AN EVALUATION OF INSECTICIDAL POTENTIAL OF *OLAX ZEYLANICA* LEAF EXTRACT MEDIATED SULFUR NANOPARTICLES TOWARDS *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE) AND *TRIBOLIUM CASTANEUM* (L.) (COLEOPTERA: TENEBRIONIDAE)

***G. D. V. A. Sandeepani* [1], *A.G.W.U. Perera* [2*], *and S.D.M. Chinthaka* [1]**

[1] *Department of Chemistry, University of Sri Jayewardenepura, Sri Lanka*
[2] *Department of Zoology, University of Sri Jayewardenepura, Sri Lanka*

Rice is a staple food for half of the world's population but is often damaged by insects during storage, namely, *Sitophilus oryzae* and *Tribolium castaneum*. Conventional insecticides, which are commonly used to control them, have adverse effects. Therefore, green synthesized nano-pesticides can be used as a safer, and ecologically sound alternative to conventional insecticides. The objective of this study was to assess the insecticidal efficacy of green synthesized sulfur nanoparticles (SNPs). Synthesis of SNPs was done by mixing sodium thiosulfate ($Na_2S_2O_3.5H_2O$) and *Olax zeylanica* leaf extract at room temperature. The resulting SNPs were then characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analyzer (TGA), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray Spectroscopy (EDX). The effect of SNPs on *S. oryzae* and *T. castaneum* was evaluated by bioassays for repellency and mortality. The UV-Vis spectroscopy showed a peak in the range of 260–280 nm, indicating the successful formation of SNPs. According to the Debye–Scherrer formula of XRD data, the average crystalline size of the SNPs counted to be 75.2 nm. All the diffraction peaks are consistent with orthorhombic sulfur (JCPDS 01-089-2600). FTIR spectrum revealed peak positions corresponding to octasulfur (S_8). TGA results show that the SNPs are thermally stable under storage facilities. The SEM and TEM images verify the particle size obtained from the XRD results, while the EDX results of SNPs confirm the existence of sulfur. According to the bioassay results, *S. oryzae* exhibited a repellency of 100% when exposed to doses of 0.0625, 0.125, 0.25, and 0.5 g kg^{-1} and *T. castaneum* demonstrated a repellency rate of 100% at the doses of 0.125, 0.25, and 0.5 g kg^{-1} within 24 hours. Also, 100% mortalities of *S. oryzae* at 1.5 g kg^{-1} dosages within 7 days and *T. castaneum* at 1.5 g kg^{-1} dosages within 6 days were shown. The results of the study indicated that using green synthesized sulfur nanoparticles will be a viable way to control insect pests in grain storage systems.

Keywords: Green-synthesized sulfur nanoparticles, *Olax zeylanica*, *Sitophilus oryzae, Tribolium castaneum*.

wathsala@sjp.ac.lk.

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G. D. V. A. Sandeepani [1], A.G.W.U. Perera [2*], and S.D.M. Chinthaka [1]

[1] Department of Chemistry, University of Sri Jayewardenepura, Sri Lanka
[2] Department of Zoology, University of Sri Jayewardenepura, Sri Lanka

INTRODUCTION

Rice is the most commonly consumed cereal among grains (Chaudhar et al.,2018). Nevertheless, rice is susceptible to infestation by insect pests at every stage of its growth, from cultivation to processing and consumption (Kumar and Kalita, 2017). Rice weevils (*Sitophilus oryzae*) and red flour beetles (*Tribolium castaneum*) are major pests that infest grains leading to significant damage during storage (Padmasri et al., 2018) which thus has encouraged the use of insecticides. Unfortunately, the most commonly used conventional insecticides are harmful chemicals, which can harm non-target organisms, making it crucial to explore alternative pest control strategies (Sabry et al., 2021; Rahman et al., 2021) to improve the overall sustainability of the food systems. In view of this background, nanopesticides may offer a promising alternative to the use of conventional insecticides.

Generally, nNanomaterials range from 1 to 100 nanometers in size (Rahman et al., 2021; Stadler et al., 2018). Nanotechnology shows promise in pest management strategies for agriculture, utilizing the unique properties of nanomaterials, such as their high surface area-to-volume ratio and reactivity. Though they have potential benefits, their associated risks cannot be overlooked. Conventional synthesis methods pose challenges such as scalability issues, hazardous chemicals, high energy requirements, and low material conversion rates (Araj et al., 2015). To mitigate these concerns, researchers have developed green synthesis methods using plant extracts to create nanoparticles. This method is an environmentally safe method that can be performed at room temperature without the need for sophisticated instruments. Also, this method is cost-effective, sustainable, and simple (Rahman et al., 2021). Additionally, nanoparticles can penetrate the cell membranes of insects, making them effective at harming pests. These particles induce harm to insects by being absorbed into the lipids of their cuticle, which serves as a defense for their water barrier. Consequently, this results in water loss and dehydration, ultimately causing insect death solely due to physical harm. As a result, nanoparticles are being used to develop new pesticides, insecticides, and insect repellents (Alisha and Thangapandiyan, 2019).

The present study aims to evaluate the insecticidal efficacy of sulfur nanoparticles (SNPs) synthesized via *Olax zeylanica* leaf extract against *Sitophilus oryzae* and *Tribolium castaneum* adults.

METHODOLOGY

Preparation of leaf extract

The *Olax zeylanica* leaf extract was used as the plant material to prepare SNPs. The leaves were washed to remove unwanted substances. Afterwards, the leaves were air-dried to remove surface moisture before being finely cut into pieces. Next, 20 g of the cut leaves were refluxed in 100 ml of distilled water at 100 ˚C for 3 hours. The boiled sample was then filtered and the resulting filtrate solution was collected as the leaf extract.

Synthesis of SNPs

To prepare the required solutions, sodium thiosulfate pentahydrate (0.78 M) and 20% citric acid were prepared according to Tripathi et al. (2018). Here, 20 ml of *Olax zeylanica* leaf extract was added to 50 ml of the prepared sodium thiosulfate pentahydrate solution and stirred for 2 minutes. Then, 5 ml of 20% citric acid was added dropwise at 5-second intervals with continuous stirring to obtain sulfur nanoparticles. The resultant mixture was then left to stir for 1 hour, and precipitation was observed. The resulting precipitates were collected via centrifugation at 8000 rpm for 10 minutes, washed with distilled water, oven-dried, and then kept at 4 ° C until use in further experimentation.

Characterization of SNPs

To confirm the formation of SNPs, UV-Vis spectroscopy was employed using a UV-1900I instrument (Shimadzu, Japan). Powder X-ray Diffraction (XRD) patterns were used to analyze the crystal structure, crystal planes, and crystalline size of the SNPs, utilizing CuKα radiation (λ= 1.54060 Å). The analysis was performed in the region of 2θ from 10 θ - 80 θ. The average particle size of the SNPs was calculated using the Debye-Scherrer formula (Tripathi et al., 2018). Fourier transform infrared (FTIR, Thermo SCIENTIFIC Nicolet iS10 SMART OMNI-TRANSMISSION) spectra were also recorded using the KBr-FTIR method to analyse the biomolecules present in the sample, as previously described by Shankar et al. (2018). The thermal stability of the prepared SNPs was evaluated using a thermogravimetric analyzer (TGA, SDT 650, Discovery, USA) under a nitrogen atmosphere (Hencz et al., 2017). The size and morphology of the green-synthesized SNPs were investigated using scanning electron microscopy (SEM, Zeiss) and transmission electron microscopy (TEM). To identify the elemental composition of the prepared SNPs, Energy Dispersive X-ray Spectroscopy (EDX) was employed.

Insect rearing

The starter adult colonies of *S. oryzae,* and *T. castaneum* were collected from heavily infested stocks of rice purchased from the local market. The insects were morphologically identified. The populations of test insects were reared for several generations over 5 years on whole, non-infested, pesticide-free rice grains in the insectary of the Insect Pest Management Unit, Department of Zoology, University of Sri Jayewardenepura, Sri Lanka in which the insect cultures were maintained under the ambient conditions of 29 ± 2℃ and 84 ± 10% R.H. with a 12 h: 12 h (Light:Dark) photoperiod. The coetaneous mixed-sex cohorts of adult insects used in all experiments were 7 d old *S. oryzae,* and *T. castaneum*.

Repellency test

The repellency effect of SNPs was evaluated using the cup bioassay, with some modifications from the method of Mohan and Fields (2002). In brief, the SNPs were mixed with 20 g of rice seeds at various dosages (0.0313 g kg^{-1}, 0.0625 g kg^{-1}, 0.125 g kg^{-1}, 0.25 g kg^{-1}, and 0.5 g kg^{-1}) in plastic cups. The upper part of the plastic cup was punctured to allow the insects to escape, and 20 individuals of adult *S. oryzae* and *T. castaneum* were separately introduced into each cup. After 5 minutes, a tape covering the holes of a cup, which contained treated grains, was removed. The cup was then placed in a glass beaker, and a muslin cloth was tightly secured around the cup's opening to prevent insect escape. A control test using untreated grains was also conducted. After 6 hours up to 24 hours, the number of insects in the glass beaker was counted as the repelled count. Five replicates were performed for each treatment and control (Perera and Karunaratne, 2016).

Mortality test

The bioassay was carried out in plastic jars that contained 20 g of rice seeds which were treated with green synthesized nanoparticles at six different dosages as 0.0625 g kg^{-1}, 0.125 g kg^{-1}, 0.25 g kg^{-1}, 0.5 g kg^{-1}, 1.0 g kg^{-1}, and 1.5 g kg^{-1} and then shaken manually to mix the particles with grains uniformly (Debnath et al., 2011). Subsequently, 20 individuals of each test insect were released in jars covered with a muslin cloth to allow ventilation. Mortality counts were recorded every 24 hours for seven days. The experiment was conducted in triplicates for each treatment and control.

RESULTS AND DISCUSSION

Characterization of SNPs

Green synthesized nanoparticles were subjected to UV-Vis spectroscopy and the absorbance was taken from 250 nm to 400 nm. The peak around 280 nm indicated the nanometer size of the particle (Suresh et al., 2015). The diffraction peaks observed from XRD are in excellent agreement with orthorhombic sulfur (JCPDS 01-089-2600). According to the Debye-Scherrer formula, the average particle size was determined to be 75.2 nm. The results obtained from FTIR analysis revealed peak positions that correspond to sulfur. According to the results of TGA, SNPs have undergone decomposition at a temperature of 293 ˚ C, suggesting that they can be effectively utilized under storage conditions for insect control. This is advantageous as the particles retain their properties regardless of temperature fluctuations. The SEM and TEM images of SNPs confirmed their nanoscale sizes obtained by XRD analysis. The EDX of SNPs further confirmed the presence of sulfur with lesser impurities.

Bioefficacy of SNPs

Based on the results, *S. oryzae* exhibited a repellency of 100% when exposed to doses of 0.0625, 0.125, 0.25, and 0.5 g kg^{-1} within 24 hours. On the other hand, *T. castaneum* demonstrated a repellency rate of 100% at doses of 0.125, 0.25, and 0.5 g kg^{-1} within 24 hours. These findings suggest that the green-synthesized sulfur nanoparticles have a stronger repellent effect on *S. oryzae* compared to their impact on *T. castaneum.* When considering the mortality test, 1.5 g kg^{-1} dosage of SNPs resulted in a 100% mortality rate for *S. oryzae* within 7 days, and for *T. castaneum* within 6 days. The mortality percentage of both insects, demonstrates the successful utilization of the 1.5 g kg^{-1} dose of these nanoparticles for controlling both insect species, better than all other doses. Thus, the present study indicates that using green synthesized sulfur nanoparticles will be a viable way to control insect pests within grain storage systems.

CONCLUSIONS/RECOMMENDATIONS

SNPs synthesized using *Olax zeylanica* leaf extract have demonstrated successful synthesis and high efficacy in controlling *S. oryzae* and *T. castaneum*. These SNPs can serve as eco-friendly insecticidal alternatives for protecting stored grain ecosystems from insect pest infestations.

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ACKNOWLEDGMENTS

I would like to acknowledge the University of Sri Jayewardenepura, grant number, ASP/01/RE/SCI/2022/19 for the financial support.