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RESEARCH ARTICLE — OPEN ACCESS

Fatty acids from soybeans: compatibility with *Panonychus citri* (Acari: Tetranychidae) and its two predators☆

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Abstract – Soybean oil is a significant alternative to synthetic chemicals for environmentally friendly and sustainable pest control. This study evaluated soybean oil and its fatty acids (palmitic, oleic, and stearic acids; individually) for their acaricidal and repellent effects against *Panonychus citri* (Tetranychidae) and its predators [*Neoseiulus californicus* and *Neoseiulus barkeri* [Phytoseiidae]) using laboratory foliar dipping and topical spray methods. The LC_{50} (Lethal Concentration) values for palmitic, oleic, stearic acids, and soybean oil against *P. citri* were 0.86%, 0.95%, 0.56%, and 0.05% for LDM (Leaf-dipping Method) and 11.92%, 5.34%, 1.46%, and 0.07% for TSM (Topical Spray Method). Soybean oil has maximum attraction (46.67 ± 3.6%) and significantly higher acaricidal effectiveness (46.25 ± 0.98%) compared to acids except oleic acid (50.83 ± 1.22%) against *P. citri*. Soybean oil showed the least toxicity, with the highest LT_{50} (Lethal Time) values for *N. californicus* (LDM: 1.19 h, TSM: 1.27 h). While soybean oil caused a less significant reduction in fertility and lower lethal efficacy (0.83 ± 0.83%) on predatory mites compared to *P. citri*. Compared to soybean oil, oleic acid reduces fertility rate (27 ± 1.60%) and is an effective repellent (65-75%) against *N. californicus* and *N. barkeri* and reduces prey consumption (15-16%) and laying eggs (0.75 ± 0.47) on treated surfaces compared to other treatments. Palmitic acid increases prey consumption (27.5 ± 3.28%) and fertility rates (5.00 ± 0.71) of *N. californicus*. Palmitic acid, despite being less toxic to *P. citri*, effectively repels predators, while soybean oil and oleic acid have stronger repellent effects. Leaf dipping was less effective than topical spray. Soybean oil, with its lower mortality rates and sublethal effects on reproduction and behavior, is a strong candidate for integrated pest management strategies. Oleic and palmitic acids, though effective, have repellency to beneficial predators.

Keywords: citrus red mite / predatory mites / soybean oil / acaricidal efficacy / repellency

Résumé – **Acides gras de soja : Compatibilité avec Panonychus citri (Acari : Tetranychidae) et ses deux prédateurs.** L'huile de soja est une alternative importante aux produits chimiques synthétiques pour une lutte antiparasitaire durable et respectueuse de l'environnement. Cette étude a évalué l'huile de soja et ses acides gras (acides palmitique, oléique et stéarique ; individuellement) pour leurs effets acaricides et répulsifs contre *Panonychus citri* (Tetranychidae) et ses prédateurs (*Neoseiulus californicus* et *Neoseiulus barkeri* (Phytoseiidae)) en utilisant des méthodes de trempage foliaire et de pulvérisation topique en laboratoire. Les valeurs de la LC50 (concentration létale) pour les acides palmitique, oléique, stéarique et l'huile de soja contre *P. citri* étaient de 0,86 %, 0,95 %, 0,56 % et 0,05 % pour la méthode de trempage foliaire (LDM) et de 11,92 %, 5,34 %, 1,46 % et 0,07 % pour la méthode de pulvérisation topique (TSM). L'huile de soja présente une attraction maximale (46,67 ± 3,6 %) et une efficacité acaricide significativement

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plus élevée contre *P. citri* (46,25 ± 0,98 %) par rapport aux acides gras, à l'exception de l'acide oléique (50,83 ± 1,22 %),. L'huile de soja a montré la toxicité la plus faible, avec les valeurs de temps létal (LT50) les plus élevées pour *N. californicus* (LDM : 1,19 h, TSM : 1,27 h). L'huile de soja a entraîné une réduction moins importante de la fertilité et une efficacité létale plus faible (0,83 ± 0,83 %) sur les acariens prédateurs par rapport à *P. citri*. Comparé à l'huile de soja, l'acide oléique réduit le taux de fertilité (27 ± 1,60 %) et est un répulsif efficace (65-75 %) contre *N. californicus* et *N. barkeri*, réduit la consommation de proies (15-16 %) et la ponte (0,75 ± 0,47) sur les surfaces traitées par rapport aux autres modalités. L'acide palmitique augmente la consommation de proies (27,5 ± 3,28 %) et les taux de fertilité (5,00 ± 0,71) de *N. californicus*. L'acide palmitique, bien que moins toxique pour *P. citri*, repousse efficacement les prédateurs, tandis que l'huile de soja et l'acide oléique ont des effets répulsifs plus importants. La technique de trempage des feuilles a été moins efficace que la pulvérisation topique. L'huile de soja, avec ses taux de mortalité et ses effets sublétaux plus faibles sur la reproduction et le comportement, est un candidat pertinent pour les stratégies de lutte intégrée contre les ravageurs. Les acides oléiques et palmitiques, bien qu'efficaces, ont un effet répulsif sur les ennemis naturels prédateurs.

Mots clés : acarien rouge des agrumes / acariens prédateurs / huile de soja / efficacité acaricide / effet répulsif

Highlights

- Soybean oil, with its low mortality rates and sublethal effects on reproduction and behavior, is a promising candidate for integrated pest management strategies.
- Oleic acid was found toxic against *Panonychus citri* and repellent against predatory mites.
- Palmitic acid attracts all mites, increasing prey consumption and fertility rates of *Neoseiulus californicus* (McGregor).
- *N. barkeri* (Hughes) exhibited a favorable response to stearic acid, demonstrating higher prey consumption and fertility rate.

1 Introduction

Food waste is a crucial global issue that affects the environment, economy, and society. Wastage of food: Almost one-third of all food produced (approx. 1.3 billion tons/year) for human consumption is lost or wasted annually (Derqui *et al.,* 2016; Falasconi *et al.,* 2019). Food waste is a major global environmental and economic challenge; according to the Food and Agriculture Organization (FAO), there are several reasons for food waste, including production, processing, retailing, and consumption leading to a lack of public awareness resulting in Greenhouse Gas (GHG) emissions and Resource Depletion likewise (Zuin and Ramin, 2018; Forster-Carneiro *et al.,* 2013; Perlatti *et al.,* 2014; Visakh *et al.,* 2023; Panzella *et al.,* 2020) etc. Several sustainable strategies like composting and bioenergy production from food waste have been suggested to overcome these challenges (Kwanyun *et al.,* 2023; Paritosh *et al.,* 2017).

Kitchen waste can be used as a natural pesticide, reducing human health risks and environmental pollution (Iqbal *et al.,* 2022). This practice aligns with sustainable farming systems, as it reduces chemical pesticides and offsets excess food waste. It also aligns with integrated pest management principles, promoting sustainability in agriculture by integrating bio-pesticides derived from kitchen waste into consistent pest control strategies (Conrad *et al.,* 2018; Stenberg, 2017). In China's restaurant industry, kitchen waste generates significant waste, with 80% used on pig farms. Soybean oil (*Glycine max*), a valuable raw material for food, feed, chemical, and healthcare industries, is consumed annually at 17.8 million tons (Patton, 2023). Burning cooking oil poses environmental and health risks, while soybean oil's long-chain fatty acids can be used as an environmentally friendly insecticidal substitute with attractive and repellent properties (Qayyoum *et al.,* 2021a; 2021b). Soybean oil bodies are capsules 250–700 µm diameter containing neutral lipid droplets, natural emulsifiers, alkaline proteins, and micro-nutrients (Zaaboul *et al.,* 2022). It is rich in saturated, monounsaturated, and polyunsaturated fats (alpha-linolenic, linoleic, stearic, palmitic, and oleic acids) (Saraiva *et al.,* 2020). Linoleic acid, a vital component of vegetable oil, led to attractive reactions (Buehlmann *et al.,* 2014). According to various studies, alpha-linolenic acid and linoleic acid found in southern cattails can alter the permeability of plant plasma membranes and destroy chloroplast membranes (Wu *et al.,* 2006). In previous studies, vegetable oil's short-chain compound (palmitic acid) gave equal repellency to synthetic chemicals (Mullens *et al.,* 2009). Oleic, stearic, and palmitic acids had significant toxic effects on *Callosobruchus maculatus* (Coleoptera: Bruchidae) and reduced longevity, fecundity, and hatchability of eggs (Aider *et al.,* 2016).

Panonychus citri, a citrus red mite, is a significant pest in citrus orchards worldwide. Current control measures include pesticide spraying, which can cause toxic effects (Karmakar, 2019). IPM strategies have been used in China since the 1970s (Liu *et al.,* 2019), but most studies focus on control effectiveness, pesticide resistance, and management (Xiao *et al.,* 2010). The focus of IPM programs should be on minimizing toxicity to natural beneficial fauna rather than causing significant harm to the target species (Tsolakis and Ragusa, 2008). Natural and naturally available predators primarily control insect pest populations, but phytoseiidae have been used as biological control agents for various phytophagous mites (Chang and Kareiva, 1999). Among the phytoseiids, *Neoseiulus californicus* and *N. barkeri* are commonly used as augmented biological control against various mites and insect pests (Mendel and Schausberger, 2011; Qayyoum *et al.,* 2021c). *N. californicus* and *N. barkeri*, commercially available since the 1980s, effectively control spider mites in crops like strawberries and tomatoes, reducing reliance on chemical pesticides (Fang *et al.,* 2013; Silva, 2023). *N. californicus* is a versatile predator known for its adaptability and ability to thrive in various environments. It can consume spider mites like *Tetranychus urticae* and *P. citri*, effectively suppressing populations of *P. citri* (Katayama *et al.*, 2006; Ebrahim *et al.*, 2014). *N. californicus* can maintain its population even with low prey densities (Abad-Moyano *et al.*, 2010; Greco *et al.*, 2005), feeding on pollen. On the other hand, *N. barkeri*, a generalist predator, exhibits different predation dynamics, particularly in intraguild interactions (Momen and Abdel-Khalek, 2021; Haghani *et al.*, 2015) (Abad-Moyano *et al.*, 2010; Çakmak *et al.*, 2006). Both species coexist and contribute to pest suppression in agricultural settings (Ahn *et al.*, 2010; Hoddle *et al.*, 2000).

Soybean oil can easily penetrate eggs, and the soft integument of *T. urticae* inhibits the rotational movement of the embryonic liquid (Oliveira *et al.*, 2017; Takeda *et al.*, 2020). It can be used in combination with predatory mites (*N. baraki* and *Typhlodromus ornatus*) (Oliveira *et al.*, 2020; Saraiva *et al.*, 2020; Teodoro *et al.*, 2020). Higher rates resulted in greater phytotoxicity (Baker *et al.*, 2018), but vegetable oils have a broad-spectrum effect against soft-bodied insect pests (Alexenizer and Dorn, 2007). However, their selection should be strictly evaluated against natural enemies and targeted pests (Guedes *et al.*, 2016; Tsolakis and Ragusa, 2008). Few studies have been conducted on evaluating fatty acids against *P. citri* and its predators, except for previous research. We tested three fatty acids (stearic acid, palmitic acid, and oleic acid) (synthetically available in the market) along with soybean oil for their toxicity and repellency (behavioral responses) effects against *P. citri*, *N. californicus*, and *N. barkeri*. The findings will provide insights into the potential use of these compounds for integrated pest management strategies.

2 Materials and methods

2.1 Mites culture

P. citri were reared under controlled laboratory conditions according to Qayyoum *et al.* (2021a, 2021b). *N. californicus* was collected from *Carica papaya* (Caricaceae) in Guangzhou, China (N 23.15, W 113.33) in 2011, as detailed in Qayyoum *et al.* (2021c). *N. californicus* was maintained using mixed stages of *T. urticae* on bean [*Phaseolus vulgaris* (Fabaceae]) leaves placed on water-saturated sponges (10 cm in diameter, 3 cm thick) in plastic boxes (15 cm × 15 cm × 6.5 cm). *N. barkeri* was reared on *Tyrophagus putrescentiae* (Acaridae), which was collected from wheat bran, and colonies were set up in Petri dishes (9 cm in diameter) with dry yeast; the Petri dishes were placed on water-saturated sponges (13 cm in diameter, 3 cm thick) in plastic circular boxes (20 cm in diameter) with water. All cultures were kept in climatic incubators (25 ± 1 °C, 65 ± 10% RH, and 16:8 h L:D) (Zheng *et al.*, 2017). All predatory mites were reared for over three generations on *P. citri* for prey-predator synchronization.

2.2 Chemicals

The Institute of Zoology, Guangdong Academy of Sciences, Guangzhou, China, provided soybean oil (*G. max*) as a trial product (Kitchen waste soybean oil) against the citrus red mite. Soybean oil contains 54% of Linoleic acid ethyl ester ($C_{20}H_{36}O_2$), 8% of Hexadecanoic acid, ethyl ester (palmitic acid = CH_3 $(CH_2)_{14}COOH$), 5% 9-Octadecenoic acid (Z)-, methyl ester (Oleic acid = $C_{19}H_{36}O_2$) and 1.8% of Octadecanoic acid, ethyl ester (Stearic acid = $C_{18}H_{36}O_2$).

Fatty acids were purchased from local markets: palmitic, 99% granular, purchased from Tianjin Fuchen chemicals reagents factory; stearic acid, 99% granular, purchased from Fuchen reagents factory and Oleic acid liquid purchased from Tian Fuyu Fine Chemicals Co. Ltd. Note: We have not used the linoleic acid because in small amounts it has maximum toxicity to predators and causes phytotoxicity in plantations.

Note: Linoleic acid is a significant part of soybean oil, but we did not select it because it is difficult to mix with water.

2.3 Chemical preparation

Two percent (0.50 mg) of acids were added to the distilled water with 1% tween and 18% ethanol. The homogenous mixture solution was made by heating it and stirring it for 2–5 min on the low-flamed burner. After attaining a solution, we used five different concentrations (2%, 1%, 0.5%, 0.25%, and 0.124%) for each fatty acid for soybean oil, followed (Qayyoum *et al.*, 2021a, 2021b) with four concentrations (0.12, 0.06%, 0.03%, and 0.013%).

All concentrations were mixed into the tap water for further use, with mortality ranging from 10% to >90%. The data was collected at 24 h, 48 h, 72 h, and 96 h intervals to obtain said mortality percentage. We also used 1% tween and 18% ethanol in water as control treatments in all experiments.

The lethal concentrations obtained through the leaf dip methods were further used for lethality effect and behavioral responses.

2.4 Experimental methodology

The toxicities of all chemicals were calculated using the modified leaf dip method (He *et al.*, 2011) and topical spray method (see supplement data). Green and healthy leaves were collected from the untreated lemon field and stored in the refrigerator for 24 hours. Before cutting leaf discs, each leaf was washed with water thoroughly and dried using a paper towel. Each leaf disc (≈3 cm^2) was fully dipped for 5 seconds and shifted on the water-saturated sponge in the plastic container. It was surrounded by wet tissue paper to avoid mites' escape. Thirty adult female mites (same age as laboratory-reared strain − ≥1-3 days old adults) were released on each leaf after 10–15 min of drying the leaf, with three replications. Water-dipped leaves were used as the control treatment. A bioassay test was performed by maintaining the 26 ± 1 °C, 16:8h (L:D) photoperiod, and 75% relative humidity in an incubator (Qayyoum *et al.*, 2021).

Each chemical's selected concentrations were also sprayed with a fine mist sprayer pump (perfume spray nozzle). A total of 4–6 times the chosen concentrations were sprayed on the leaf discs. The data of dead mites and the number of eggs were counted with specific time intervals using both methods.

For lethality effectiveness, we used lethal doses obtained by leaf dipping. The topical spray method provided high concentrations of all fatty acids (more than 1%) that can have a phytotoxic effect on plantations. The lethality of each LC_{50} was checked against both predators and prey (*P. citri*), and data were recorded at 4 h, 8 h, 12 h, 24 h, 48 h, 72 h, and 96 h intervals. We also inspected the impact of lethality on the total number of eggs, eggs hatching %, and % offspring reached the adult stages (Adultery %). We used 40 eggs to check the hatching % and % adultery of offspring.

We also used the leaf dip method for behavioral experiments, a lethal dose of each chemical against *P. citri*. The behavioral responses of *N. californicus* and *N. barkeri* were evaluated on the repellency of treated leaf discs, and prey consumption was recorded. Two plastic strips (1 cm in diameter) were used to make an "X-shaped" cross-bridge between four equally spaced circular cells. Ten female adults of ≥1–3 days old (He *et al.,* 2011) were released in each cell. Ten *N. californicus* and *N. barkeri* were individually released on the cross point for different experimentations. The above methodology was performed to compare the chemical with the control treatment in a choice test using two experimental methods by replicating four times. The number of predatory mites present and the number of prey consumed were recorded after 24 hours of predatory mites' release.

The behavioral response of choice test was used as described by Roh *et al.,* (2013). We used more than 7 months of citrus plantation with almost 1–2 feet in height, which bears the insect and phytotoxic effect. We clipped all leaves except two leaves from different sides. One leaf was dipped with solution (LC_{50}) of each solution for 10 seconds, and the second leaf was left blank. After air-drying, 30 mites of female *P. citri* adults were released on the stem. The adult mite mortality and the total number of eggs on the treated and untreated were recorded after 24-hour mite inoculation. This section of the experiment was replicated four times.

2.5 Statistical analysis

The five selected concentrations of each acid (2%, 1%, 0.5%, 0.25%, and 0.124%) were used to calculate the lethal concentrations (LC_{50}) along with lethal time (LT_{50}). The confidence interval of 95% was also estimated with a log-probit regression model using SPSS version 22.0 (IBM-Corp., 2013). The data between the two treatments (treated *vs.* untreated (Choice test)) was compared using an independent samples *t*-test, suggesting that both treatments were unequal for behavioral response experiments.

ANOVA was analyzed using treatment and time as fixed factors (lethal efficacy of soybean and fatty acids against *Panonychus citri* and its predators to check mortality, number of eggs, hatching percentage, and eggs reached adultery). In contrast, the number of mites was a dependent variable. If the interaction was significantly observed, the data was sliced by time. All analysis was done using SPSS version 22.0 (IBM-Corp., 2013).

3 Results

3.1 Toxicity against *Panonychus* citri and its predators

The toxic effect of different concentrations of each acid resulted in lethal and sublethal effects, as shown in Table 1. The LC_{50} of palmitic, oleic, stearic acids, and soybean oil

Table 1. Toxicity of fatty acids of soybean plant against *Panonychus citri* in two application methods. All units are used in percentage V/V.

Treatments	Leaf dip method							
	LC_{30} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope ± SE	Z	χ^2 (*df*=3)	P1	P2
Palmitic acid	0.137 (0.003-0.309)	0.861 (0.208-1.748)	76.205 (11.008-539200188.2)	0.658 ± 0.248	2.656	0.437	0.008	0.933
Oleic Acid	0.193 (0.023-0.374)	0.949 (0.507-4.200)	46.831 (7.483-203445.94)	0.757 ± 0.251	3.019	0.282	0.003	0.963
Stearic acid	0.180 (0.058-0.302)	0.564 (0.345-0.974)	9.224 (3.379-174.164)	1.056 ± 0.256	4.118	1.306	0	0.728
Soybean oil	0.025 (0.013-0.035)	0.051(0.037-0.077)	0.298(0.156-1.421)	1.676 ± 0.376	4.454	0.294	0	0.863
	Topical spray method							
Palmitic acid	0.667 (0.082-191865.849)	11.922 (2.231-2.078E+46)	2008.321 (32.566-4.743E+195)	0.519 ± 0.260	1.996	0.045	0.046	0.997
Oleic Acid	0.465	5.336	2070.177	0.495 ± 0.255	1.941	0.885	0.052	0.829
Stearic acid	0.442 (0.364-0.526)	1.464(1.178-1.941)	27.361(6.890-1916.773)	1.008 ± 0.268	3.762	1.369	0	0.713
Soybean oil	0.031 (0.015-0.046)	0.074 (0.050-0.155)	0.615 (0.239-11.449)	1.389±0.373	3.725	0.755	0	0.685

Note: The concentrations were obtained using 2%, 1%, 0.5%, 0.25%, and 0.125% solutions except 0.12%, 0.06%, 0.03%, and 0.0125% for Soyabean Oil.

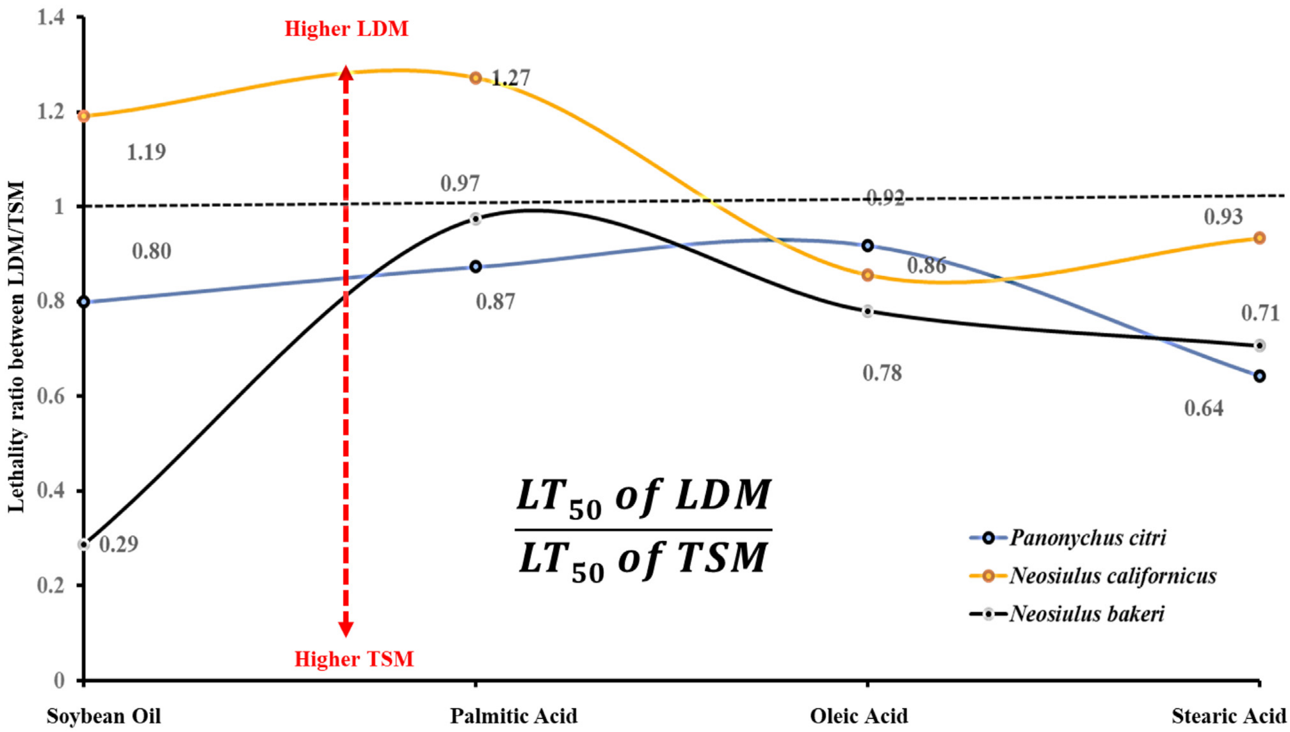

Fig. 1. Lethal time (LT_{50}) ratio between the leaf dip method and topical spray method of exposure to soybean oil and fatty acids at different concentrations of each treatment over observational time (24 h, 48h, 72h, and 96h) against *Panonychus citri* and its predators.

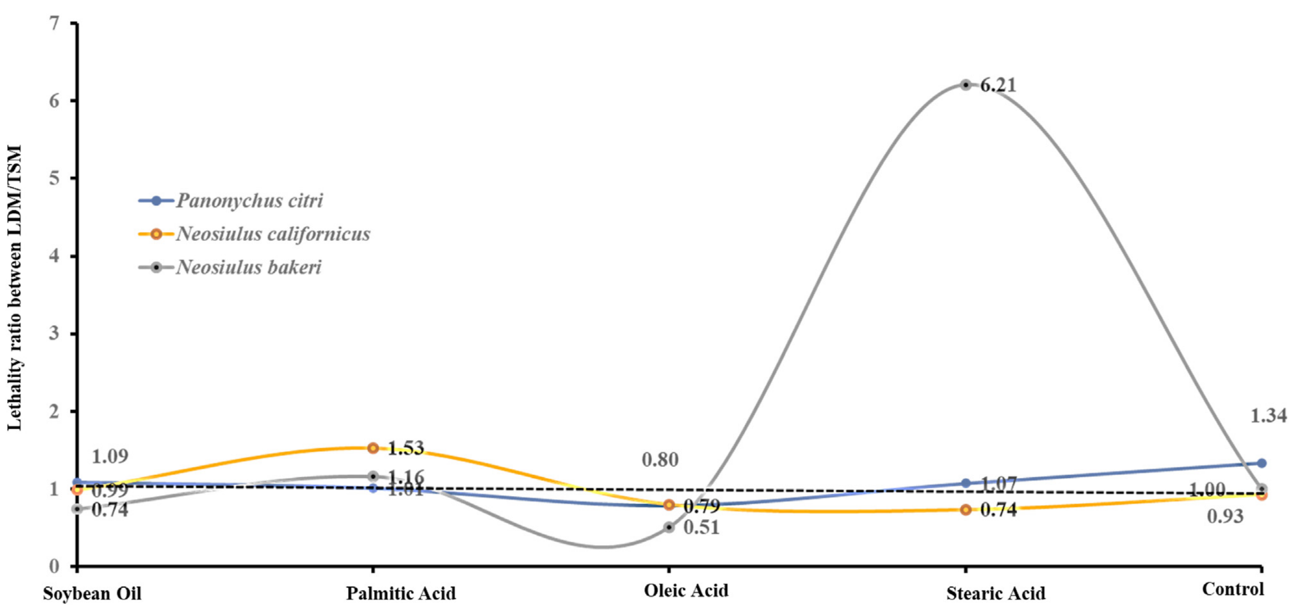

Fig. 2. Lethal time (LT_{50}) ratio between the leaf dip method and topical spray method of exposure to soybean oil and fatty acids at a lethal concentration of each treatment over observational time (4 h, 8 h, 12 h, 24 h, 48 h, 72 h, and 96 h) against *Panonychus citri* and its predators.

were valued at 0.86%, 0.95%, 0.56%, and 0.05%, respectively, for the leaf dipped method (LDM) and 11.92%, 5.34%, 1.46%, and 0.07%, respectively for the topical spray method against *P. citri* (Tab. 1). The leaf dipping method resulted in quick mortality compared to the topical spray method (TSM) with lower lethal time (LT_{50}) except for soybean oil (1.19) and palmitic acid (1.27) against *Neoseiulus californicus*. Soybean oil resulted lower LT_{50} comparison value (0.29) for LDM (Slope ± SE = 1.49 ± 2.21, χ^2 (df = 2) = 0.02, $P = 0.98$) compared to TSM (Slope ± SE = 0.80 ± 1.24, χ^2 (df = 2) = 0.01, $P = 0.99$) which mean TSM resulted lowest effectiveness against *N. barkeri* (Fig. 1).

Table 2. The lethal efficacy of soybean and fatty acids against *Panonychus citri* and its predators was exposed using leaf dip and topical spray after 24 h of exposure.

Treatments	Leaf dip method		Topical Spray method	
	Mortality %	Total no. eggs	Mortality %	Total no. eggs
Panonychus citri (McGregor) (n=30)				
Soybean oil	46.25 ± 0.983a	27.5 ± 0.567b	43.75 ± 0.983a	29.5 ± 0.567b
Palmitic acid	32.917 ± 1.598c	30.75 ± 1.319b	39.583 ± 1.598ab	29.75 ± 1.319b
Oleic acid	50.833 ± 1.22a	27 ± 1.604b	29.583 ± 1.327c	26 ± 1.604b
Stearic acid	38.333 ± 0.63b	30.75 ± 1.319b	35 ± 0.63b	27.75 ± 1.319b
Control	5 ± 1.409d	79.625 ± 2.146a	2.5 ± 1.045d	95 ± 2.557a
Statistics at df = 4,35	F = 218.54, P = <.001	F = 234.83, P = <.001	F = 196.12, P = <.001	F = 345.99, P = <.001
Neoseiulus californicus (McGregor) (n=15)				
Soybean oil	0.833 ± 0.833b	12.375 ± 0.905b	0.833 ± 0.833a	14.375 ± 0.905b
Palmitic acid	4.167 ± 1.22a	8.25 ± 0.62c	0.833 ± 0.833a	6.25 ± 0.62d
Oleic acid	0 ± 0b	12.125 ± 0.666b	0 ± 0a	10.125 ± 0.666c
Stearic acid	0 ± 0b	19.875 ± 1.043a	2.5 ± 1.754a	17.875 ± 1.043ab
Control	0 ± 0b	21.875 ± 1.043a	0 ± 0a	19.875 ± 1.043a
Statistics at df = 4,35	F = 7.48, P = <.001	F = 43.08, P = <.001	F = 1.17, P =0.342	F = 40.66, P = <.001
Neoseiulus barkeri Hughes (n=15)				
Soybean oil	2.5 ± 1.22ab	7.125 ± 0.833c	3.33 ± 1.26bc	7.625 ± 0.73c
Palmitic acid	5.833 ± 1.511a	5.25 ± 0.62c	7.5 ± 0.833a	11.125 ± 0.693c
Oleic acid	1.667 ± 1.091ab	7.625 ± 0.73c	4.167 ± 1.22ab	7.625 ± 0.73c
Stearic acid	1.667 ± 1.091ab	17.875 ± 1.043b	5 ± 1.091ab	15.875 ± 1.043b
Control	0 ± 0b	27.25 ± 1.77a	0 ± 0c	25.25 ± 1.77a
Statistics at df = 4,35	F = 3.78, P = 0.012	F = 75.07, P = <.001	F = 7.49, P = <.001	F = 47.33, P = <.001

The ratio between LDM and TSM was higher (1.27) for palmitic acid, as LDM responded slower than TSM. Soybean oil had significantly different effects across methods and mites used, while oleic acid had a more significant impact. Stearic acid (20.26 h, Slope ± SE = 2.37 ± 0.58, χ^2 (df = 2) = 2.12, P = 0.35) and palmitic acid (111.06 h, Slope ± SE = 3.51 ± 1.38, χ^2 (df = 2) = 0.29, P = 0.86) resulted maximum impacted against *P. citri* and *N. barkeri* respectively. Soybean oil was less effective against predatory mites than fatty acids used, as shown in figure 1 (see Tab. S1).

We also checked the lethal effect against *P. citri* and its predators (*N. californicus* and *N. barkeri*) with a time interval of 4, 8, 12, 24, 48, 72, and 96 h after exposure (Tab. S2, Fig. 2, Fig. S4). The lethal time (LT_{50}) of oleic acid was shortest (Slope ± SE = 2.33 ± 0.26, χ^2 (df = 5) = 8.22, P = 0.15) during leaf dip method with 0.79 compared value between LDM (20.73 h) and TSM (26.36 h), followed by soybean oil (1.09), stearic acid (1.074), and palmitic acid (1.33) against *P. citri*. The lethality activity of oleic acid was more toxic against *N. californicus* and *N. barkeri*, with TSM yielding 20% and 50% lower efficacy than LDM.

In contrast, stearic acid resulted in more effectiveness using LDM, which resulted in more than 6 times less (6.21) efficacy against *N. barkeri*. Oleic acid seems more effective against *P. citri* with a shorter lethal time, but its effectiveness against both is unsuitable for our recommendations against both predators. Palmitic acid has the lowest effectiveness compared to other treatments with values above 1 (although LDM was a better choice than TSM). Control treatment performs a similar pattern as soybean oil but with a higher lethal time than other treatments except against *N. californicus* compared to stearic acid and palmitic acid (Tab. S2, Fig. 2).

The lethal impact of acids (fatty acids) and soybean oil was tested against *P. citri*, *N. californicus*, and *N. barkeri*, with mortality (%) after 24 hours and a total number of eggs laid by females. Lethality resulted significantly differently within treatments for prey and predators except for the mortality of *N. californicus* during the topical spray method (Tab. 2). All the treatments used gave significant mortality % after 24 hours against *P. citri* compared to the control treatment. Oleic acid showed 50% mortality using the leaf-dipped method, which decreased to 29.5% using the tropical spray method. Soybean oil efficiency was like oleic acid but slightly decreased mortality from 46.25% to 43.75% (LDM to TSM). The mortality trend after 24 hours for LDM was Oleic acid = soybean oil > stearic acid > palmitic acid > control. In contrast, TSM, soybean oil > palmitic acid > stearic acid > oleic acid > control (Tab. 2). The impact of the lethality of treatments used on the next generations was checked by examining the egg-hatching percentage and reached adultery. Soybean oil had a more substantial impact on the eggs of infected females hatching (75%), and hatched individuals reached adultery (77.6%). All acids perform similarly to each other with significantly different control treatments. Compared to LDM, the TSM section gave slightly different results, as soybean oil significantly impacted the hatching percentage. Still, the adultery percentage was more impacted by palmitic acid (Tab. S3).

Higher mortality (%) in adult females resulted in fewer eggs being laid. Higher mortality (%) resulted in more eggs for *P. citri*, *N. californicus*, and *N. barkeri* during the topical spray method. Still, it lowered than controlled treatments (Tab. 2). Palmitic acid also showed slightly higher (1%) phytotoxicity than other treatments. Soybean oil similarly impacted the egg

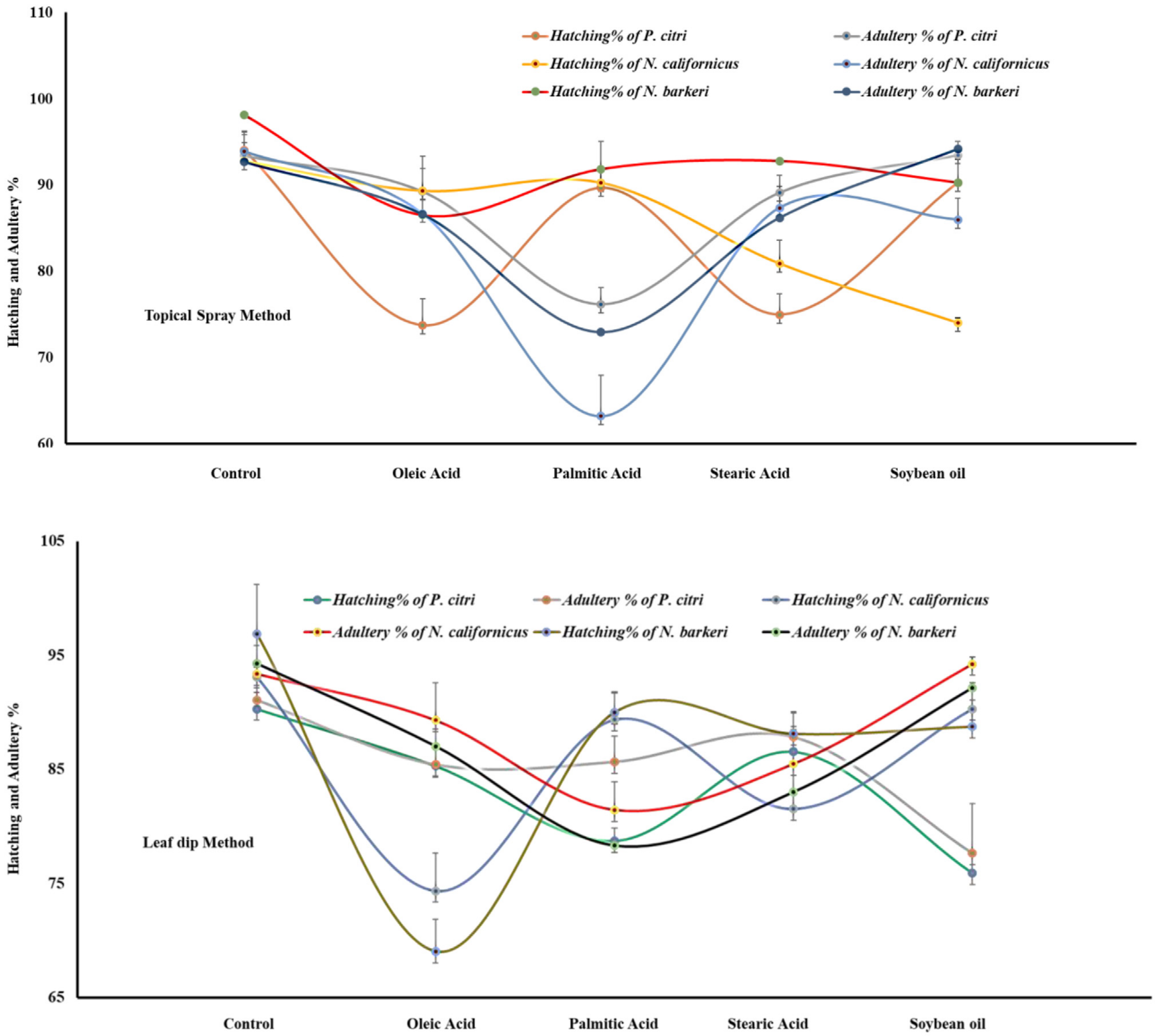

Fig. 3. The lethal efficacy of soybean and fatty acids impact egg hatching percentage and the percentage of hatched eggs reached adultery when exposed to the parental population of *Panonychus citri* and its predators using leaf dip and topical spray methods.

hatching percentage out of 40 eggs against predatory and citrus red mites. In contrast, the rate of adultery was more affected by the leaf dip method (Fig. 3, Tab. S3). Palmitic acid maximum impacted *P. citri* using LDM while less effective against *N. barkeri* while palmitic impacted less by using LDM compared to TSM. The lowest hatching percentage was observed against *N. barkeri* during LDM, while the maximum was observed against *P. citri* during TSM. In contrast, the rate of adultery was observed to be almost similar in the methods used and prey and predators. All treatments impacted predators significantly and *P. citri* compared to the control treatment (Fig. 3, Tab. S3).

3.2 Behavioral responses

The behavioral responses were checked by the movement of mites towards (attractiveness) and away (repel) from treated surfaces. The attractiveness and repellency of palmitic (t-value: −5.83, p-value: 0.01) and stearic (t-value: −2.67, p-value: 0.04) acids significantly affected the movement of *P. citri*. Both acids attracted 41 to 42% of adults towards them. In comparison, they repelled 58.33% (Tab. 3). This attraction was increased from 40 to 45% to attract predatory mites compared to other treatments (Tab. 4). The repellent effect of soybean oil and oleic acid was significantly higher (For *N. californicus*: (Soybean oil: t-value: −5.28, p-value: 0.002, Oleic acid: t-value: −4.66, p-value: 0.003), and for *N. barkeri* (Soybean oil: t-value: −3.57, p-value: 0.012; Oleic acid: t-value: −5.82, p-value: 0.01)) than palmitic and stearic acids. The study found that *P. citri* showed a more significant, non-significant attraction to treated surfaces than palmitic and stearic acids (Tabs. 3 and 4).

The total number of eggs laid by adult females of *P. citri* was significantly lower on leaves dipped with oleic acid, followed by palmitic acid, soybean oil, and stearic acid

Table 3. The numbers of female *Panonychus citri* attracted to, and the numbers of eggs oviposit on the citrus leaves treated (LC50) or untreated with soybean oil and its fatty acids in choice tests.

Treatments	% adults movement				Total no. of eggs			
	Treated	Untreated	t-value	P-value	Treated	Untreated	t-value	P-value
Panonychus citri (McGregor) (n=30)								
Soybean oil	46.667 ± 3.6	53.33 ± 3.6	-1.309	0.238	30.5 ± 2.466	62.25 ± 4.385	-6.311	$<.001$
Palmitic acid	40.833 ± 2.097	58.33 ± 2.152	-5.824	0.001	21.75 ± 2.213	42.75 ± 2.357	-5.797	0.001
Oleic acid	46.667 ± 2.357	53.33 ± 2.357	-2	0.092	18.25 ± 1.25	44.50 ± 3.50	-7.063	$<.001$
Stearic acid	41.667 ± 4.41	58.33 ± 4.41	-2.673	0.037	34.75 ± 1.377	54.50 ± 4.173	-4.494	0.004

(Tab. 3). Oleic acid also considerably affected the total number of egg production by both adult females of *N. californicus* and *N. barkeri* with trends of oleic acid > soybean oil > stearic acid > palmitic acid and oleic acid > soybean oil > palmitic acid > stearic acid, respectively (Tab. 4).

The lethal effect of soybean oil and oleic acid (leaves dipped) has a significant impact on the movement of *N. californicus* (by repelling 65% more mites away from treated surfaces), with the lowest prey (*P. citri*) consumption rate (%) on the treated surfaces. Due to non-significant movement between treated and untreated surfaces of palmitic and stearic acids, *N. californicus* consumes more *P. citri*. The movement of *N. barkeri* on treated leaves with palmitic acid and untreated leaves was not significant compared to the other treatment results. In contrast to *N. californicus*, *N. barkeri* led to significantly lowest prey consumption after 24 h on palmitic-treated surfaces, even though the movement was non-significantly different between treated and untreated surfaces (Tab. 4).

4 Discussion

In the search for novel plant-based compounds for IPM programs of different pests, we selected long-chained saturated and unsaturated fatty acids from soybean oil. This work demonstrated that soybean oil and fatty acids (palmitic acid, oleic acid, and stearic acid) exhibited acaricidal activity as well as reducing the total number of eggs of *P. citri* and its predators (*N. californicus* and *N. barkeri*). In our previous work, we documented that soybean oil acts as an alternative to synthetic chemicals by impacting the dispersal and behavioral response of *P. citri* (Qayyoum *et al.,* 2021a, 2021b). Although a few articles have been published related to the impact of fatty acids on acaricidal or insecticidal activity (Li, 2016; Mohamad *et al.,* 2013; Sims *et al.,* 2014; Twining *et al.,* 2018; Zhu *et al.,* 2018). The first time, we demonstrated the impact of three fatty acids against *P. citri* and its two common predators.

In the acaricidal lethal efficacy test, oleic acid impacted maximum on the *P. citri* with the lowest LT_{50}, while palmitic acid was found to be less toxic against all tested mites. Oleic acid gave 25% mortality against *Callosobruchus maculatus* instead of 50% against *P. citri* (Tab. 3). The fecundity rate of *P. citri* was also significantly reduced compared to findings with higher toxic effects. It stated that the toxicity of fatty acid increased with the increase of carbon atoms in its molecular weight (Sims *et al.,* 2014) as oleic acids ($C_{19}H_{36}O_2$) have a higher number of carbon atoms than palmitic acid ($C_{18}H_{36}O_2$). Still, this statement failed when compared with the molecular formula of stearic acid ($C_{20}H_{40}O_2$). Sims *et al.* (2014) also showed that more toxicity was observed in a compound with an odd number of carbon atoms, as in oleic acid in a recent study.

We also found that the topical spray method needs more time to kill 50% of the offered pest population than the leaf-dipped method, which differs from (Sims *et al.,* 2014) results (similar toxicity in both application methods). The variation of results from previous studies depends on the nature of the chemical and pest species. Soybean oil toxicity was significantly higher after oleic acid against *P. citri* due to a mixture of many fatty acids, as observed by Hatem *et al.* (2009). Due to the presence of Linoleic acid and other along-chain unsaturated fatty acids in soybean oil (Oliveira *et al.*, 2017), soybean oil acts as toxic and has a repellent effect on arthropods (Hieu *et al.*, 2015). A similar effect was observed against *Typhlodromus ornatus* (Acari: Phytoseiidae) (Saraiva *et al.,* 2020) and *Stomoxys calcitrans* (Diptera, Muscidae) (Hieu *et al.,* 2015) after 24 hours of exposure.

Maximum toxicity with lower egg production is a common phenomenon in the case of *P. citri*. Still, oleic acid revealed no toxicity or less toxicity against both predatory mites, which also induces the production of their eggs compared to other stearic acid or controlled treatments. However, the number of eggs remains almost similar in both application methods. We concluded that with maximum time availed with a maximum number of mites, the production of eggs also increased (Sabelis, 1985).

Most fatty acids are part of plant cuticles, which play an essential role by interfering with plant and phytophagous insects. Applying fatty acids alone or a combination of different fatty acids changes the behavior of different insects and mites (Qayyoum *et al.,* 2021a, 2021b). The role of attraction or deterring any arthropod depends on its structure and length: long-chain unsaturated fatty acids inhibited the settlement of *Myzus persicae* (Hemiptera: Aphididae), but long-chain saturated fatty acids showed an attractive effect (Santana *et al.,* 2012).

Due to the above qualities, we try to confirm the repellency and attractiveness of fatty acids and soybean oil against *P. citri*, *N. californicus*, and *N. barkeri.* We resulted in significant mites repelled from treated surfaces (58.33%) except for soybean oil (Qayyoum *et al.,* 2021a, 2021b) and oleic acid against *P. citri*. This non-significant repellency and attractiveness were also confirmed against *T. urticae* by using Myrtaceae essential oils (Roh *et al.,* 2013) and against *M. persicae* by using

Table 4. The numbers of female *Neoseiulus californicus* (McGregor) and *Neoseiulus barkeri* Hughes attracted to, and the numbers of eggs oviposition on the citrus leaves treated (LC50) or untreated with soybean oil and its fatty acids in choice tests.

Treatments	% adults movement				% prey consumption				Total no. of eggs			
	Treated	Untreated	t-value	P-value	Treated	Untreated	t-value	P-value	Treated	Untreated	t-value	P-value
Neoseiulus californicus (McGregor) (n=10)												
Soybean oil	22.5 ± 4.787	65 ± 6.455	-5.288	0.002	13.75 ± 6.575	43.75 ± 4.27	-3.827	0.009	3.5 ± 0.645	6.00 ± 0.408	-3.273	0.017
Palmitic acid	45 ± 6.455	50 ± 7.071	-0.522	0.62	27.5 ± 3.277	53.75 ± 5.154	-4.317	0.005	5.00 ± 0.707	9.75 ± 1.031	-3.8	0.009
Oleic acid	27.5 ± 4.787	65.00 ± 6.455	-4.666	0.003	15.00 ± 2.041	58.75 ± 6.25	-6.654	<.001	0.75 ± 0.479	5.5 ± 0.645	-5.911	0.001
Stearic acid	45.00 ± 6.455	55.00 ± 6.455	-1.095	0.315	25.00 ± 2.041	51.25 ± 5.543	-4.444	0.004	4.75 ± 0.629	12.25 ± 0.946	-6.599	<.001
Neoseiulus barkeri Hughes (n=10)												
Soybean oil	37.5 ± 4.787	60 ± 4.082	-3.576	0.012	23.75 ± 3.75	48.75 ± 5.543	-3.735	0.01	1.50 ± 0.289	8.75 ± 0.854	-8.043	<.001
Palmitic acid	45.00 ± 2.887	47.5 ± 4.787	-0.447	0.67	15.00 ± 2.041	40.00 ± 2.041	-8.66	<.001	2.00 ± 0.707	5.75 ± 0.479	-4.392	0.005
Oleic acid	22.5 ± 6.292	75.00 ± 6.455	-5.824	0.001	16.25 ± 3.75	56.25 ± 4.27	-7.039	<.001	0.75 ± 0.479	3.50 ± 0.645	-3.422	0.014
Stearic acid	40.00 ± 4.082	60.00 ± 4.082	-3.464	0.013	28.75 ± 2.394	58.75 ± 2.394	-8.863	<.001	4.00 ± 0.913	6.00 ± 1.08	-1.414	0.207

Medium-Chain Fatty Acids from *Eugenia winzerlingii* (Myrtaceae) (Cruz-Estrada *et al.,* 2019). In contrast, soybean oil resulted in more significant repellency against *Neoseiulus baraki* after one hour of exposure (Oliveira *et al.,* 2017) and *T. ornatus* (Saraiva *et al.,* 2020). Like the results of *N. baraki* (Oliveira *et al.,* 2017) and *T. ornatus* against predatory mites, soybean oil and oleic acid forced the majority of *N. californicus* and *N. baraki* away from treated surfaces (Tab. 4).

This response against different insects due to their anti-feeding and reduction in oviposition depends on species-specific chemoreceptors like sensilla in insect antennae (Seenivasagan *et al.,* 2013). This repellent behavior also reduced the number of eggs on the treated surfaces (Tab. 4) as the impact of Datura stramonium against *T. urticae* (Kumral *et al.,* 2010) as fatty acids penetrate the integument of mites like *T. urticae*, which inhibits the rotational movement of embryonic liquid by resulting in quick toxicity and less egg production (Takeda *et al.,* 2020; Tsolakis and Ragusa, 2008).

The attractive behavior of predatory mites is due to the presence of detoxifying enzymes (monooxygenases (Roush and Plapp Jr, 1982), esterases (Anber and Oppenoorth, 1989), and glutathione-S-transferases (Fournier *et al.,* 1987)). Attraction toward prey is a social olfactory interaction cue, which is a predator-borne chemosensory cue for the HIPVs (herbivore-induced plant volatiles) recognition (Hettyey *et al.,* 2015; Sabelis and Dicke, 1985; Saraiva *et al.,* 2020; Schausberger *et al.,* 2020). As a long-chain saturated fatty acid, palmitic acid has an attractive effect (Castillo *et al.,* 2010; Santana *et al.,* 2012). In contrast, we find neither significant attractiveness nor repellency of predatory mites towards treated (palmitic acid) and untreated surfaces with an equal number of prey (Tab. 4). However, this phenomenon significantly impacted the prey consumption and fecundity rates, which need more detailed explanations (Cisak *et al.,* 2012).

5 Conclusion

This study provides valuable insights into the toxic effects of three fatty acids—palmitic, oleic, and stearic acids—on *Panonychus citri* (the citrus red mite) and its predatory mites (*Neoseiulus californicus* and *Neoseiulus barkeri*). Each fatty acid exhibited distinct lethal and sublethal effects, with oleic acid demonstrating the most effective toxicity against *P. citri*, followed by stearic and palmitic acids. Notably, soybean oil, which contains a unique combination of these fatty acids, had a more pronounced impact on *P. citri* compared to the individual acids, suggesting that the composition of fatty acids in soybean oil may enhance its overall toxicity. However, its effect on the predatory mites was less favorable, with soybean oil showing lower efficacy than the individual fatty acids in terms of both lethal impact and predator mortality.

Considering the balance between pest control and predator preservation, oleic acid emerged as a potentially more effective agent for targeting *P. citri* due to its shorter lethal time, though it had a more significant repellent effect on the predators, making it less ideal for integrated pest management. On the other hand, palmitic acid, while less effective against *P. citri*, demonstrated a more balanced impact on both pests and predators. Stearic acid also exhibited notable efficacy but was more variable in its effects depending on the application method.

In terms of alternative treatments, this study highlights the potential for using soybean oil, but a combination of two or more fatty acids could offer a more tailored solution, balancing toxicity against *P. citri* while minimizing the impact on beneficial predatory mites. Further research is needed to explore the use of other oils with different fatty acid compositions that may be more suited for this purpose. Oils rich in specific fatty acids may offer better control over pest populations while preserving beneficial species, ensuring the sustainability of pest management strategies. Thus, while soybean oil shows promise as an effective pest control agent, a more nuanced approach, potentially incorporating specific fatty acids or blends, may provide optimal results for both controlling *P. citri* and protecting predatory mites.

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Conflicts of interest

The authors declare they have no competing interests.

Supplementary material

Figure S1. Toxicity of all treatments against *Panonychus citri*.

Figure S2. Toxicity of all treatments against *Neosiulus californicus*.

Figure S3. Toxicity of all treatments against *Neosiulus barkeri*.

Figure S4. Lethality of treatments against *Panonychus citri, Neoseiulus californicus*, and *N. barkeri*.

Table S1. Lethal time (LT_{50}) of *Panonychus citri* and its predators exposed to soybean oil and fatty acids by using leaf dip method and topical spray method.

Table S2. Lethal time (LT_{50}) of *Panonychus citri* and its predators exposed to LC_{50} soybean oil and fatty acids using leaf dip and topical spray methods.

Table S3. Lethal efficacy of soybean and fatty acids impact egg hatching percentage and percent hatched eggs reached to the adultery when exposed to the parental population of Panonychus citri and its predators exposed by leaf dip method and topical spray method.

The Supplementary Material is available at https://www.ocl-journal.org/10.1051/ocl/2024035/olm.

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