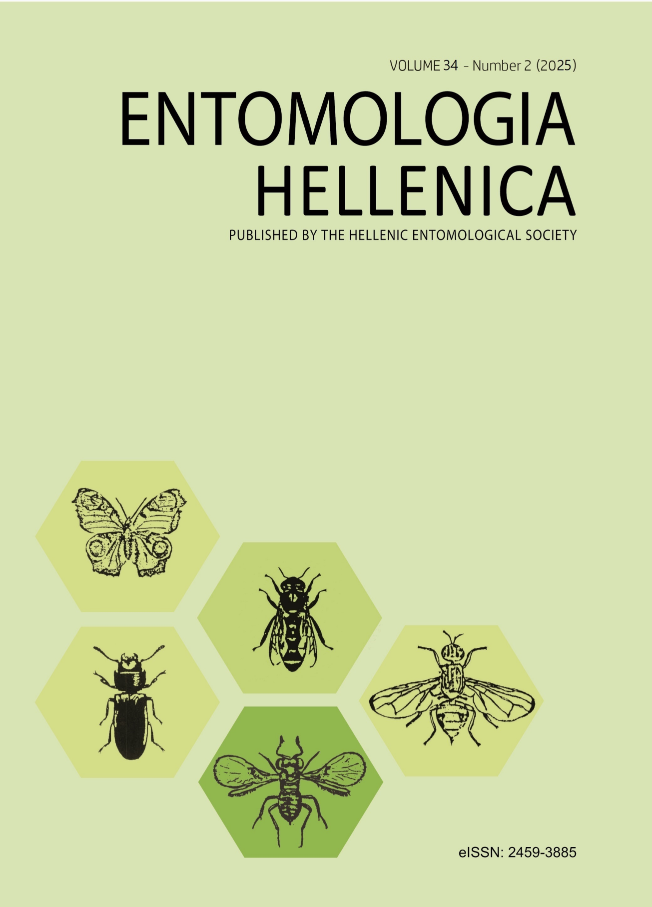

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Optimizing Pest Control and Reducing Pesticide Consumption in Citrus Orchards against citrus soft scale, *Pulvinaria aurantii* Cockerell (Hemiptera: Coccidae)

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ABSTRACT

The citrus soft scale, *Pulvinaria aurantia* Cockerell (Hemiptera: Coccidae), is a major citrus pest. In this study, the efficacy of chlorpyrifos, acetamiprid, and Palizin, both alone and in combination with mineral oils and CitraPlus oils, were investigated against first and second instar nymphs of citrus soft scale under laboratory conditions. Results showed that the combination of acetamiprid with CitraPlus oil exhibited a synergistic effect, resulting in lower dose of acetamiprid required to achieve the same level of control compared to only acetamiprid application. However, in chloropyrifus treatment a dose of 9 ml/L resulted in only 75% mortality of the second nymph stage of *P. aurantii*. This indicates that *P. aurantii* now requires higher doses of the insecticide for effective population control. Overall, the findings of this research demonstrated that the combination of mineral oils and CitraPlus oils with pesticides significantly improved their effectiveness while reducing the amount of pesticide needed.

KEY WORDS: Citrus soft scale, Acetamiprid, Synergistic effect, Mineral oils, Palizin.

Introduction

Citrus fruits are among the most valuable crops globally, renowned for their high nutritional value, wide use in the food and beverage industry, production of essential oils, and potential health benefits (Saini et al. 2022). They are rich in vitamins, minerals, fiber, and bioactive compounds that exhibit antioxidant, anti-inflammatory, and antimicrobial properties, potentially reducing the risk of chronic diseases such as cardiovascular ailments, cancer, and diabetes (Codoñer-franch and Valls-Belles 2010; Saini et al. 2022; Borghi and Pavanelli 2023). According to the FAO's Statistical Yearbook 2021, the total area under citrus cultivation worldwide in 2019 was 9.34 million hectares, with top producers including Brazil, China, India, the United States, Mexico, Egypt, Spain, Turkey, Iran, and Italy (FAO 2021). However, citrus crops face several challenges, notably climate change, and agricultural pests (Hossain et al. 2024; Tourani and Abbasipour 2019).

Various pests infest citrus fruits, including scale insects, mites, mollusks, and citrus leafminor (Fotouhi and Fattahi Moghaddam

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2006; Toorani et al. 2020). Amongst these, the citrus soft scale, *Pulvinaria aurantii* Cockerell (Hemiptera: Coccidae), is particularly significant due to its widespread distribution and high economic impact. (Amozegar et al. 2015). *P. aurantii* is a polyphagous pest, and is commonly found on citrus trees and other ornamental plants, feeding on plant sap using its piercing-sucking mouthparts (CABI 2021). Its distribution includes countries in the Australian, Oriental, and Palaearctic regions, with notable presence in Iran's Gilan, Golestan, Mazandaran, and Sistan and Balouchestan provinces (Moghaddam 2013).

Infestations by *P. aurantii* cause both direct and indirect damage to host plants. Direct damage results from sap depletion due to the feeding activity of nymphs and adult females, leading to reduced plant vigor, stunted growth, and premature leaf drop. Indirect damage arises from the excretion of large amounts of sugary honeydew, which supports the growth of sooty molds on plant surfaces, further diminishing photosynthesis and fruit quality (Esmaeili 1996). Additionally, the honeydew attracts ants that protect the scales from natural predators, complicating control efforts (Camacho and Chong 2015).

Traditionally, chemical insecticides have been employed to manage *P. aurantii* populations (Damavandian 2007). However, the use of certain pesticides, such as chlorpyrifos, has faced regulatory challenges. The European Union banned chlorpyrifos in 2020 due to concerns over human health, particularly its potential neurotoxic effects on children (European Commission 2020). This regulatory shift underscores the need for alternative pest management strategies.

Botanical insecticides, deriving from natural plant sources, offer a more environmentally friendly alternative to synthetic chemicals. These compounds typically exhibit lower toxicity to non-target organisms and reduce environmental contamination risks (Isman 2020). Their mechanisms include disrupting insect nervous systems, interfering with growth and development, and acting as repellents or feeding deterrents. Common examples include pyrethrin, rotenone, neem oil, and Spinosad (Bucur et al. 2014; Dively et al. 2020). In contrast, chemical insecticides are synthetic compounds designed to control insect pests. While effective in reducing crop damage and improving yields, they can negatively impact the environment and human health (Ansari et al. 2014). These compounds may persist in the environment, contaminating soil and water, and harming non-target organisms (Beketov et al. 2013). Human exposure has been linked to cancer, reproductive and developmental disorders, and neurological damage (Van Wendel de Joode et al. 2016).

In this study, a selection of insecticidal products with different modes of action and levels of environmental compatibility was used. Chlorpyrifos is a broad-spectrum organophosphate insecticide (Ubaid ur Rahman et al. 2021), while acetamiprid belongs to the neonicotinoid group known for systemic activity (Elbert et al. 2008). Palizin is a natural contact insecticide containing coconut oil, commonly used for the control of soft-bodied pests in environmentally sensitive settings. The use of these oils with insecticides is known to improve the efficacy of insecticides by enhancing their penetration and coverage on plant surfaces (Amiri-Besheli et al. 2020). Mineral oil is a petroleum-derived product that works by suffocating pests through physical coating (Helmy et al. 2012). CitraPlus is a plant-based adjuvant derived from food-grade gums and citrus extract derivatives, used to enhance insecticide efficacy by improving surface coverage and pest contact (Seyyedi-Sahebari et al. 2021).

The aim of this study was to evaluate the efficacy of these pesticides (acetamiprid, Palizin, and chlorpyriphos), both alone and in combination with CitraPlus and mineral oils, in controlling citrus soft scale *P. aurantii.* By comparing the efficacy of different treatments, the study can help identify the most effective and sustainable pest management strategies for citrus orchards. Furthermore, the use of alternative insecticides in combination with oils can reduce the reliance on synthetic insecticides. Therefore, the findings of this study can contribute to the development of more sustainable and environmentally friendly pest management practices for citrus crops.

Materials and Methods

Insect rearing. The original population of *P. aurantii* was collected from an abandoned and unsprayed citrus orchard located in Kiakola, Mazandaran Province, Northern Iran. This region has a humid subtropical climate, with citrus orchards widely distributed throughout the area. The selected orchard had not been treated with any chemical pesticides for at least the past five years, making it a suitable source for collecting a natural population. Adult females at the egg -laying stage were carefully transferred onto healthy orange seedlings to initiate colony establishment. Biennial orange seedlings were cultivated in a greenhouse at the Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University (SANRU), and maintained under controlled conditions (25 ± 1 °C, 75 ± 5% RH, and a photoperiod of 16:8 h light: dark). Once the eggs hatched, the resulting first and second instar nymphs were used for the laboratory bioassays to ensure age uniformity and reduce variability in insect susceptibility.

Compounds Used. The characteristics of the commercial products used in this study, including their formulation types and used dosages are summarized in Table 1.

Table 1. Characteristics and concentrations of the compounds studied in this research.

Pesticides	Active compound	Trade name	Producing company	Formulation	Doses tested for the 1st nymphs	Doses tested for the 2nd nymphs*
Pesticide	Acetamiprid	Mospilan	Meshkfam	SP20%	0.02 0.04 0.2 0.1 0.5	0.05 0.09 0.19 0.38 0.75
	Chlorpyrifos	Dorsban	Mahan	EC40.8%	1.0 1.45 2.2 3.2 5.0	2.0 2.95 4.26 6.16 9.0
	Palizin	Palizin	Kimia Sabzavar	SL65%	0.1 0.2 0.5 1.1 2.5	0.25 0.59 1.41 3.36 8.0
Synergist	Oils	Mineral oils	Shimi Keshavarz	EC80%	5.0 6.0 7.0 8.5 10.0	7.0 9.33 12.02 15.48 20.0
	Oils	CitraPlus	Kimia Sabzavar	EC90%	1.0 1.45 2.2 3.2 5.0	2.5 3.54 5.01 7.07 10.0

*All doses are expressed in milliliters per liter of water (ml/L) except for acetamiprid, which is expressed in grams per liter (g/L).

Bioassay of acetamiprid, chlorpyrifos, and Palizin pesticides on the first instar nymphs. The first instar nymphs were used for this purpose; whereby infected leaves were separated from seedlings. Approximately 10 nymphs were kept on each leaf, and three leaves (3 × 10 nymphs) were used per concentration (the surplus nymphs were removed from the leaf using a brush). In the first phase, during the preliminary experiments, the concentrations that cause mortality of about 25 to 75% in the population were used in the final experiment. For each experiment, a high concentration solution was prepared, and subsequent concentrations were prepared from the bulk solution using the equation N1 × V1 = N2 × V2. In this equation N1 and N2 are high and low concentrated solutions and V1 and V2 are high and low concentrated solution volumes. In this experiment, five concentrations were prepared for all insecticides. Approximately 100 ml of each concentration was prepared and to prevent the solutions from settling, with the solution being thoroughly stirred before dipping the leaves. The leaves were fully immersed in the insecticide solution for approximately 10 seconds and then allowed to air dry at room temperature for about 15 minutes. Nevertheless, to better adhere the insecticides to the leaves, Tween 80 emulsifier (0.02%) was used. At least 30 nymphs were used for each concentration. The petioles were then covered with cotton and placed in disposable containers. The samples were stored in a germinator at 25±1°C, light period of 8:16 (light-dark), and relative humidity of 75±5% mortality was assessed after 24 hours by gently probing the nymphs with a fine needle under a stereomicroscope; those that showed no movement were recorded as dead.

Bioassay of Mineral oils and CitraPlus oils on the first stage of nymph. In order to determine the toxicity of mineral oils and CitraPlus oils, bioassay was performed same as above, with the difference that the mortality rate of nymphs was calculated after 48 hours.

Bioassay of acetamiprid, Palizin, and chlorpyrifos pesticides in the second stage of nymph. The concentrations that caused mortality of about 25 to 75% in the preliminary experiments, were used in the final experiment. At this stage, the bulk solution was prepared from the equation N1 × V1 = N2 × V2. In this equation N1 and N2 represent high and low of dilute solution concentrations and V1 and V2 denote concentrated of dilute solution volumes. In this experiment, five concentrations were prepared for all insecticides. Approximately 100 ml of each concentration was prepared and to prevent the solutions from settling, the solution was thoroughly stirred before dipping the leaves. Nevertheless, to better adhere the insecticides to the leaves, Tween 80 emulsifier with a rate of 0.02% was used. Mortality was assessed 6 days after treatment and nymphs were considered dead if they did not move when gently probed with a fine brush. Biometric experiments, after modification in the Abbott's formula (Abbott 1925), were analyzed by SAS software in the Probit formula. The lethal concentrations of LC_{50} were calculated. The Abbott equation is as follows:

$$P = (\frac{(P' - C)}{(1 - C)} \times 100$$

where:

P = Modified mortality rate

P' = Percentage of mortality observed in pesticide concentration

C = Percentage of the mortality in Control

Mortality counts were performed at optimized times of 48 hours, 60 hours, and 72 hours. The nymphs that were dried and dark in color as well as easily separated from the leaves were considered dead. Three replications for each concentration were conducted, and the value of LC50 of toxins per population was calculated in milliliters per liter.

Statistical analysis. Bioassay data were corrected using Abbott's formula (Abbott 1925) and subsequently analyzed using the Probit model in SPSS software to estimate LC_{50} values. Data visualization and supplementary analysis were performed using Microsoft Excel. Mean comparisons were conducted using Duncan's multiple range test at a 5% significance level ($P \leq 0.05$).

Results

Lethal effect of chlorpyrifos pesticide, acetamiprid, Palizin, mineral oils, CitraPlus oil, and their synergistic effect on the first instar nymph of *P. aurantii*

The effect of Palizin treatment on the mortality of first instar nymph of *P. aurantii* was investigated. The results showed that a concentration of 0.1 ml/L of Palizin resulted in 25% mortality, while a higher concentration of 2.5 ml/L was required to achieve 75% mortality. However, when lower doses of Palizin (1.25 ml/L and 0.95 ml/L) was combined with mineral oils (5 ml/L) or CitraPlus (5 ml/L), the mortality of the first nymph of *P. aurantii* reached 75%.

The results showed that a concentration of 0.04 g/L of acetamiprid caused 25% mortality, while a concentration of 0.5 g / l caused 75% mortality. To reduce the amount of acetamiprid used, a combination treatment of acetamiprid with CitraPlus oil and mineral oils was tested. The results indicated that a lower concentration of acetamiprid (0.25 and 0.35 g/L) was required to achieve 75% mortality when used in combination with the oils, demonstrating the high efficiency of this approach (Table 2, Fig. 1).

The findings presented in Table 2 demonstrate the efficacy of chlorpyrifos treatment in addressing pest infestations. Specifically, at concentrations of 1 ml/L and 5 ml/L, the mortality rates for pests were observed to be 25% and 75%, respectively. Moreover, when used in conjunction with CitraPlus oil at a concentration of 3 ml/L, the required dose of chlorpyrifos was lowered to 0.9 ml/L, resulting in comparable levels of pest mortality. When used individually, chlorpyrifos at a concentration of 4.1 ml/L caused slightly higher levels of pest mortality, with rates of 25% and 75% observed, respectively.

Table 2. Lethal effect of pesticides: Chlorpyrifos, Acetamiprid, Palizin, Mineral oils, CitraPlus oil and their synergistic effect on the first nymph of *P. aurantii*.

Treatment	Dose causing 25% mortality (ml/L)	Dose causing 75% mortality (ml/L)	Time after treatment (hours)
Palizin	0.1	2.5	60
Acetamiprid	0.04	0.5	72
Mineral oils	5.0	10	72
CitraPlus	1.0	5.0	60
Chloropyrifus	1.0	5.0	48
Palizin + Mineral oils	Palizin 0.05 Mineral oils 3.0	Palizin 1.25 Mineral oils 3.0	60
Palizin + CitraPlus	Palizin 0.03 CitraPlus 3.0	Palizin 0.95 CitraPlus 3.0	60
Acetamiprid + Mineral oils	Acetamiprid 0.015 Mineral oils 3.0	Acetamiprid 0.35 Mineral oils 3.0	60
Acetamiprid + CitraPlus	Acetamiprid 0.012 CitraPlus 3.0	Acetamiprid 0.25 CitraPlus 3.0	60
Chloropyrifus + Mineraloils	Chloropyrifus 0.85 Mineraloils 3.0	Chloropyrifus 3.6 Mineraloils 3.0	48
Chloropyrifus + CitraPlus	Chloropyrifus 0.9 CitraPlus 3.0	Chloropyrifus 4.1 CitraPlus 3.0	48

*The dose of mineral oils and CitraPlus oil in all combined treatments was equal to 3 ml/L.

When Acetamiprid and Palizin were used alone, they had LC50 values of 0.28 ml/L and 1.33 ml/L, respectively. These values were the lowest and second lowest among all the pesticides tested. The LC90 value for Acetamiprid alone was 0.80 ml/L, while for Palizin alone, it was 3.04 ml/L. When Acetamiprid was used with Mineral oil or CitraPlus, its LC50 values were slightly lower than when used alone, with values of 0.18 ml/L and 0.13 ml/L, respectively. However, the LC90 values for Acetamiprid with Mineral oil or CitraPlus were higher than when used alone, with values of 0.71 ml/L and 0.68 ml/L, respectively. When Palizin was used with Mineral oil or CitraPlus, its LC50 values were similar to when used alone, with values of 0.68 ml/L and 0.47 ml/L, respectively. However, the LC90 values for Palizin with Mineral oil or CitraPlus were much higher than when used alone, with values of 3.04 ml/L and 0.88 ml/L, respectively (Table 3).

Table 3. Probit analysis of different pesticides on the 1st nymph of citrus soft scale *P. aurantii.*

Pesticides	Total	LC_{50} (ml/L) (CL*)	LC_{90} (ml/L) (CL)	Slope (±SE)	χ^2	p-value
Chlorpyrifos	900	3.2 (2.94 - 3.46)	2.68 (1.89 - 4.6*)	1.71 (0.19)	3.03	0.38
Chlorpyrifos + Mineral oil	900	2.20 (2.12 - 2.32)	1.84 (1.646 -2.212)	5.152 (0.587)	2.911	0.406
Chlorpyrifos + CitraPlus	900	2.31 (2.23 - 2.39)	4.94 (4.72 - 5.34)	4.69 (0.53)	3.92	0.27
Palizin	900	1.33 (1.24 - 1.42)	3.04 (1.47 - 9.81)	0.77 (0.09)	0.66	0.88
Palizin + Mineral oil	900	0.68 (0.66 - 0.70)	0.98 (0.92 - 1.09)	8.22 (0.96)	0.54	0.90
Palizin + CitraPlus	900	0.47 (0.44 - 0.50)	0.88 (0.83 - 0.95)	10.30 (1.18)	2.06	0.56
Acetamiprid	900	0.28 (0.26 - 0.30)	0.80 (0.10 - 1.10)	0.64 (0.09)	12.98	0.005
Acetamiprid + Mineral oil	900	0.18 (0.14 - 0.22)	0.71 (0.69 - 0.75)	18.79 (2.45)	2.73	0.43
Acetamiprid + CitraPlus	900	0.13 (0.10 - 0.16)	0.68 (0.66 - 0.70)	31.48 (3.73)	3.43	0.32
Mineral oil	900	7.56 (7.46 - 7.66)	15.83 (15.43- 16.63)	3.51 (0.45)	1.50	0.68
CitraPlus	900	3.03 (2.98 - 3.08)	7.57 (7.73 - 7.97)	1.45 (0.19)	1.53	0.67

*Denote confidence limit.

Lethal effect of chlorpyrifos pesticide, acetamiprid, Palizin, mineral oils, CitraPlus oil and their synergistic effect on the second instar nymph of *P. aurantii.*

The data presented in Table 4 reveals that the use of Palizin at different concentrations resulted in varying mortality rates for the second instar nymph of *P. aurantii*. A concentration of 0.25 ml/L caused 25% mortality, while a higher concentration of 8 ml/L caused 75% mortality. When Palizin was mixed with mineral oils at 5 ml/L at a concentration of 0.15 ml/L, a 25% mortality rate was observed. Similarly, when Palizin was mixed with CitraPlus at a concentration of 0.11 ml/L, a mortality rate of 25% was observed, which was the same as Palizin with mineral oils. It shows that Palizin with CitraPlus have a better effect than Palizin with mineral oils, and the reason may be that both have plant sources, while mineral oils have a mineral source.

The use of acetamiprid alone at different concentrations resulted in varying levels of pest mortality, with 0.05 g/L causing

25% mortality and 0.75 g/L causing 75% mortality. When mineral oils were added to the treatment at a concentration of 5 ml/L, lower amounts of acetamiprid were needed to achieve the same level of pest mortality, with 0.032 g/L and 0.59 g/L causing 25% and 75% mortality, respectively. Furthermore, when combined with CitraPlus, even lower amounts of acetamiprid were required, with 0.42 g/L achieving the same level of pest mortality as 0.59 g/L with mineral oils. These findings suggest that the combination of acetamiprid with mineral oils or CitraPlus can be more effective in killing pests compared to using acetamiprid alone (Table 4, Fig. 1).

According to the results presented in Table 5, the LC50 value of chlorpyrifos alone was 4.58 ml/L, whereas when combined with mineral oil or CitraPlus, it reduced to 3.3 and 3.5 ml/L, respectively. These findings suggest that the combination of chlorpyrifos and CitraPlus is more effective than the other two pesticides. Similarly, the LC50 value of Palizin alone was 2.4 ml/L, but when combined with mineral oil or CitraPlus, it decreased to 2.01 and 0.7 ml/L, respectively, indicating that CitraPlus plus Palizin was the most effective pesticide. Finally, the LC50 value of acetamiprid alone was 0.32 ml/L, but when combined with mineral oil or CitraPlus, it decreased to 0.3 and 0.2 ml/L, respectively, demonstrating that CitraPlus plus acetamiprid was the most effective pesticide among the three combinations mentioned.

Table 4. Lethal effect of pesticides: Chlorpyrifos, Acetamiprid, Palizin, Mineral oils, CitraPlus oil and their synergistic effect on the second nymph of *P. aurantii*.

Treatment	**Dose Causing 25% mortality (ml/L)**	**Dose Causing 75% mortality (ml/L)**	**Time after treatments (hours)**
Palizin	0.25	8.0	60
Acetamiprid	0.05	0.75	72
Mineral oils	7.0	20	72
CitraPlus	2.5	10	60
Chloropyrifus	2.0	9.0	48
Palizin + Mineral oils	Palizin 0.15 Mineral oils 5.0	Palizin 3.20 Mineral oils 5.0	60
Palizin + CitraPlus	Palizin 0.15 CitraPlus 5.0	Palizin 1.20 CitraPlus 5.0	60
Acetamiprid + Mineral oils	Acetamiprid 0.032 Mineral oils 5.0	Acetamiprid 0.59 Mineral oils 5.0	60
Acetamiprid + CitraPlus	Acetamiprid 0.02 CitraPlus 5.0	Acetamiprid 0.42 CitraPlus 5.0	60
Chloropyrifus + Mineraloils	Chloropyrifus 1.49 Mineraloils 5.0	Chloropyrifus 5.08 Mineraloils 5.0	48
Chloropyrifus + CitraPlus	Chloropyrifus 1.7 CitraPlus 5.0	Chloropyrifus 6.15 CitraPlus 5.0	48

*The dose of mineral oils and CitraPlus oil in all combined treatments was equal to 5 ml/L.

Discussion

Synergistic effects of combining chemical or botanical insecticides with adjuvants have been investigated to improve their efficacy. Given the increasing difficulty in controlling *P. aurantii* with conventional insecticides, exploring strategies that enhance insecticide performance while reducing chemical input has become essential. This study addressed this need by evaluating the potential of adjuvants to improve efficacy against resistant nymphal stages.

The results clearly demonstrate that combining insecticides with adjuvants such as mineral oil and CitraPlus significantly enhances their efficacy against both first and second instar nymphs of *P. aurantii*. Among the tested combinations, acetamiprid paired with CitraPlus exhibited the most pronounced synergistic effect, achieving high mortality at notably lower concentrations. This pattern was consistently observed across both mortality rates and LC50 values, suggesting that CitraPlus may improve insecticide penetration or retention on the pest's cuticle. In contrast, Palizin alone required relatively high concentrations to achieve sufficient mortality, but its combination with CitraPlus markedly improved its performance, particularly against second instar nymphs. These findings highlight the potential of oil-based adjuvants, especially those of botanical origin, to optimize pest control strategies while reducing chemical input.

In the current study, Palizin at a concentration of 0.95 ml/L with 5 ml/L CitraPlus oil caused the death of 75% of the nymphs, while 1.25 ml/L Palizin with 5 ml/L CitraPlus oil resulted in 75% mortality of two instar nymphs of citrus cushions. These results indicate that Palizin is effective in controlling both first and second instar nymphs of citrus cushions, especially when used in combination with CitraPlus oil, which enhances its lethal effect on the pest and reduces its consumption. However, the slight difference in results between this study and others could be due to differences in experimental conditions, as this study was conducted in a laboratory setting while others were performed in natural field conditions. In the present study, it was shown

Table 5. Probit analysis of different pesticides on the 1st nymph of citrus soft scale *P. aurantii.*

Pesticides	Total	LC_{50} (ml/L) (CL*)	LC_{90} (ml/L) (CL)	Slope (±SE)	χ^2	p-value
Chlorpyrifos	450	4.589 (3.928 - 5.435)	23.817 (15.729 - 50.144)	1.792 (0.271)	2.890	0.409
Chlorpyrifos + Mineral oil	450	3.317 (2.967 - 3.730)	8.145 (6.719 - 11.076)	6.447 (0.938)	1.750	0.626
Chlorpyrifos + CitraPlus	450	3.541 (3.146 - 3.948)	8.817 (7.429 - 11.447)	6.134 (0.811)	2.626	0.453
Palizin	450	2.443 (1.794 - 3.547)	51.394 (23.789 - 184.061)	0.969 (0.128)	1.009	0.799
Palizin + Mineral oil	450	2.014 (1.749 - 7.348)	5.183 (4.292 - 11.781)	7.914 (0.974)	0.721	0.868
Palizin + CitraPlus	450	0.746 (0.656 - 0.852)	1.703 (1.463 - 2.095)	19.151 (2.293)	1.613	0.657
Acetamiprid + Mineral oil	450	0.330 (0.279 - 0.392)	0.875 (0.732 - 1.126)	30.309 (4.190)	3.280	0.350
Acetamiprid + CitraPlus	450	0.277 (0.242 - 0.322)	0.626 (0.533 - 0.780)	46.189 (5.927)	3.404	0.333
CitraPlus	450	5.975 (5.228 - 7.007)	22.858 (16.130 - 41.900)	2.199 (0.316)	2.179	0.536
Acetamiprid	450	0.322 (0.254 - 0.432)	2.884 (1.639 - 7.221)	1.346 (0.173)	2.513	0.473
Mineral oil	450	14.881 (13.643 - 16.576)	32.744 (26.746 - 45.249)	3.742 (0.466)	0.632	0.889

*Denote confidence limit.

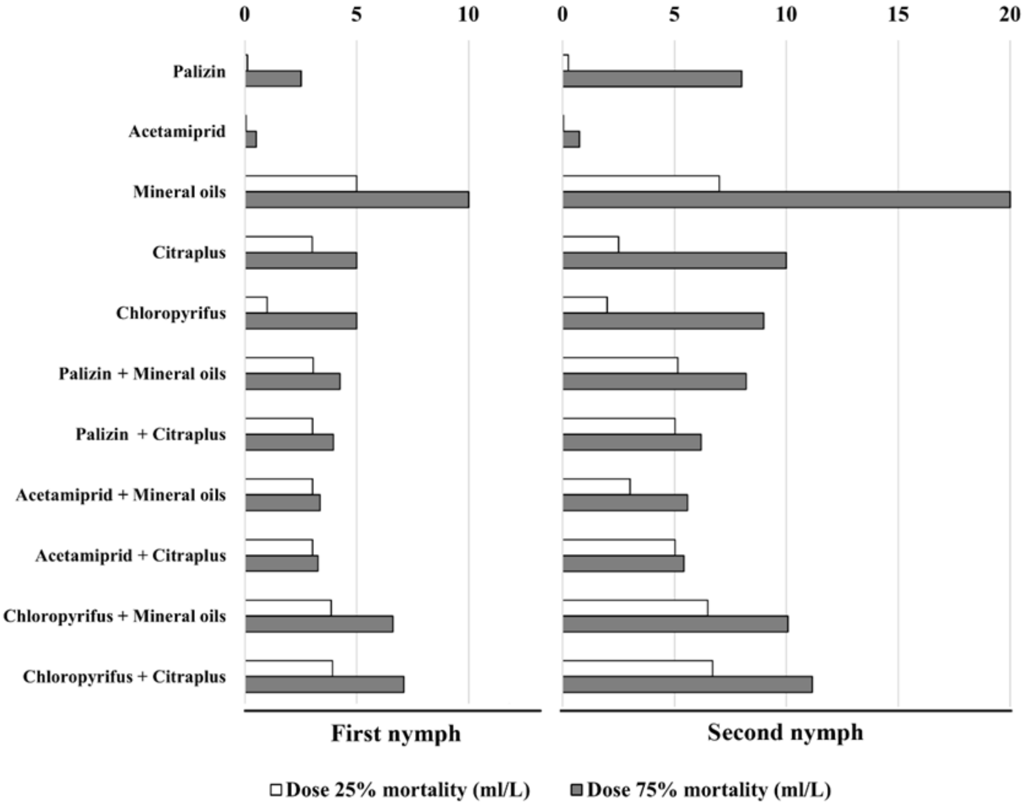

FIG. 1: Investigation of the lethal effect of pesticides: Chlorpyrifos, Acetamiprid, Palizin, Mineral oils, CitraPlus oil and their synergistic effect on the nymphs of *P. aurantii*.

that when Palizin botanical insecticide is combined with CitraPlus oil, less amount of Palizin is required compared to when this insecticide is combined with mineral oil as both Palizin and CitraPlus oil are plant-based and have better compatibility (Hummelbrunner and Isman, 2001; Ravela, 2015). This supports our conclusion that botanical adjuvants offer enhanced compatibility and efficacy in integrated pest management programs.

Toorani et al. (2017) reported that Palizin, a plant-based insecticide, at a concentration of 2.5 ml/L, caused 53% mortality in the nymph stage and 42% in the second nymph stage of *P. aurantii* after 24 hours. In contrast, the current study found that the same concentration resulted in 75% mortality in one stage of citrus cushion nymphs, with mortality assessed 60 hours after spraying. The difference in mortality rates may be attributed to variations in testing conditions, particularly the timing of mortality assessment.

According to research conducted by Shahnazari (2012), chlorpyrifos insecticide is effective in killing first-instar nymphs of P. aurantii, which is consistent with the results of the current study. Chlorpyrifos is widely used as an insecticide in citrus orchards in Mazandaran province, but its excessive use can pose environmental risks. Furthermore, Chlorpyrifos can also negatively affect insect predators, which are important natural enemies of pest insects. For example, Tourani et al. (2017) examined the effects of chlorpyrifos, a widely used insecticide, on both beneficial and target insect species in citrus orchards.

Their findings showed that a concentration of 2 ml/L caused 83.3% ± 8.8% mortality in *Cryptolaemus montrouzieri*, a key predatory ladybird, and 90% mortality in overwintering female adults of *P. aurantii* within 24 hours, highlighting the non-selective toxicity of chlorpyrifos and the need for safer alternatives. However, in our recent study, even a higher concentration (9 ml/L) resulted in only 75% mortality in the second-instar nymphs of *P. aurantii*, suggesting the development of resistance. This resistance is likely driven by prolonged and repeated exposure to chlorpyrifos, leading to strong selection pressure. Reported resistance mechanisms in armored scales include increased detoxification enzyme activity and reduced cuticular penetration (Kanga et al. 2018; Tian et al. 2018). Such resistance justifies the need for combining insecticides with effective adjuvants to restore control.

In recent studies, CitraPlus and mineral oil have been used as adjuvants with insecticides to control various pests in crops. Hu et al. (2024) investigated the enhanced efficacy of synthetic insecticides (chlorpyrifos, thiamethoxam, and pyriproxyfen) when combined with mineral oil (Lvying) against the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), under varying rainfall conditions. Mineral oil improved rainfastness and enhanced deposition by lowering surface tension. Scanning electron microscopy showed physical damage to mouthparts and spiracles. These results highlight the dual function of mineral oils as adjuvants and insecticidal agents, reinforcing our current findings.

Seyyedi-Sahebari et al. (2021) evaluated Palizin alone and with CitraPlus oil under field conditions against cabbage aphid. Both treatments were effective, supporting their use in integrated pest management (IPM). They also reported that winter foliar sprays using 2% CitraPlus with Palizin or recommended pesticides could eliminate overwintering pests like soft scales and moth eggs. Moreover, CitraPlus at 9 ml/L with 1.9 ml/L Palizin effectively controlled mites and citrus soft scale. These findings are consistent with our results, suggesting the broader applicability of CitraPlus-based treatments in citrus pest management.

Sepasi et al. (2018) demonstrated that mineral oil was among the most effective treatments in reducing *Helicella candeharica* Pfeiffer L (Pulmonata: Hygromiidae) snails in citrus orchards. Its cost-effectiveness and consistent performance make it a promising sustainable tool, aligning with our findings on mineral oil's efficacy in integrated citrus pest management.

Amiri-Beshli et al. (2019) tested several insecticides on aphids and the predator *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae). Palizin with Citrol oil and Dayabon (7–10 ml/L) caused high aphid mortality, particularly *Aphis spiraecola* Patch (Hemiptera: Aphididae) and *A. citricidus* van der Goot (Hemiptera: Aphididae). Malathion caused complete ladybird mortality, while Dayabon had the least impact. This supports the selectivity advantage of botanical insecticides, a key consideration for maintaining beneficial insect populations.

In a greenhouse study on cut roses in various regions of Iran, Arbabi et al. (2022) found that flufenzin and Dayabon-3 were effective against *Tetranychus urticae* Koch (Acari: Tetranychidae) on roses. Flufenzin performed better in Tehran and Esfahan. They emphasized rotating pesticides and adjusting dosages and timing to delay resistance—a strategy that complements our approach of combining botanicals and oils to enhance control.

Raza et al. (2017) reviewed citrus pest management strategies in China, emphasizing non-chemical (green) methods. They stressed the growing demand for high-quality, pesticide-free citrus, highlighting the need for integrated pest management. Their review covered agricultural practices like pruning, ground cover, and winter orchard sanitation, as well as biological control through conservation and augmentation of natural enemies. They also discussed microbial and mineral pesticides, including mineral oils, whose role in sustainable pest control supports our current findings.

Amiri-Besheli (2008) evaluated eco-friendly insecticides—including *Bacillus* *thuringiensis*, mineral oil, and plant-based emulsions—against *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in lab conditions. All treatments significantly increased larval mortality compared to the control, though differences among them were not significant. Mineral oil caused 37.7% mortality, indicating moderate efficacy, with peak mortality observed 72–96 hours post-treatment. These findings support the use of mineral oil in integrated management, though chemical control may still be necessary under heavy infestations.

This study confirms the effectiveness of Palizin in pest control, as it had a significant impact on the first and second instar nymphs of *P. aurantii*. The results showed that Palizin at a dose of 2.5 ml had a mortality rate of 71%, with an LC50 of 2.44 ml/L. Combined with the synergistic effects observed with CitraPlus oil, our findings support the potential for reducing pesticide input without compromising efficacy.

Conclusion

This experiment demonstrated that combining plant-based insecticides such as Palizin with CitraPlus oil demonstrate synergistic effect, allowing for effective control at lower doses. This combination also presents reduced environmental impact. The citrus cushion scale showed resistance to chemical insecticides like chlorpyrifos, with limited changes in mortality even at higher concentrations. In contrast, the use of natural enemies and biological control strategies proved effective and are considered among the most suitable approaches for the ecological conditions of northern Iran. Given the adverse effects of conventional insecticides on the environment and human health, employing insect growth regulators (IGRs) and oil-based products that reduce reliance on chemical insecticides is highly beneficial. Overall, this study aimed to maintain pest control efficacy while minimizing pesticide use and preserving natural enemy populations.

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