Preprint Title	Sulfur nanocomposites with insecticidal effect for the control of *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae)
Authors	Lany S. Araujo, Juan O. Tigrero, Vicente A. Delgado, Vladimir A. Aguirre and Josue N. Villota
Publication Date	14 Apr. 2023
Article Type	Full Research Paper
ORCID® iDs	Lany S. Araujo - https://orcid.org/0000-0003-0087-7710; Juan O. Tigrero - https://orcid.org/0000-0001-6309-6415

Sulfur nanocomposites with insecticidal effect for the control of *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae)

Lany S. Araujo*[1], Juan O. Tigrero*[1], Vicente A. Delgado‡[2], Vladimir A. Aguirre‡[2] and Josue N. Villota‡[1]

Address: [1]Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas ESPE, Av. General Rumiñahui 171-5-231B, Sangolquí PO Box 171-5-231B, Ecuador and [2]Centro de Nanociencia y Nanotecnología (CENCINAT), Universidad de las Fuerzas Armadas ESPE, Av. General Rumiñahui 171-5-231B, Sangolquí, PO Box 171-5-231B, Ecuador.

Email: Lany Araujo*- lanyaraujoyepez@gmail.com

Juan Tigrero*-jotigrero@yahoo.com

* Corresponding author

‡ Equal contributors

Abstract

The purpose of this research was to synthesize nanocomposites formed by sulfur nanoparticles, coated with eucalyptus and rosemary essential oil. To determinate the insecticidal effect in the control of nymphs of paratrioza (*Bactericera cockerelli* Sulc) in potato crops. A solution of thiosulfate was reduced to zero valent sulfur, the sulfur nanoparticles were coated with eucalyptus and rosemary essential oil at three concentrations: 0.25%, 0.5% and 0.75%. The samples were characterized by UV-visible spectroscopy, energy dispersive spectroscopy, transmission electron microscopy and scanning electron microscopy. Insecticidal efficacy was evaluated at 24, 48 and 72 hours after application. Furthermore, efficacy was compared versus the commercial insecticide thiamethoxam 0.25% and a control. The results show that eucalyptus nanocomposites at concentrations: 0.25%, 0.5% and 0.75% and rosemary nanocomposites at concentration of 0.5%, have an insecticidal efficacy of 100% for the control of insect nymphs 24 hours after application. While the insecticidal efficacy of rosemary nanocomposites at concentrations of 0.25% and 0.75% increases over time, reaching 100% at 24 and 72 hours respectively. The synthesized nanocomposites are more effective in controlling nymphs of paratrioza than the commercial insecticide thiamethoxam. Could be used for the development of new insecticides.

Keywords

Paratrioza control; eucalyptus; nanoinsecticide; nanotechnology, rosemary

Introduction

Paratrioza (*B. cockerelli* Sulc) is one of the most dangerous pest of potatoes, tomatoes, pepper and other crops of the Solanaceae family [1]. The insect is one of the most destructive potato pests in the western hemisphere, New Zealand, and Australia [2]. It is native to North America, however, due to its aggressiveness, the susceptibility of cultivated species varieties and favorable climatic conditions for its development, it has been distributed to México, Central America, and recently to South American countries [3,4,5].

In Ecuador, potato cultivation is one of the main agricultural activities, due to the generation of income for producers and the importance in the daily diet of the population [6]. The main problems for producers are pests and diseases that severely affect the crop [7]. When high densities of the insect feed prior to flowering in potato crops can result in higher numbers and weights of unmarketable tubers [8]. Additionally, when it has been associated with the bacterium *Candidatus Liberibacter solanacearum* cause abnormal development and early death of the plant reducing the quality and yields in potato, tomato and pepper crops [2].

B*. cockerelli* tends to be difficult to manage, synthetic insecticides such as organophosphates, organochlorines, carbamates, and pyrethroids are used to combat this pest [9]. But the insect has been shown to develop resistance due to the high fecundity and short generation [10]. Also, persistence, bio-accumulation, toxicity, misuse and overuse of synthetic insecticides has led to deterioration of soil, air pollution, contamination of water bodies, degradation of agroecosystems, and damages to human health for their directly or indirectly exposure [11,12,13]. Therefore, new methods should be considered to control the pest. Thus, nanotechnology has emerged as a technological advance that can enhance the modern agriculture [8]. Helping in the development of new nanoinsecticides to combat pests in a more productive, cost-effective and eco-friendly way [12].

Nano-agricultural products are developing using nanotechnology, such as nanopesticides, nanoinsecticides, nanoemulsions, and nanoparticles to reduce the use of toxic chemicals [14]. Furthermore, different kinds of polysaccharides (e.g. chitosan, alginates, polyethylene glycol and others) have been used for synthesis of nanoinsecticides [15]. While, others form of polymer and non-polymer based nanoformulations like nanofiber, nanocapusles, nanogels, nanomicelles and nanospheres, have been used for encapsulation of nanoinsecticides [16].

Nanoencapsulation technique is used to improve the insecticidal assessment, the nanometer-sized active ingredient is encapsulated by a thin-walled sac to allow the controlled release of the active ingredient [14]. Also, improve the efficiency and reduce the amount of pesticide input and environmental hazards [16].

Sulfur is considered one of the oldest pesticides used in agriculture for the treatment of a wide range of plant diseases [17]. Elemental sulfur is now available from nanoparticles forms that can be generated by different chemical methods [18,19]. Elemental sulfur nanoparticles (SNPs) have already demonstrate evidence of significant insecticidal, fungicidal and bactericidal activity [20,21]. By manipulating particle size and surface area, SNPs can have greater absorption, increase the efficacy of new insecticides formulations and reduce the amount of insecticide required for control [22]. Nanoparticles are known insecticidal properties, interacting with the cell membrane of the insect [23]. Causing the denaturation of organelles and enzymes, oxidative stress and cell death [24].

Essential oils are potential botanical sources for developing new insecticides [25]. Their active components act as strong repellents against target pest species including toxicant and repellent effects, developmental and behavioral alterations, and sterility/infertility of insects [26]. Technologies such as nanoformulations or microencapsulation of essential oils protect their active components from degradation and losses by evaporation, thereby enhancing their stability and solubility [27].

In this framework, this research shows the synthesis of nanocomposites formed by elemental sulfur nanoparticles. Coated with essential oil of eucalyptus and rosemary at different concentrations. Characterized by UV-visible spectroscopy, energy dispersive spectroscopy (EDS), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). With the aim of evaluating the insecticidal efficacy of nanocomposites for the control of nymphs of paratrioza.

Results and Discussion

Sulfur Nanoparticles

Figure 1 shows the UV-visible spectrum of the synthesized SNPs; a maximum absorption peak was observed at 253 nm indicating their successful formation. SNPs have been reported to show maximum absorption peak in the range of 250 to 400 nm [28,29,30].

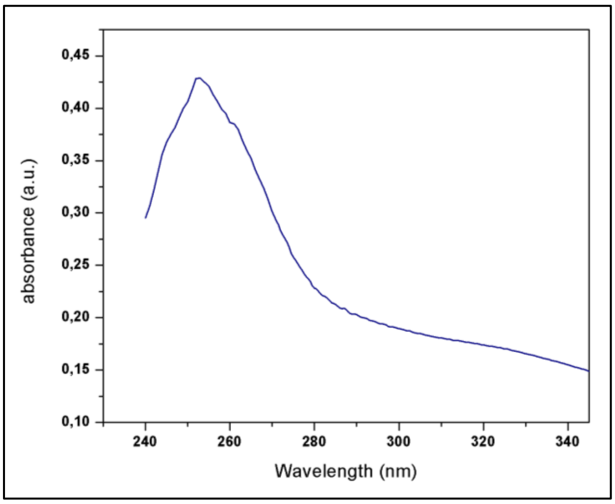

Figure 1: UV-vis spectra of SNPs.

EDS analysis (Figure 2) shows the presence of sulfur at a mass percent of 28%. Furthermore, other elements (Na, Cl, and O) corresponding to the by-products of the reduction reaction (Equation 1) of sodium thiosulphate to obtain sulfur were found [19,28,31]. The presence of the element carbon is due to the substrate used in the EDS analysis [32,33].

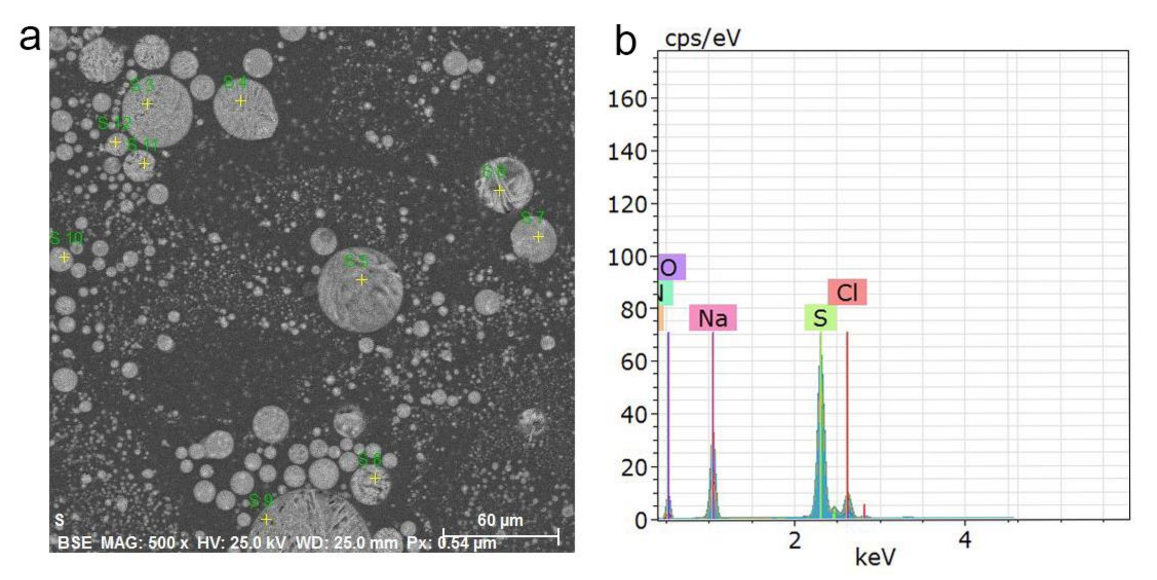

Figure 2: a) SEM micrograph and b) EDS of synthesized SNPs.

TEM image (Figure 3a) shows spherical SNPs with a tendency to agglomerate with an average diameter of 28 nm and a narrow size distribution (standard deviation of ±4.5 nm, see histogram in Figure 3b). The diameter agrees with other research which used the same method of SNPs synthesis by chemical precipitation from sodium thiosulfate as a sulfur source [28,29,30].

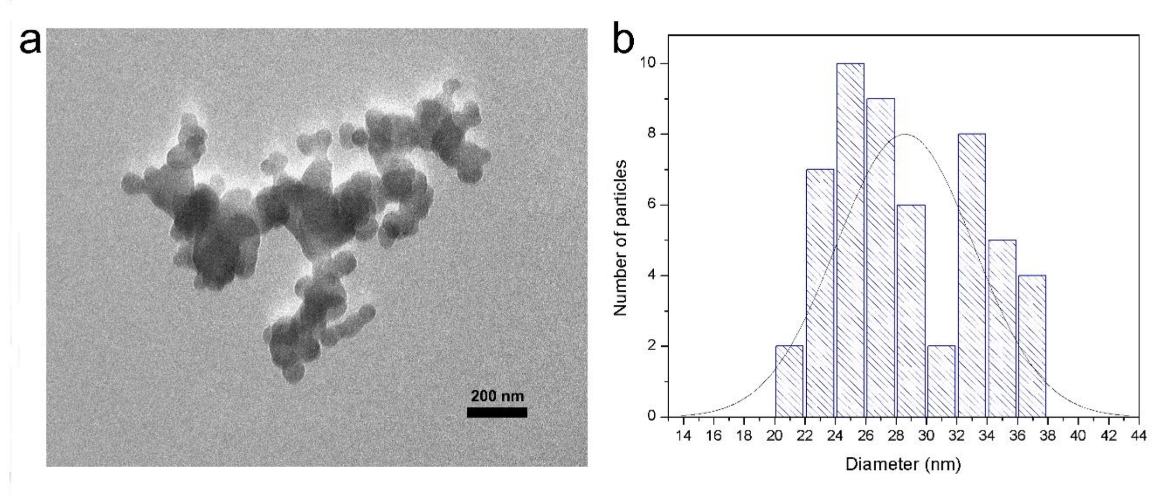

Figure 3: TEM of SNPs samples a) 28 nm synthesized SNPs b) particle size analysis.

Eucalyptus nanocomposites

Figure 4 shows STEM micrographs of eucalyptus nanocomposites (NCMPs), all of them tend to agglomerate and present a wide particle size distribution. At the concentration of 0.25% they presented an average diameter of 97nm (standard deviation of ±17.70 nm on 130 analyzed particles, see histogram Figure 4b). At the concentration of 0.5% they showed an average diameter of 140 nm (standard deviation of ±31 nm on 107 analyzed particles, see histogram Figure 4d) and at the concentration of 0.75% they presented an average diameter of 147 nm (standard deviation of ±29.90 nm on 133 analyzed particles, see histogram Figure 4f).

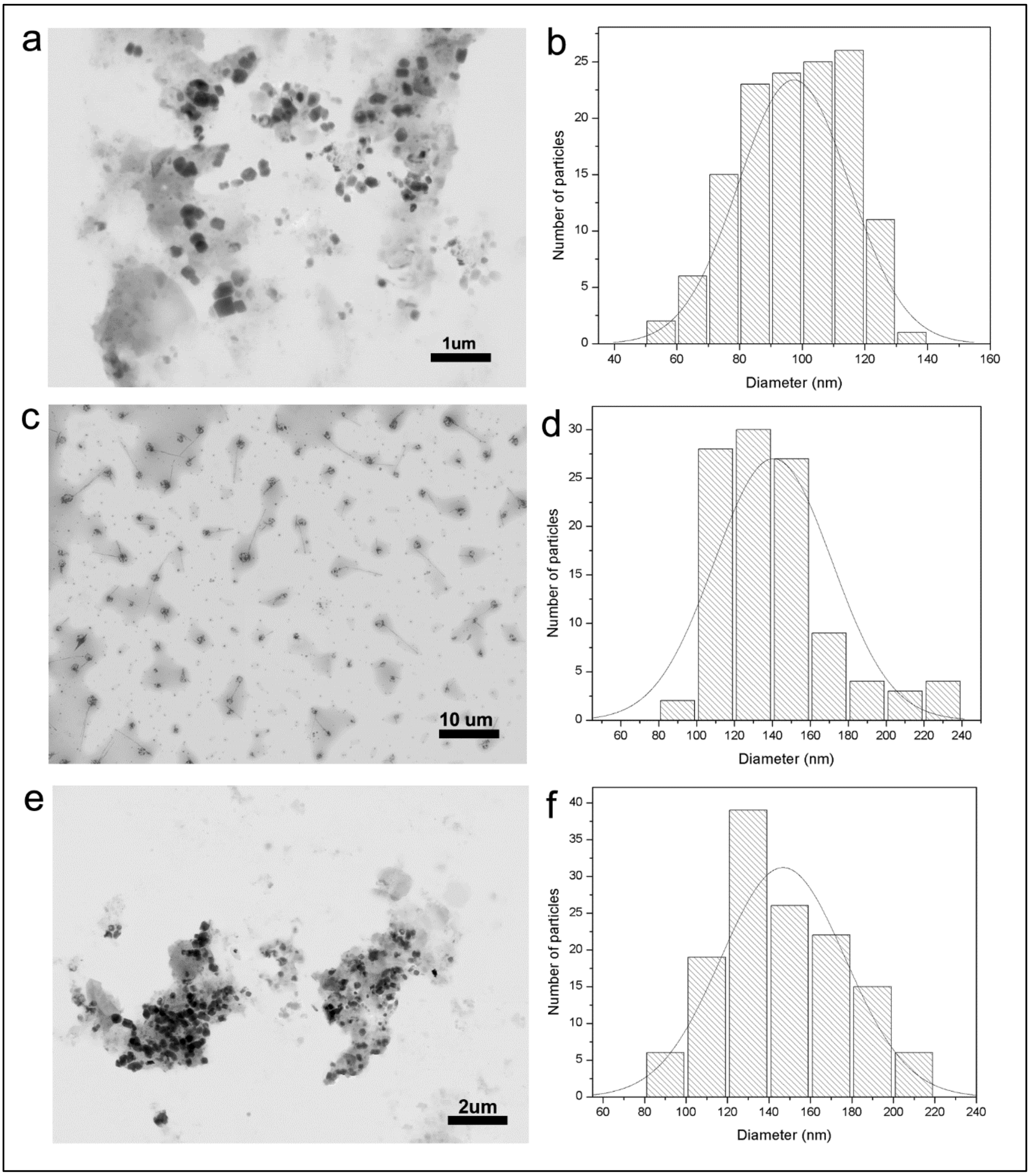

Figure 4: STEM micrographs of eucalyptus NCMPs a) 0.25% concentration b) particle size analysis of 0.25% concentration c) 0.5% concentration d) particle size analysis of 0.5% concentration e) 0.75% concentration and f) particle size analysis of 0.75% concentration.

Rosemary nanocomposites

Figure 5 shows STEM micrographs of the rosemary NCMPs, all of them presented a spherical shape with a tend to agglomerate and a broad particle size distribution. At the 0.25% concentration they presented an average diameter of 106 nm (standard deviation of ±24.54 nm on 115 analyzed particles, see histogram 5b). At the 0.5% concentration they showed an average diameter of 190 nm (standard deviation of ±43.38 nm on 120 analyzed particles, see histogram 5d) and the 0.75% concentration they exhibited an average diameter of 208 nm (standard deviation of ±48 nm on 118 analyzed particles, see histogram 5f).

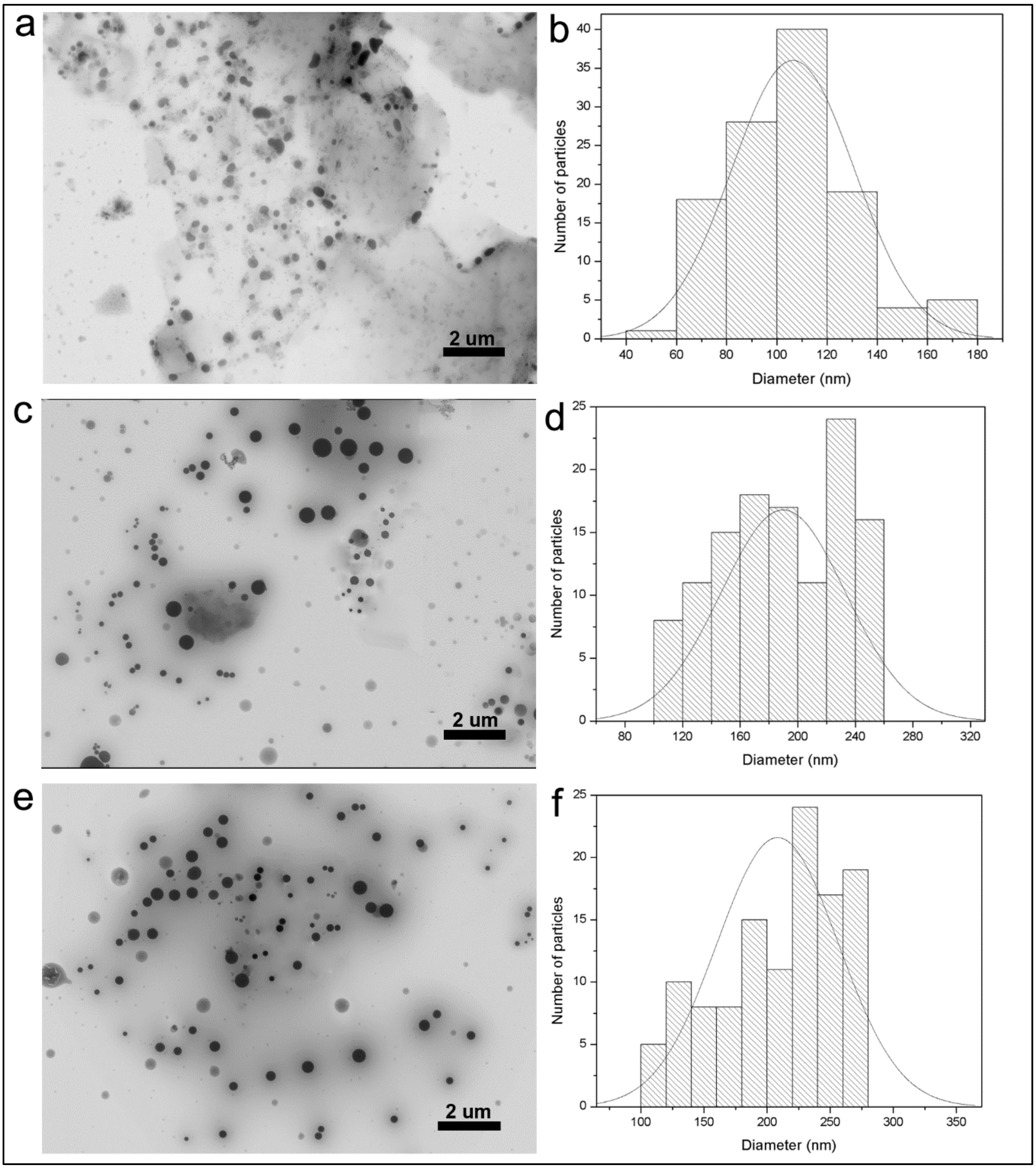

Figure 5: STEM micrographs of rosemary NCMPs a) 0.25% concentration b) particle size analysis of 0.25% concentration c) 0.5% concentration d) particle size analysis of 0.5% concentration e) 0.75% concentration and f) particle size analysis of 0.75% concentration.

Figure 6 shows a STEM micrograph of the structure of the rosemary NCMPs corresponding to a nanomicelle, composed of two immiscible phases: (a) the aqueous formed by the sulfur nanoparticles and (b) the oily formed by the essential oils of rosemary. The ethanol used for synthesis acts as a cosurfactant since being an amphiphilic molecule with a hydrocarbon chain and a hydroxyl group, it is able to reduce the interfacial tension between the two immiscible phases [34,35].

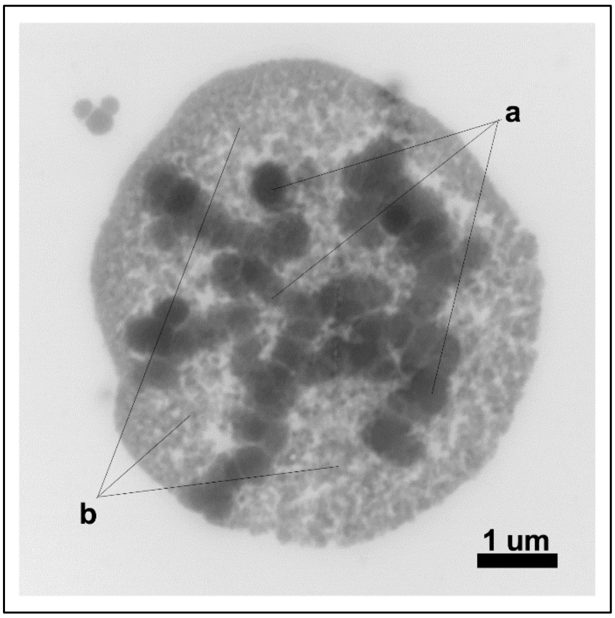

Figure 6: STEM micrographs of rosemary NCMPs at 0.75% concentration a) aqueous face b) oily phase.

Moreover, as observed in the histograms (Figure 4 and Figure 5) the size of the NCMPs depends on the increase in the concentration of the eucalyptus and rosemary essential oil respectively. Agreeing with similar studies where the increase in the essential oil concentration influences the viscosity of the oily phase of the nanomicelle, increasing the diameter of the particle [36,37].

Evaluation of insecticidal efficacy of nanocomposites

The results of the treatments evaluated are shown in Table 1. 24 hours after application, treatments T1, T2, T3 and T5 showed an insecticidal efficacy of 100%. As time went through, the insecticidal efficacy of the other treatments was increasing. At 48 hours, the T4 treatment reached an efficacy of 100% and after 72 hours the T6 treatment exhibited 100% efficacy. Treatment with thiamethoxam (T7) had significantly lower insecticidal efficacy than nanocomposite treatments in the three days evaluated. On the other hand, the control group (T8) showed the lowest values of insecticidal efficacy among all the treatments evaluated.

Table 1: Insecticidal efficacy of treatments for the control of paratrioza nymphs.

Treatment	Insecticidal efficacy[a] ± SD[b]		
	24 h	**48 h**	**72 h**
T1: Eucalyptus 0.25%	100±3.33	100±4.86	100±1.67
T2: Eucalyptus 0.5%	100±3.33	100±4.86	100±1.67
T3: Eucalyptus 0.75%	100±3.33	100±4.86	100±1.67
T4: Rosemary 0.25%	90±3.33	100±4.86	100±1.67
T5: Rosemary 0.5%	100±3.33	100±4.86	100±1.67
T6: Rosemary 0.75%	96.67±3.33	96.67±4.86	100±1.67
T7: Thiamethoxam 0.25%	50±3.33	70±4.86	83.33±1.67
T8: Control	13.33±3.33	26.67±4.86	43.33±1.67

[a]Values are mean ± SD of three trials; [b]SD: standard deviation

Also, nanoencapsulation is known to improve insecticidal efficacy due to a larger surface area and specificity, providing greater contact of the active substance with the insect [38]. On the other hand, the action mechanism of nanocomposites may be due to their effective penetration through the pores and microfibrils of the insect's cuticle [38]. Releasing the essential oil and sulfur nanoparticles, interfering with the biology, physiology and nervous system [39].

Also, the use of elemental sulfur as an insecticide in the control of parasitic insect nymphs has been reported [40]. Other authors report the use of sulfur nanoparticles in the mortality of larvae, pupae and adults of the fruit fly *Drosophila melanogaster* [41].

In Addition, nanoencapsulated essential oils have chemical activity and increased mobility, allowing penetration into insect tissues through the cuticle or by ingestion through the digestive tract [42]. Essential oils are lipophilic and thus can enter the insect and cause biochemical dysfunction and mortality [43]. Rosemary essential oil-laden nanoformulations have been found to show significant insecticidal activity for the effective management of the red beetle *Tribolium castaneum* [44]. Another study claimed that eucalyptus essential oil-laden nanoemulsions had insecticidal activity against *Sitophilus oryzae* in rice crops [45].

Conclusion

In summary, nanocomposites with a nanomicellar structure were synthesized. Composed of an aqueous phase, made up of elemental sulfur nanoparticles with an average diameter of 28±4.5 nm and an oily phase made up of eucalyptus and rosemary essential oils at three concentrations: 0 .25%, 0.5% and 0.75% respectively. Furthermore, the increase in essential oil concentration influenced the diameter size of the NCMPs. In addition, the insecticidal efficacy of the synthesized NCMPs was evaluated; at 24 hours after application, eucalyptus NCMPs at concentrations of 0.25%, 0.5%, 0.75% and rosemary at 0.5% present an insecticidal efficacy of 100%. The insecticidal efficacy of rosemary nanocomposites at 0.25% and 0.75% increases with time, reaching 100% at 24 and 72 hours, respectively. The treatment with thiamethoxam at 0.25% had a significantly lower efficacy than the treatments with NCMPs in the three days evaluated. Concluding that the synthesized nanocomposites are more effective for the control of paratrioza nymphs than the commercial insecticide thiamethoxam. Nanocomposites can be used as potential treatments for integral pest management programs and development of new insecticides.

Experimental

Materials

The reagents: sodium thiosulfate pentahydrate $Na_2S_2O_3 \cdot 5H_2O$, ACS reagent, ≥99.5%, CAS number: 10102-17-7), hydrochloric acid (HCl, ACS reagent, 37%, CAS number: 7647-01-0), triethanolamine ($(HOCH_2CH_2)_3N$, ACS reagent, ≥99.5%, CAS number: 102-71-6), ethanol (CH_3CH_2OH, CAS number: 64-17-5), and polyethylene glycol (PEG, $H(OCH_2CH_2)_nOH$, Wt:6000, CAS number: 25322-68-3), were acquired from Sigma-Aldrich. The chemical insecticide thiamethoxam ($C_8H_{10}ClN_5O_3S$, CAS number 153719-23-4, concentration 0.25%) was acquired from Syngenta. The distilled water was obtained in the laboratory.

Essential oil extraction

2 Kg of rosemary leaves and stems and 2 kg of eucalyptus leaves were purchased in a local market located in Sangolquí-Ecuador. The extraction of essential oils was carried out by steam distillation method. Using a Clevenger type apparatus and an extractor, following the protocol described by the literature [46,47]. The oils obtained were stored in amber glass bottles at 4°C for later use.

Synthesis of sulfur nanoparticles

In 25 ml of a 0.01M solution of thiosulfate pentahydrate, 250 uL of a 2M solution of hydrochloric acid with stirring at 25 °C was added. The ratio of molar concentration of thiosulfate to HCl was 1:2 for all assays. After 30 minutes, the redox reaction to form sulfur reached equilibrium (Equation 1).

$$Na_2S_2O_3+2HCl \rightarrow 2NaCl+SO_2+S\downarrow+H_2O \qquad (1)$$

Synthesis of nanocomposites

Solutions of ethanol-essential oil of eucalyptus and rosemary were prepared at three concentrations: 0.25%, 0.5% and 0.75%. Subsequently, the solution of sulfur nanoparticles previously synthesized as shown in Table 2 was added. Finally, as a stabilizing agent, 1 mL of 1% PEG was placed in each solution.

Table 2: Amount of sulfur nanoparticles added to ethanol-essential oil solutions.

Solution of ethanol-essential oil	**Added sulphur nanoparticles (mL)**
Ethanol-eucalyptus 0.25	25
Ethanol-eucalyptus 0.5%	18
Ethanol-eucalyptus 0.75%	20
Ethanol-rosemary 0.25%	37
Ethanol-rosemary 0.5%	29
Ethanol-rosemary 0.75%	26

Characterization techniques and equipment

UV-visible spectroscopy was performed on an Analytik Jena SPECORD® S 600 spectrophotometer. The size and morphology of the SNPs was obtained using an FEI Tecnai G2 Spirit Twin transmission electron microscope. Energy dispersive spectroscopy analysis of SNPs was performed on a Phenom ProX scanning electron microscope equipped with a QUANTAX-EDS detector, using a voltage of 25 kV and Prosuite software. The size and morphology of NCMPs was obtained with a Tescan MIRA3 scanning electron microscope.

Sampling of paratrioza nymphs

For experimentation with nymphs of the paratrioza insect, sampling trials and evaluation of insecticidal efficacy were conducted in accordance with the pertinent laws and institutional guidelines of the technical cooperation agreement between the Phytosanitary Regulation and Control Agency-AGROCALIDAD and the Universidad de las Fuerzas Armadas-ESPE.

Leaves infested with paratrioza nymphs were collected from a potato plantation located at the IASA I campus of the Universidad de las Fuerzas Armadas-ESPE. Samples were taken to the entomology laboratory under standard insectary conditions (27 ± 1°C temperature, 80 ± 10% relative humidity and 12 h light/12 h dark photoperiod) [48].

Evaluation of insecticidal efficacy of nanocomposites

The evaluation was *in vitro* in the entomology laboratory of the Universidad de las Fuerzas Armadas-ESPE with 8 treatments (Table 1). The experimental unit was a 250 mL polypropylene jar containing 10 nymphs of the insect placed on a potato leaf on absorbent paper moistened with distilled water. The test was repeated three times and distilled water was use like control. All the treatments were applied with a fine-drop sprayer. Mortality was recorded 24, 48 and 72 hours after application. Nymphs were considered dead when after touch stimulation they did not move at all.

Data analysis

Based on mortality data, the percentage efficacy of treatments was calculated using the Henderson-Tilton formula [49].

$$\%\text{Effectiveness}=\frac{b-k}{100-k} \qquad (3)$$

Where:

b: percentage of dead individuals of the treatments.

k: percentage of dead individuals of the control.

Data were analysed with InfoStat software followed by a Fisher's LSD significance test. Results were expressed as means (± SD) of data and were considered significantly different at $p<0.05$.

Acknowledgements

We are grateful with the Phytosanitary Regulation and Control Agency-AGROCALIDAD to helping in the sampling and experimentation with the nymphs of paratrioza, and with the Biotechnology Engineer Gabriel Guaman, for the contributions in the development of the research.

ORCID® iDs

Lany Araujo- https://orcid.org/0000-0003-0087-7710

Juan Tigrero- https://orcid.org/0000-0001-6309-6415

Vicente Delgado- https://orcid.org/0000-0001-8040-2947

Vladimir Aguirre- https://orcid.org/0000-0002-1954-0918

Josué Villota- https://orcid.org/0000-0003-3819-0017

References

1. Vereijssen, J.; Smith, G.; Weintraub, P. G. *Journal of Integrated Pest Management* **2018,** 9, 1. doi:10.1093/jipm/pmy007
2. Vereijssen, J. *Bactericera cockerelli* (tomato/potato psyllid), Version 1.0.0, CABI Compendium. CABI International, **2022** https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.45643. doi:10.1079/cabicompendium.45643
3. Prager, S. M.; Trumble, J. T. Psyllids: Biology, Ecology, and Management. In *Sustainable management of arthropod pests of tomato;* Wakil, W.; Brust, G. E.; Perring T. M., Eds.; Academic Press: New York, ***2018***, Vol. 2, pp 163-181. doi:10.1016/B978-0-12-802441-6.00007-3
4. Wan, J.; Wang, R.; Ren, Y.; McKirdy, S. *Insects* **2020**, 11, 5, 298.

doi:10.3390/insects11050298

5. Carrillo, C.; Fu, Z.; Burckhardt, D. First record of the tomato potato psyllid *Bactericera cockerelli* from South America. *Bulletin of Insectologyl* **2019,** *72*, 1, 85-91.
6. Soltani, A.; Stoorvogel, J. J.; Veldkamp, A. *European Journal of Agronomy* **2013,** 48, 101-108. doi:10.1016/j.eja.2013.02.010
7. Navarrete, I.; Panchi, N.; Andrade, J. *Revista Latinoamericana de la Papa* **2017**, 21, 2, 69-88. doi:10.37066/ralap.v21i2.280
8. Furlong, J. N; Vereijssen, J.; Pitman, A. R.; Butler, R. *New Zealand Plant Protection* **2017**, No. 70, 320. doi:10.30843/nzpp.2017.70.84
9. Abubakar, Y.; Tijjani, H.; Egbuna, C.; Adetunji, C.; Kala, S.; Kryeziu, T.; Kala, S.; Ifemeje, J.; Iwuanyanwu, K. Pesticides, History, and Classification. In *Natural Remedies for Pest, Disease and Weed Control*; Egbuna C.; Sawicka B., Eds.; Academic Press: Amsterdam, **2020**; Vol. 1, pp 29–42. doi:10.1016/B978-0-12-819304-4.00003-8
10. Buchman, J.; Heilman, B.; Munyaneza, J. *Journal of Economic Entomology* **2011**, 104, 6, 1783-1792. doi:10.1603/ec11146
11. Mahmood, I.; Imadi, S. R.; Shazadi, K.; Gul, A.; Hakeem, K. R. Effects of Pesticides on Environment. In *Plant, Soil and Microbes;* Hakeem, K.; Akhtar, M.; Abdullah, S., Eds.; Springer, Cham: Switzerland, **2016**; Vol. 1, pp 253–269. doi:10.1007/978-3-319-27455-3_13
12. Pathak V. M.; Verma V. K.; Rawat B. S.; Kaur B.; Babu N.; Sharma A.; Dewali S.; Yadav M.; Kumari R.; Singh S.; Mohapatra A.; Pandey V.; Rana N.; Cunill J. M. *Front. Microbiol.* **2022***,* 13, No. 10. doi:10.3389/fmicb.2022.962619
13. Qu, C.; Albanese, S.; Lima, A.; Hope, D.; Pond, P.; Fortelli, A.; Romano N.; Cerino, P.; Pizzolante A.; De Vivo B. *Environ. Int.* **2019**, 124, 89–97.

doi:10.1016/j.envint.2018.12.031

14. Suresh C. M.; Shani R.; Rohini T. *Biochemistry and Biophysics Reports* **2020***,* 24. doi:10.1016/j.bbrep.2020.100821
15. Shah M. A.; Wani S. H.; Khan A. A. Nanotechnology and insecticidal formulations. *J. Food Bioengineering and Nanoprocessing* **2016**, 1, 285–310.
16. Nuruzzaman M.; Rahman M. M.; Liu Y.; Naidu R. *J. Agric. Food Chem.* **2016**, 64, 1447–1483. doi:10.1021/acs.jafc.5b05214
17. Shankar, S.; Jaiswal, L.; Rhim, J.W. *Critical Reviews in Environmental Science and Technology* **2020**, 51, 2329-2356*.* doi:10.1080/10643389.2020.1780880
18. Abuyeva, B.; Burkitbayev, M.; Mun, G.; Uralbekov, B.; Vorobyeva, N.; Zharlykasimova, D.; Urakaev, F. *Materials Today: Proceedings* **2018**, 5, 11, 22894–22899. doi:10.1016/j.matpr.2018.07.107
19. Chaudhuri, R. G.; Paria, S. *Journal of Colloid and Interface Science* **2011**, 354, 2, 563–569. doi:10.1016/j.jcis.2010.11.039
20. Saedi, S.; Shokri, M.; Rhim, J. W. *Arabian Journal of Chemistry* **2020**, 13, 8, 6580-6588. doi:10.1016/j.arabjc.2020.06.014
21. Kim, Y. H.; Kim, G. H.; Yoon, K. S.; Shankar, S.; Rhim, J. W. *Microbial Pathogenesis* **2020***,* 144, 104178*. doi:*10.1016/j.micpath.2020.104178
22. Griffith, C. M.; Woodrow, J. E.; Seiber, J. N. *Pest Management Science* **2015**, 71, 11, 1486–1496. doi:10.1002/ps.4067
23. Benelli, G.. *Environmental Science and Pollution Research* **2018**, 25, 12329-12341. doi:10.1007/s11356-018-1850-4
24. Saranya, S.; Selvi, A.; Babujanarthanam, R.; Rajasekar, A.; Madhavan, J. Insecticidal Activity of Nanoparticles and Mechanism of Action. In: *Model Organisms to Study Biological Activities and Toxicity of Nanoparticles*; Siddhardha,

B.; Dyavaiah, M.; Kasinathan, K., Eds.; Springer: Singapore, **2020**; Vol 1, pp 243-266. doi:10.1007/978-981-15-1702-0_12

25. Palermo, D.; Giunti, G.; Laudani, F.; Palmeri, V.; Campolo, O. *Sustainability* **2021**, *13*, 17, 9746. doi:10.3390/su13179746
26. Li, Y.; Fabiano-Tixier, A. S.; Chemat, F. Essential Oils as Insecticides. In *Essential Oils as Reagents in Green Chemistry*; Sharma S. K., Ed.; SpringerBriefs in Molecular Science, Vol. 1; Springer, Cham: New York, **2014**; pp 41-53. doi:10.1007/978-3-319-08449-7_5
27. Giunti, G.; Campolo, O.; Laudani, F.; Zappalà, L.; Palmeri, V. *Industrial Crops and Products* **2021**, 162, 113257*.* doi:10.1016/j.indcrop.2021.113257
28. Massalimov, I. A.; Khusainov, A. N.; Zainitdinova, R. M. *Russ. J. Appl. Chem.* **2014**, 87, 700–708. doi:10.1134/S1070427214060068
29. Tripathi, R. M.; Rao, R. P.; Tsuzuki, T. *RSC Adv.* **2018**, 8, 36345–36352. doi:10.1039/C8RA07845A
30. Ghotekar, S.; Pagar, T.; Pansambal, S.; Oza, R. *Advanced Journal of Chemistry-Section B: Natural Products and Medical Chemistry* **2020**, *2*, 3, 128-143. doi:10.22034/ajcb.2020.109501
31. Shankar, S.; Pangeni, R.; Park, J. W.; Rhim, J. W. *Materials Science and Engineering: C* **2018,** 92, 508–517, 2018, doi:10.1016/j.msec.2018.07.015
32. Wassilkowska, A.; Czaplicka, A.; Bielski, A.; Zielina, M. An analysis of the elemental composition of micro-samples using EDS technique. In *Technical* Transactions*;* Gawlik J., Ed.; Cracow University of Technology: Krakow, **2014**; pp 133-148. doi:10.4467/2353737XCT.14.283.3371
33. Ghisleni, R.; Rzepiejewska, K.; Philippe, L.; Schwaller, P.; Michler, J. *Microscopy Research and Technique* **2009**, 72, 242-249. doi:10.1002/jemt.20677
34. Beyki, M.; Zhaveh, S.; Khalili, S. T.; Rahmani, T.; Abollahi, A.; Bayat, M.;

Mohsenifar, A. *Industrial crops and products* **2014**, 54, 310-319. doi:10.1016/j.indcrop.2014.01.033

35. Pavoni, L.; Perinelli, D. R.; Bonacucina, G.; Cespi, M.; Palmieri, G. F. *Nanomaterials (Basel)* **2020**, 10, 135. doi:10.3390/nano10010135
36. Aziz, Z. A. A.; Nasir, H. M.; Ahmad, A. Enrichment of Eucalyptus oil nanoemulsion by micellar nanotechnology: transdermal analgesic activity using hot plate test in rats' assay. *Sci. Rep*. **2019,** 9, 13678. doi:10.1038/s41598-019-50134-y
37. Khalili, S. T.: Mohsenifar, A.; Beyki, M.; Zhaveh, S.; Rahmani T.; Abdollahi, A; Tabatabaei, M. *LWT-Food Science and Technology* **2015**, 60, 502-508. doi:10.1016/j.lwt.2014.07.054
38. Goswami, A.; Roy, I.; Sengupta, S.; Debnath, N. *Thin Solid Films* **2010**, 519, 1252-1257. doi:10.1016/j.tsf.2010.08.079
39. Rai, M.; Ingle, A. *Applied microbiology and biotechnology* **2012**, 94, 287-293. doi:10.1007/s00253-012-3969-4
40. Guillén A.;. Azufres de uso Agrícola (fungicidas); El Cid Editor: Argentina, 2015.
41. Araj, S. E.; Salem, N. M.; Ghabeish, I. H; Awwad, A. M. *Journal of Nanomaterials* **2015**, 4, 1-9. doi:10.1155/2015/758132
42. Werdin J. O.; Gutiérrez, M. M.; Ferrero, A. A.; Fernández, B. *Chemosphere* **2014**, 100, 130-138. doi:10.1016/j.chemosphere.2013.11.056
43. Isman, M. *Zeitschrift für Naturforschung C* **2020**, 75, 179-182. https:10.1515/znc-2020-0038
44. Khoobdel, M.; Ahsaei, S. M.; Farzaneh, M. *Entomological Research,* **2017**, 47, 175–18. doi:10.1111/1748-5967.12212
45. Adak, T.; Barik, N.; Patil, N. B.; Govindharaj, G.; Gadratagi, B. G.; Annamalai, M.; Mukherjee, A.; Rath, P. C. *Industrial Crops and Products* **2020**, 143, 111849 doi:10.1016/j.indcrop.2019.111849

46. El Kharraf, S.; Faleiro, M. L.; Abdellah, F.; El-Guendouz, S.; El Hadrami E. M.; Miguel M. G. *Molecules* **2021,** 26, 5452. doi:10.3390/molecules26185452
47. Božović, M.; Navarra, A.; Garzoli, S.; Pepi, F.; Ragno, R. *Natural Product Research* **2017,** 31, 2387–2396. doi:10.1080/14786419.2017.1309534
48. Subcommittee on Plant Health Diagnostic Standards. *Diagnostic Protocol for the detection of the Tomato Potato Psyllid, Bactericera cockerelli (Šulc)*; Australia, 2017.
49. Henderson C. F.; Tilton E. W. *Journal of Economic Entomology* **1955**, 48, 157-161.doi:10.1093/jee/48.2.157