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Ethanolic Extracts from Tangerine (*Citrus reticulata* L.) Peels as an Eco-Friendly Botanical Pesticide for Small-Farm Potato (*Solanum tuberosum* L.) Cultivation

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Abstract: Background: Modern agriculture relies heavily on chemicals to ensure high yields and food security, but their overuse has led to health issues and pest resistance. Researchers are now exploring natural, eco-friendly alternatives for pest control. Methods: This study evaluated two ethanol-based formulations (12.5% and 25% v/v) derived from the tangerine peel (*Citrus reticulata* L. var 'Clementina') against conventional chemical treatments and untreated control in potato (*Solanum tubevar*. L. var. 'Capiro') cultivation. A randomised block design with three blocks per treatment, each containing 45 plants, was used during the wet season (February–April 2023). Results: Visual inspections and yellow traps followed weekly application from day 30 to 105 post-planting to monitor pest (e.g., *Frankliniella occidentalis*, Aphididae) and beneficial insect (e.g., Coccinellidae, *Aphis mellifera*) populations. The 25% formulation performed similarly to chemical treatments against pests but was harmless to beneficial insects. Post-harvest analysis showed that the formulations achieved 73% of conventional yields, with comparable tuber damage and *Premnotrypes vorax* larvae levels. Conclusions: Toxicological tests confirmed the formulations' eco-friendliness, making them suitable for small-scale Andean "chakras" for organic farming and honey production without chemicals.

Keywords: Botanical pesticide; *Citrus reticulata* L.; *Solanum tuberosum* L.; Citrus peel ethanolic extract; beneficial insects

1. Introduction

The large-scale use of chemical substances in agriculture to maintain soil fertility and prevent the harmful effects of pests and diseases on major crops has fostered high yields and led to the food security that modern agriculture exhibits today [1]. For the first time, there were enough food supplies, at least from production, to nourish the growing global population [2]. Uncontrolled and overused chemical agents harm agriculture's long-term sustainability and global food security [3,4].

Although very effective, some chemical substances may also cause various health disorders for humans and the surrounding flora and fauna. Additionally, the effectiveness of some pesticides declined over time as the pests they were combating mutated and acquired various resistance genes that allowed them to survive contact with such agents.

Imidacloprid, a widely used popular insecticide, effectively controls many insects, many of which are pests. However, studies have shown that workers who handle this chemical insecticide are

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at serious risk of health issues due to prolonged exposure [5]. It causes the near annihilation of a significant group of beneficial insects, such as the honeybee. [6,7]. Raising awareness and acting could work towards a future where these risks are minimised.

While botanical pesticides may not be as potent at stopping pests that harm crops, they show promise for the future of agriculture [8–10]. They have the advantage of having a lower impact on human health and a less adverse effect on agroecosystems. In some cases, they have been incorporated into integrated pest management programs, aiming to reduce the negative impact of agrochemicals without significantly affecting current agricultural yields [11,12].

One of the examples of botanical insecticides that has caught attention is based on the use of essential oils from citrus peels [13,14]. These peels contain a relative abundance of monoterpenes, such as limonene and linalool, which have proven insecticidal action [14].

In a previous study, essential oil from the peels of tangerines (*Citrus reticulata* L.) controlled whiteflies (*Trieurodes vaporariorum* W.) [15]. These extracts were obtained with nonpolar organic solvents (petroleum ether and n-hexane), whose toxicity and flammability make them challenging to use in broader contexts.

The work will use ethanol extracts from the tangerine peel (*Citrus reticulata* L.) with a new, safer solvent-ethyl alcohol. This choice of solvent is much less toxic, flammable, and safer than petroleum ether and n-hexane, providing reassurance about the safety of the research methods [16].

Other authors have also reported that essential oils obtained from C. reticulata possess antimicrobial activity [17–21], including some insect-transmitted infections, such as 'cucumber wilt', a serious illness caused by the bacteria *Erwinia tracheiphila* [22].

Additionally, alcoholic extracts of *C. reticulata* peel have been found to contain some flavonoids, especially poly-methoxy-flavonoids (PMFs), such as tangeretin and nobiletin, which are attributed antifungal properties against certain fungal pests like *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Fusarium oxysporum* [22,23].

This study used potatoes (Solanum tuberosum L.) as a model crop. The study will be conducted in a small field plot under controlled conditions, using two levels of ethanolic extract of tangerine. The impact of the mentioned formulations will be meticulously evaluated and contrasted with the conventional chemical treatment and with a variant that did not receive any pesticide treatment. The dynamics of the different groups of insects in the agroecosystem will be observed with the utmost care, and the yields obtained from the potato will be compared.

2. Materials and Methods

2.1. Raw Materials

Citrus reticulata L. var. 'Clementine' employed here was obtained from the Pimampiro Canton (0°24′0″N, 77°58′12″W), situated in northern Ecuador. The gathered fruits reached maximum ripeness when the skin detached rapidly from the pulp [24].

Before peeling, the tangerines were cleansed with abundant tap water to eliminate dust collected during shipping. Each fruit was manually dried using a cloth. Following peeling, the objects were cubed into small pieces (1-2 cm) to optimise ethanolic extraction, as suggested by previous studies [15,24].

2.2. Pilot-Plant Extraction-Concentration Ethanolic-Extract of Tangerine Peels

The tangerine peel extract was extracted and concentrated in a pilot unit for discontinuous solvent extraction, coupled with a condenser for solvent separation (Armfield Limited, Hampshire, England BH24 1DY, UK. http://www.armfield.co.uk).

Near 10 kg of fresh, washed mandarin peel was weighed. Then, the material was mixed with 20 litres of 96 % ethyl alcohol, technical grade (for a solvent/solid ratio of about 2 l/kg), and left in contact overnight for about 12 hours.

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The next day, the ethanolic extract began to evaporate, condense, and recirculate for about 5-6 hours in the pilot unit. Afterwards, the alcohol was evaporated and separated from the extract for 2 hours, ensuring the condenser temperature remained below 90 °C. The operation was halted when the temperature rose above 90 °C, concluding the material concentration process and the safe removal of the solvent.

The material, resulting from a precise and controlled process, was stored in a 1-litre amber bottle at 4 °C, ensuring its preservation until its use in field experiments and analyses.

2.3. Characterization of Ethanolic-Extract of Tangerine Peels

2.3.1. Phytochemical Screening of Ethanolic Tangerine Peel Extract

The ethanolic extract was evaluated for its phytochemical analysis using standard procedures reported in the literature with minor modifications.

2.3.1.1. Protein and Amino Acids Determinations (Ninhydrin Assay)

A mixture of equal volumes of tangerine peel ethanolic extract and 0.2 % (m/v) ninhydrin solution (freshly prepared) in a test tube was heated for 1-2 minutes, resulting in a blue to dark purple product, demonstrating the existence of amino acids and proteins [25].

2.3.1.2. Test for Phenols and Tannins

In a test tube, equal amounts of ethanolic extract and 2 % (m/v) $FeCl_3$ solution were mixed to give a blue-green or black colouration, indicating that phenols and tannins are present [25].

2.3.1.3. Test for Carbohydrates

An equal volume of ethanolic extract was combined with Benedict's reagent in a test tube and subsequently heated to boiling. The emergence of a reddish-brown precipitate signifies the presence of carbohydrates [26].

2.3.1.4. Flavonoid Assays (Alkaline Reagent Test)

An equal volume of ethanolic extract was combined with a 2% NaOH solution in a test tube, resulting in a pronounced yellow colouration. The presence of flavonoids results in a colourless solution upon the addition of several drops of concentrated hydrochloric acid [27].

2.3.1.5. Flavonoid Assays (Shinoda Test)

In a test tube, 1 ml of ethanolic extract and 10 drops of diluted hydrochloric acid were mixed, followed by 500 mg of magnesium. The presence of flavonoids results in the production of reddish, pink, or brown colouration [28].

2.3.1.6. Test for Steroids (Libermann Test)

In a test tube, 1 ml of ethanolic extract was combined, and 1 ml of ethanolic extract and 2 ml chloroform + 2 ml acetic acid. Then, the mixture was cooled using ice, and 0.5 ml of concentrated sulphuric acid was added incrementally and precisely. If, after that, the colour turns into violet, blue, or green, it implies the presence of steroidal molecules [27].

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2.3.1.7. Test for Cardiac Glycosides

In a test tube, an equal volume of ethanolic extract from tangerine peels was combined with glacial acetic acid, which contained two drops of a 2 % (m/v) $FeCl_3$ solution. The solution was transferred to a distinct test tube containing 1 ml of strong sulphuric acid. The presence of glycosides is indicated by a brown ring at the interphase or a blue colouration in the acetic acid layer, along with a red colour in the interphase of the two acids [25].

2.3.2. FTIR Study of Ethanolic Tangerine Peel Extract

Ethanolic extract from tangerine peels was analysed using infrared spectrometry (Agilent Cary 630 FTIR, Agilent Technologies Inc., CA, USA) across a wavenumber range of 400 to 4,000 cm^{-1}, performing 32 scans at a resolution of 4 cm^{-1}. Additionally, an ATR sampling method was employed to analyse a single rebound diamond crystal.

2.3.3. Reversed-Phase HPLC

The samples were analysed using the RP-HPLC system (Ultimate 3000 HPLC, with a reverse-phase C-18 column (150 × 4.6 mm) from HPLC Hypersil GOLD™, an autosampler, a Thermo Scientific™ quaternary pump, a column compartment, and a photodiode array detector (PAD)). The chromatograms were produced at a wavelength of 205 nm using a linear gradient of H_2O/MeCN (95:5) to (40:60) for 8 min.

2.4. Toxicity Evaluation on Caenorhabditis Elegans Model

2.4.1. Caenorhabditis Elegans Strain Culture

C. elegans wild-type N2 strain worms were cultured on NGM agar plates using *Escherichia coli* strain OP50 as feed. The nematode culture was maintained at 20 °C and sub-cultured every two weeks [29]. Each culture was synchronised by bleaching with sodium hypochlorite solution treatment and then culture for two days at 20 °C until the L2 nematodes stage. [30,31].

2.4.2. Toxicity Assay and LC_{50} Estimation

Toxicity assays were performed in 96-well culture plates in a final volume of 200 µL of liquid S-basal culture media dosed with 1, 6, 12.5, 25 and 75 % of the tangerine peel ethanolic-extract (Eth-E) [29]. Twenty L2 nematodes were collected individually with a worm picker in each well. Four wells were prepared for each treatment in triplicate on one culture plate. Ivermectin (6.0 mg/mL) was used as a positive control. Liquid S-basal culture media was used as the negative control, and ARPON solution (0.99 mg/mL) was used as the solvent control [32].

Plates were incubated at 20 °C for 24, 48 and 72 hours. After exposure, the live and dead worms were counted by visual inspection under a dissection microscope. The dead condition was considered when the worms did not respond to light and mechanical stimuli. Results were presented as the mean survival percentage of three replicates with t-student analysis for independent samples. Finally, the lethal concentration (LC50) was calculated for PROBIT analysis.

2.5. Field Experiments on Small Potato (Solanum tuberosum L.) Cultivation

The field experiments were conducted between January 2023 and April 2023 on the grounds of the experimental farm “La Pradera”, belonging to the “Universidad Técnica del Norte” and located in the canton of Chaltura, province of Imbabura, Ecuador (0°22’31.3" N; 78°21’20.5" W). During a rainy period, the most persistent insect and fungal pests proliferate over the crops.

The “Capiro” variety of *Solanum tuberosum* L. was used for the small-scale potato cultivation experiment. A completely randomised block design of experiments was used. Four treatments (formulae at 12.5 % (v/v) (F-12.5%) and 25.0 % (v/v) (F-25%), conventional management (‘conv. treat’),

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and 'untreated') were carried out, with three blocks per treatment. In each block, three rows of five plants were planted, separating the rows by about 0.4 m.

The ethanolic extract formulated at 12.5 % (v/v) and 25 % (v/v) (F-12.5% and F-25%) was applied every seven days. To each formulation, 1 ml·l^{-1} of polyether polymethyl-siloxane was added as an adjuvant to promote the formation of a homogeneous emulsion. Adjuvant was also included in the conventional chemical treatment.

Each treatment was supplemented with fertilisers during the experiment, as described in Table 1.

Table 1. Different chemicals are used for '*conv. treat.*'.

Name	Function	*'conv. treat.'*	F-25%	F-12.5%	*'untreated'*	Dose	Frequency	Mode of action[1]
'Biol'	organic fert.	✓	✓	✓	✓	5 l	Every month	-
Compost	organic fert.	✓	✓	✓	✓	300 kg	Start of cultivation	-
Calcium carbonate	Soil pH adjust.	✓	✓	✓	✓	50 kg	Start of cultivation	-
NPK (13-40-13)	inorganic fert.	✓	✓	✓	-	7.46 kg	Every two months	-
Imidacloprid	insecticide	✓	-	-	-	0.5 ml/l	Every two weeks	(i)
Thiamethoxam + lambda cyhalothrin	insecticide	✓	-	-	-	125 ml/l		(ii)
Acephate	insecticide	✓	-	-	-	100 g/l		(iii)
Fipronil	insecticide	✓	-	-	-	2 ml/l		(iv)
Methomyl	insecticide	✓	-	-	-	3 ml/l		(iii)
Malathion	insecticide	✓	-	-	-	2 ml/l		(iii)
Fluopicolide + Propamocarb chlorhydrate	fungicide	✓	-	-	-	1.6 ml/l	Every two weeks	(v)
Propineb + Fluopicolide	fungicide	✓	-	-	-	2 ml/l		(vi)
Carboxin + Captan	fungicide	✓	-	-	-	3 g/l		(vii)

(i) Nicotinic acetylcholine receptor (nAChR) competitive modulators; (ii) Sodium channel modulators + Receptor (nAChR) competitive modulators; (iii) Acetylcholinesterase (AChE) inhibitors; (iv) GABA-gated chloride channel blockers; (v) Disrupting the function of spectrin-like proteins in the fungal cytoskeleton + targeting their phospholipid biosynthesis; (vi) Multiple metabolic processes +Disrupting the function of spectrin-like proteins in the fungal cytoskeleton; (vii) Disrupting fungal respiration + hindering their energy production.

Direct and indirect monitoring were implemented to measure the variables. In indirect monitoring tracking, the number of eggs and nymphs of *Bactericera cockerelli* S., aphids, and Lepidoptera larvae were counted on the 15 plants. Each plant was divided into three-thirds, and in each third, the number of these insects was counted on three leaves.

Yellow traps (dim. 10 x 25 cm) were used to monitor the units. These were changed every 15 days, and the number of *F. occidentalis*, *B. cockerelli*, *Epitrix* spp., aphids, and leaf miners were recorded.

2.6. Post-Harvest Analysis

Finally, after harvesting the three blocks of each variant, the yields of each treatment block (in ton·ha^{-1}) were determined. Additionally, a random sample of 10 potato tubers from each block was taken to determine which showed observable external damage, thus determining the percentage of damaged tubers in the sample. The damaged tubers were also carefully cut to count the white worms they contained.

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2.7. Statistical Analysis of Experiments

The statistical language R (RStudio 2024.12.0+467) was used for statistical analysis and part of the graphs. The Tukey test was used to compare normally distributed samples, while the non-parametric Friedmann and Dunn tests were used to compare non-normal samples.

3. Results

3.1. Pilot-Scale Solid-Liquid Extraction

In the extraction-concentration process, 992 mL of tangerine peel ethanolic extract (with a dark brown colour and characteristic smell) was obtained from 9.8 kg of initial tangerine peels and 20 litres of ethanol at 96 % (technical degree' ethanol). The whole extraction-concentration yield of the process was 9.9 % (w/w).

3.2. Characterization of Ethanolic Extract from Tangerine Peels

Phytochemical screening of tangerine peel ethanolic extract was conducted through various chemical assays to illustrate the existence of secondary metabolites (e.g., phenols, tannins, flavonoids, proteins, amino acids, reducing sugars, saponins, carbohydrates, steroids, terpenoids, cardiac glycosides, alkaloids, etc.) (Table 2). Certain substances have demonstrated beneficial bioactivity for human health and plant protection.

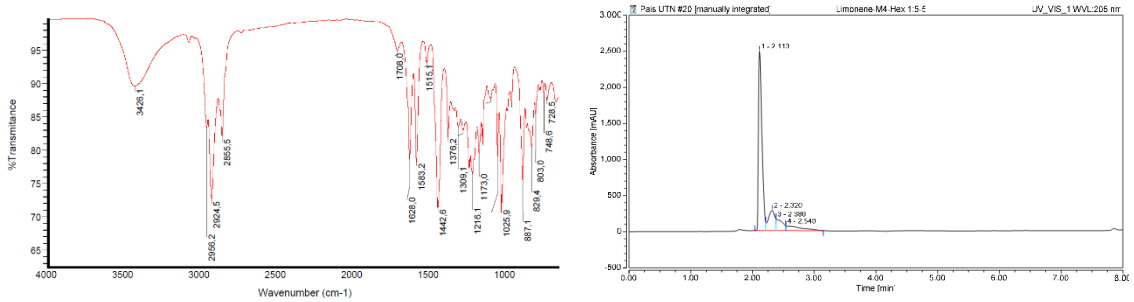
Table 2. Phytochemical analysis of tangerine peel ethanolic extract.

Test	Tangerine peel ethanolic extract
Amino acids and Proteins	-
Phenols and Tannins	+
Carbohydrates	-
Flavonoids (Alkaline test)	+
Flavonoids (Shinoda test)	+
Steroids	+
Cardiac Glycosides	+

The FTIR spectral peaks indicate the primary interactions among the atoms concerning the extract composition (Figure 1a). The initial peak is around 3,426 cm^{-1}, indicative of the stretching vibration of the O-H bond. The 3,000 to 2,800 cm−1 peaks correspond to the asymmetric stretching of aliphatic C-H bonds in ethyl (-CH_3) and methylene (-CH_2) groups. The pronounced peak at 1,627 cm^{-1} indicates the C = C bond stretching, likely associated with the cyclohexene limonene ring. Peaks at roughly 1,442 cm^{-1} indicate the doublet stretching of the C-H bond in the methylene groups.

As a result, the ethanolic extract of tangerine peel records the presence of functional groups commonly found in secondary metabolites such as limonene, phenols, flavonoids, and steroids.

The HPLC detects significant compounds susceptible to UV/Vis in the ethanolic tangerine peel extract (Figure 1b).



(**a**) (**b**)

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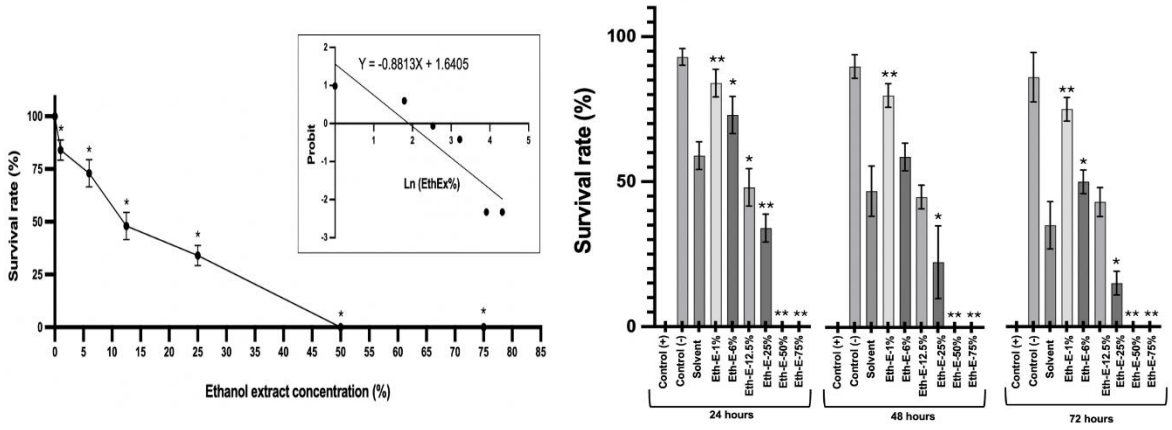
Figure 1. Spectral characterisation of ethanolic extract of tangerine peels. (**a**) Scanning of FTIR between 4000 – 400 cm-1; (**b**) RP-HPLC profile at 205 nm.

The HPLC chromatogram shows one prominent peak in the sample at retention times of 2.113 minutes, representing 70 % of this compound in the entire sample. The remaining peaks in the chromatogram profile represent 30 % of the mixture content.

3.3. Toxicity Evaluation of Ethanolic-Extracts of Tangerine Peels on Caenorhabditis elegans Model

The results exhibited a dose-dependent effect of the ethanolic extract (solved on ARPON) on the L2-nematodes' mortality after 24 hours of exposure, as shown in Figure 2a. The PROBIT analysis demonstrated an LC_{50} value of 6.43 %.

Data exhibits that the tangerine peel ethanol extract causes an interesting anthelmintic effect in *C. elegans* models. It produces significant mortality from 25 to 75 % at 24 hours of testing and progressively increases over time. Qualitative microscopic analysis of the digestive nematode integrity revealed interesting disruptions in its structure and functionality. These findings suggest that the extract and the solvent generate a direct acute toxic effect on the digestive system and the cuticle of the nematodes.



(**a**) (**b**)

Figure 2. Toxicology effect of ethanolic extract of tangerine peels on Caenorhabditis elegans.
(**a**) Concentration-effect curve of the nematodes exposed to extract for 24 h. Each point represents the average of three experiments. (*) represents a significant difference between each treatment and the control (-). 1% ($p = 0.00032$), 6% ($p = 0.01411$), 12.5% ($p = 0.03117$), 25% ($p = 0.00032$), 50% ($p = 0.00015$) and 75% ($p = 0.00015$) according to Student's t-test. The Probit regression analysis reflects $R2 = 0.8044$ and $p = 0.0154$. (**b**) Survival of C. elegans in different concentrations of extract at different experimental times. (*) $p < 0.05$, (**) $p < 0.01$ represents a significant difference compared to each treatment with the solvent.

Interestingly, the solvent used in the assay reduces nematode survival (compared to control (-) with solvent; $p = 6.88 \cdot 10^{-5}$). Some evidence suggests that the silicones in ARPON (polyether polymethyl siloxane copolymer) can inflame and irritate epithelia in the long term [33], justifying the effect on the cuticle observed in *C. elegans* (Figure 2b). However, the combined use of tangerine essential oil shows a protective effect, improving nematode survival (Eth-E-1% and Eth-E-6%).

The observed effect could be due to the antioxidant and anti-inflammatory capacity reported for essential oils from plants, including different species of citrus [34–37].

Finally, the toxic effect of the ethanolic extracts is significant, starting from the F-25% dose in combination with the solvent at all the experimental times, highlighting the safe use of tangerine peel ethanolic extract from F-25% in the agricultural field.

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3.4. Small-Scale Field Experiments in Potato Cultivation (Solanum tuberosum L.)

The application results with the formulations show more *Bactericera cockerelli* eggs than the other pests. Additionally, in the treatment with the ethanol extract formulation at 12.5% (v/v) (F-12.5%) and the untreated variant (further as 'untreated'), a greater quantity was observed compared to the other treatments throughout the entire experiment.

The four treatments had significantly different average numbers of eggs, nymphs, aphids, and caterpillars (according to the Friedmann test, $p < 0.001$).

In all cases, the lowest values were always from the conventional treatment ('conv. treat.') and the highest from the 'untreated' variant. The intermediate ranges were the F-12.5% and F-25% treatments, with the latter being closer to 'conv. treat.', except for the aphids (Figure 3c), where F-12.5% was closer to 'conv. treat.'.

However, the most significant difference between these two treatments was observed on day 72, when the control surpassed the F-12.5% treatment with double the oviposition. In contrast, F-25% reached 21 eggs, and the conventional treatment ('conv. treat.') exceeded 23 eggs.

On the other hand, the results were similar for the F-25% treatment and the 'conv. treat.' both show the lowest oviposition (Figure 3a).

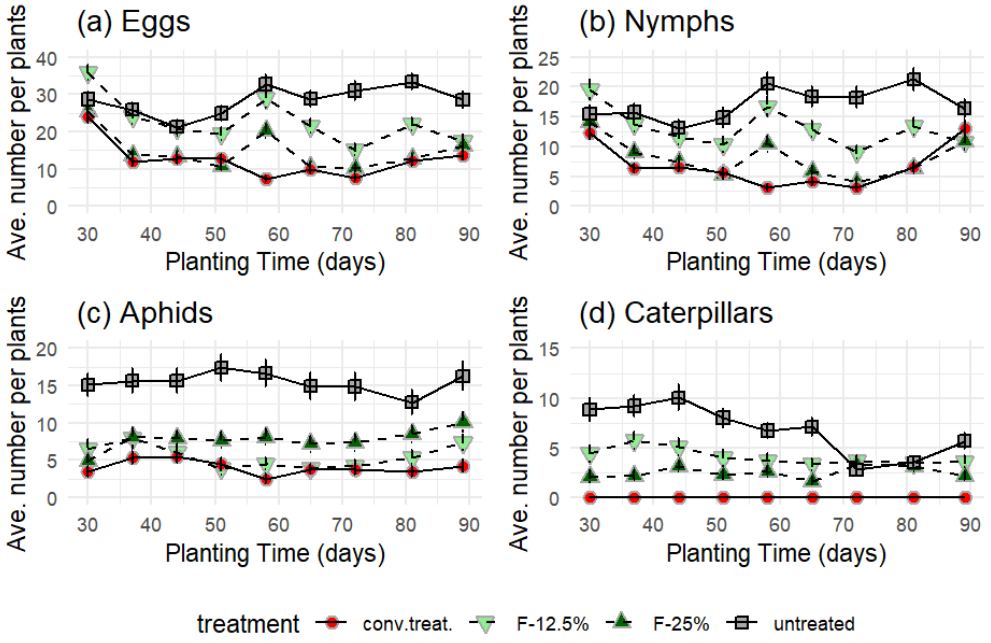

Figure 3. Direct count of (**a**) eggs, (**b**) nymphs, (**c**) aphids, and (**d**) caterpillars observed before each treatment. The value represents the average of three treatment blocks, and the bar represents the standard deviation.

The number of nymphs about the number of eggs was reduced by 45 % with the application of F-25% and 'conv. treat.' while with the application of F-12.5%, it was only 42 % and with the 'untreated', it was reduced by 40 %.

The effect of treatments on nymphs was very similar to that on eggs, with F-25% and 'conv. treat.' showing the lowest number of nymphs (Figure 4b).

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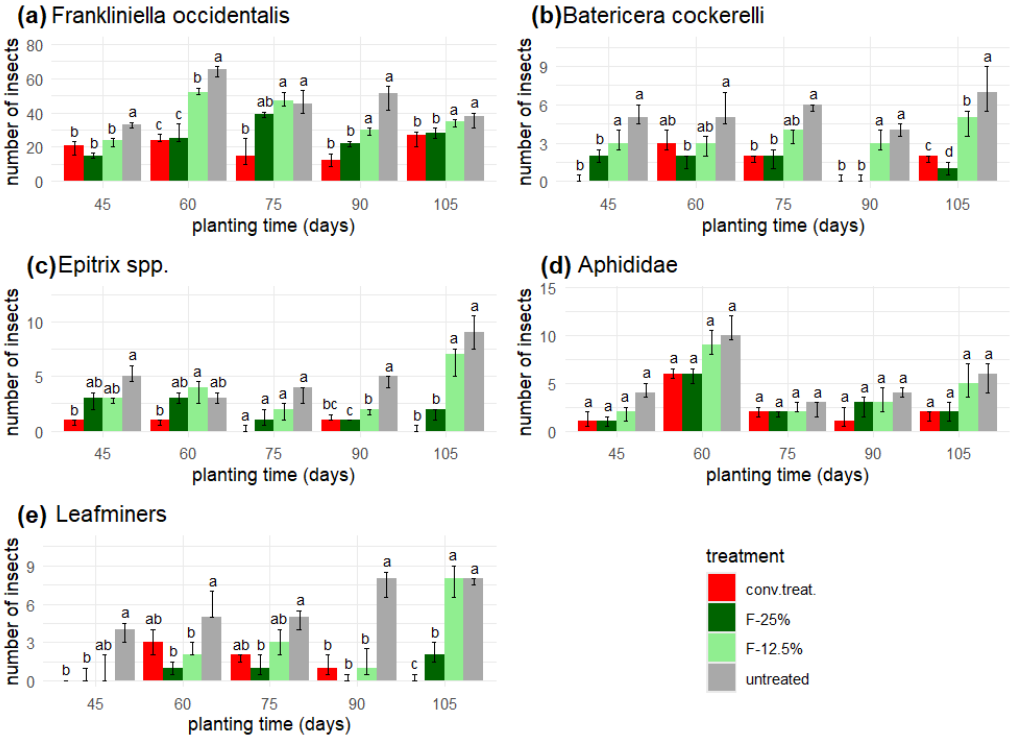

Figure 4. Yellow traps: Insect pests. (**a**) *Franklinella occidentalis*, (**b**) *Bactericera cockerelli*, (**c**) *Epitrix* spp., (**d**) Aphididae, (**e**) Leafminers in yellow traps: insect pests. The bar above each column represents the interquartile range, and the unequal letters represent statistically different median values ($p < 0.05$) within each planting time, according to the Friedmann test.

The insect pest with the highest presence in the yellow traps was thrips, which showed a lower population with the 'conv. treat.' In some weeks, it was identical to the application of F-25%.

Similar populations were observed for the aphids on days 75 and 90 when applying 'conv. treat.', F-12.5%, and F-25%, while for the last count, a lower population was noted with F-25% and 'conv. treat.'. Similarly, the population of *Bactericera cockerelli* was reduced by F-25% and 'conv. treat.' in the last three measurements (Figure 4b).

Unlike *Epitrix* spp., the most significant effect on its population was achieved with the applications of F-12.5% and the 'conv. treat.'. The application of F-25% surpassed these last two by around 5-6 insects, while compared to the 'untreated', it surpassed them by around 13-15 individuals (Figure 4c).

The results show that in the plots with 'conv. treat.' fewer than three individuals of ladybugs and wasps were present, and honeybees (*Aphis mellifera*) were absent from this treatment.

However, the extracts' application did not affect the populations of beneficial insects, with the F-25% and 'untreated' doses showing eight more specimens of Hymenoptera compared to the F-12.5% applications (Figure 5).

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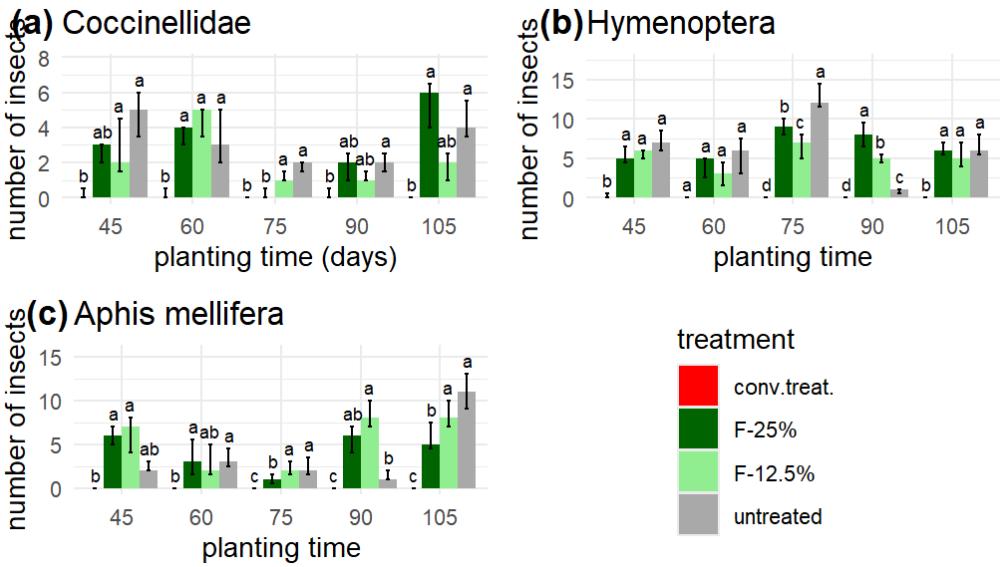

Figure 5. Yellow traps: beneficial insects or natural enemies. (**a**) Coccinellidae, (**b**) Hymenoptera (except *Aphis mellifera*), and (**c**) *Aphis mellifera*. In concordance with the Friedman test, identical letters within each planting time indicate no significant differences ($p > 0.05$).

Unlike honeybees, their population showed no difference between applying extracts and without treatments. The number of ladybugs was lower; however, the oil application reduced their population by 4 to 5 specimens compared to the 'untreated' group.

3.5. Postharvest Analysis

Conventional management reached ~36 tons·ha^{-1}, the highest yielding unit, surpassing the experimental units F-25% and F-12.5% by more than 10 tons·ha^{-1}. The plots without applications showed 24 tons·ha^{-1} less than conventional management, demonstrating the damage caused by different pests on yield (Figure 6).

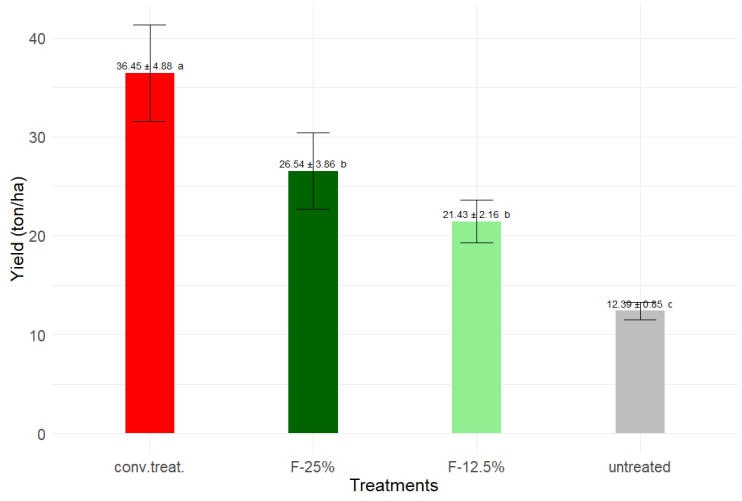

Figure 6. The average potato harvest yields per treatment. The values represent the average ± standard deviation of three blocks of experiments. According to the Tukey test, the letters denote statistically significant differences ($p > 0.05$).

Finally, the selected samples of tubers (n = 10) from each treatment block were thoroughly examined and cut. The number of damaged tubers was detected, and then the number of white grub

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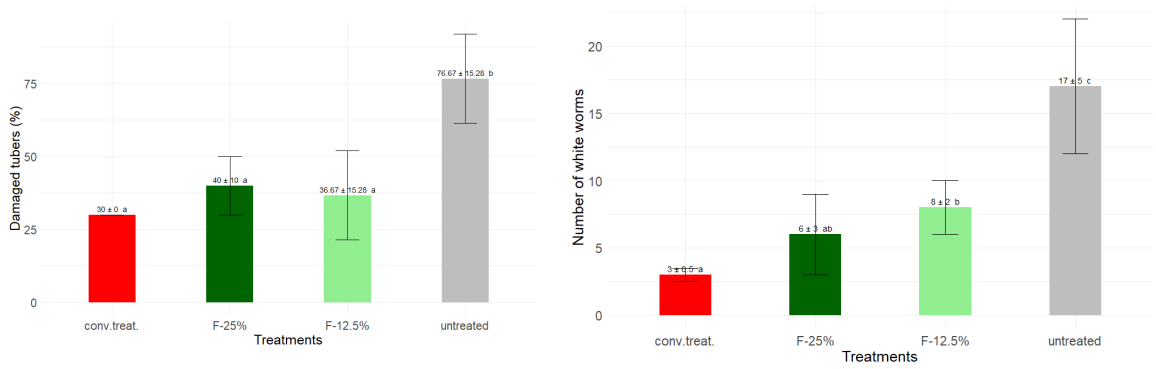
larvae (*Premnotrypes vorax* Hustache (Coleoptera: Curculionidae)) present in each damaged tuber was counted, determining the average number of white grub larvae in each treatment (Figure 7).



(**a**) (**b**)

Figure 7. Post-harvest analysis of samples (n = 10 per block and three blocks per treatment) of the different treatments. (**a**) Percentage of damaged tubers; (**b**) Average white worm (*P. vorax* H.) larvae detected in the damaged tubers. According to the Dunn test, unequal letters indicate significant differences ($p > 0.05$).

The 'untreated' variant was observed always to have significantly ($p > 0.05$) higher values than the other treatments. Additionally, no differences were observed between the 'conv. treat.', F-12.5% and F-25% treatments regarding the average value of damaged tubers, while, concerning the average number of counted white worms, no differences existed between the 'conv. treat.' and F-25%, nor between the F-25% and F-12.5% treatments, although there were differences between the latter and the 'conv. treat.' (Figure 7).

This result suggests that the treatments studied based on alcoholic extracts of mandarin peel can extend their protective action to harmful soil insects such as *P. vorax*, the cause of the 'Andean potato weevil'.

4. Discussion

As was expected [38], the phytochemical analysis confirmed the presence of several secondary metabolites, such as limonene, phenols, steroids, and flavonoids.

IR analysis allowed us to corroborate the phytochemical studies by detecting the most representative functional groups for the compounds in the extracts. The signals of peaks at 3,426, 2,924, 2,855, and 1,627 cm^{-1} suggest the presence of limonene and flavonoids, which show the existence of -OH, -CH_3, and -C=C—groups present in molecules of the terpene family (such as limonene) and flavonoids (Figure 1a).

According to the previous research [15], the RP-HPLC spectrum peaks at 2.113 min, corresponding to limonene as the significant primary compound (70 %). The rest of the mixture probably corresponds to the other secondary metabolites (flavonoids, tannins, and steroids) (Figure 1b).

The existence of mixtures within the extract is confirmed since the natural complexity of the plant constituents generates the detection of multiple peaks in the RP-HPLC profile and FTIR chromatogram (Figure 1a,b).

Some authors have identified the presence of D-limonene with the insecticidal activity of the ethanolic extract [39–43] and the flavonoids with the antifungal activity of ethanolic extract of *Citrus reticulate* peels [19,22,23,44].

Besides, cardiac glycosides are promising therapeutics in treating and managing congestive heart failure, arrhythmias and heart failure [45].

The results suggest that the overall toxicity of the F-12.5% and F-25% formulations is significantly lower than that of conventional chemical treatment, as evidenced by the almost

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negligible presence of beneficial pollinator insects in the yellow traps of this treatment. A similar result has been reported for other botanical insecticides [12,46–48].

Despite the lower yields, reaching roughly 73 % of the yield of the chemical 'conv. treat.' obtained, a formulation of 25 % ethanolic extract (F-25%) could be a good candidate for a botanical pesticide and could partially replace the use of chemical insecticides and fungicides.

Under the concepts of circular bioeconomy [49,50], utilisation and valorisation of agro-industrial waste can not only provide innovative solutions to the by-products and waste of agro-industry but also, as in this case, allow for the production of a botanical pesticide that is more environmentally friendly and effective against a group of insects and fungal pests that damage agricultural products, such as potatoes, and reduce their yields.

It could be a good alternative for small farms that wish to produce healthy organic products and organic bee honey [51] on their farms [9,52].

Various experiments are underway to validate the results shown here. Some are planned to be carried out in the "dry" season (from May to September), where the rainfall regime is less intense and the climate consequently less humid. Additionally, a more thorough characterisation of the different chemical compounds and their concentration in the ethanolic extract of tangerine peels, which were only qualitatively characterised here, is planned.

5. Conclusions

The formulation based on ethanolic extract from tangerine peels (F-25%) demonstrated considerable efficacy as an environmentally sustainable botanical pesticide. It attains pest control levels comparable to conventional chemical methods while preserving beneficial insect populations, including Coccinellidae and *Aphis mellifera*. This formulation significantly reduced infestations of key pests, including *Bactericera cockerelli* and *Frankliniela occidentalis*, in alignment with sustainable pest control goals.

Phytochemical analyses indicated that limonene, flavonoids, and polymethoxy-flavonoids are crucial bioactive constituents that augment their pesticide and antifungal properties.

The F-25% treatment attained 73 % of conventional production levels, maintaining identical tuber quality and mitigating damage by *Premnotrypes vorax* larvae. Its low environmental toxicity, validated by *Caenorhabditis elegans* assays, underscores its safety for agroecosystems.

This approach improves the utilisation of citrus waste, conforming to circular bioeconomy principles and offering small-scale Andean farmers a sustainable substitute for petrochemical pesticides in organic potato cultivation and honey production.

Future research must increase extraction efficiency and field application procedures to enhance yield equivalency with existing methods. Integrating tangerine peel extracts into pest management systems may reduce chemical dependence, improve ecological resilience, and promote sustainable agricultural practices globally.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1.

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