Synthesis of Nano Sulfur/Chitosan-Copper Complex and Its Nematicidal Effect against *Meloidogyne incognita* In Vitro and on Coffee Pots

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Sulfur is one of the inorganic elements used by plants to develop and produce phytoalexin to resist certain diseases. This study reported a method for preparing a material for plant disease resistance. Sulfur nanoparticles (SNPs) stabilized in the chitosan-Cu²⁺ (CS-Cu²⁺) complex were synthesized by hydrolysis of Na₂S₂O₃ in an acidic medium. The obtained SNPs/CS-Cu²⁺ complex consisting of 0.32% S, 4% CS, and 0.7% Cu (w/v), contained SNPs with an average size of ~28 nm as measured by transmission electron microscopy images. The X-ray diffraction pattern of the SNPs/CS-Cu²⁺ complex showed that SNPs had orthorhombic crystal structures. Interaction between SNPs and the CS-Cu²⁺ complex was also investigated by ultraviolet-visible. Results *in vitro* nematicidal effect of materials against *Meloidogyne incognita* showed that SNPs/CS-Cu²⁺ complex was more effective in killing second-stage juveniles (J2) nematodes and inhibiting egg hatching than that of CS and CS-Cu²⁺ complex. The values of LC₅₀ in killing J2 nematodes and EC₅₀ in inhibiting egg hatching of SNPs/CS-Cu²⁺ complex were 75 and 51 mg/l, respectively. These values were lower than those of CS and the CS-Cu²⁺ complex. The test results on the nematicidal effect against *M. incognita* on coffee pots showed that the SNPs/CS-Cu²⁺ complex was 100% effective at a concentration of 150 mg/l. Therefore, the SNPs/CS-Cu²⁺ complex could be considered as a biochemical material with potential for agricultural applications to control root-knot nematodes.

**Keywords**: chitosan, chitosan-copper complex, *Meloidogyne incognita*, nano sulfur, nematodes

Today, nanotechnology has been applied in many fields including medicine, energy, electronics, and agriculture (Wypij et al., 2023). Nanomaterials, particularly nanocomposites have been used in agriculture as a plant protection agent because of their antibacterial, antifungal, and antiviral properties (Elbeshey et al., 2015; Krishnaraj et al., 2012; Mishra et al., 2018; Pasha et al., 2022; Wong and Liu, 2010), and/or nano-nutrient fertilizer properties (Du et al., 2019; Tomadoni et al., 2019). Nanoparticles capable of inhibiting plant diseases are metal, metal oxide, metallicloid, and carbon nanoparticles (Elmer et al., 2018). Nanomaterials have the ability to resist a variety of plant pathogens such as nano SiO₂/oligochitosan, nano Cu₄O-Cu/alginic resistant to *Neoscytalidium dimidiatum* (Du et al., 2019; Tuan et al., 2019), nano SiO₂ resistant to *Rhizoctonia solani* (Abdelrhim et al., 2021), nano Ag resistant to *Fu-
sarium oxysporum (Kaman and Dutta, 2019), nano MgO, nano Cu$_2$O–Cu, nano ZnO, MnO, resistant to Xanthomonas oryzae pv. oryzae (Abdallah et al., 2019; Ngoc et al., 2021; Ogunyemi et al., 2020), and nano Ag resistant to yellow bean mosaic virus (Elbeshley et al., 2015).

In addition to harmful microorganisms, plants are also harmed by root-knot nematodes (Meloidogyne spp.) which are parasitic on plants such as coffee, tomatoes, peppers, carrots, potatoes, eggplants, watermelons, cucumbers, etc. (El-Ashy et al., 2022; Fan et al., 2020; Hooper, 1990; Jiang et al., 2018). Nematode diseases can reduce the yield of agricultural products by 15-25%, or up to 75% in some cases (Jiang et al., 2018). Metal, metal oxide, and metalloid nanoparticles such as nano Ag (Heflish et al., 2021), nano Cu/Fe (Gkanatsiou et al., 2019), nano SiO$_2$, nano ZnO (Khalil et al., 2018), nano CuO (Khan et al., 2022a), nano TiO$_2$ (Khan et al., 2022b), and Se (Udalova et al., 2018) have the ability to kill root-knot nematodes and inhibit egg hatching. To the best of our knowledge, sulfur nanoparticles (SNPs) have only been reported to be effective against the *M. incognita* by Al Banna et al. (2020). The use of SNPs in plant disease control is encouraged because they are non-toxic, the most abundant nonmetal on earth, and a by-product of the petroleum industry (Saedi et al., 2020; Yuan et al., 2021).

The SNPs/chitosan-Cu$^{2+}$ (SNPs/CS-Cu$^{2+}$) complex was made up of single materials including SNPs, CS, and Cu$^{2+}$, all of which are resistant to root-knot nematodes. Sulfur is an element with a high fungicidal activity that has been used in the treatment of cancer cells and plant diseases from ancient times (Shankar et al., 2018).

CS prepared from shrimp shells is a non-toxic biopolymer with anticancer (Kuppusamy and Karuppaiyah, 2012), antibacterial (Benhabiles et al., 2012; Li and Zhuang, 2020), antifungal (Meng et al., 2020; Qiu et al., 2014), and nematicidal properties (Khalil and Badawy, 2012; Makhayeva et al., 2020). Therefore, CS has the potential to be used as a plant-disease control agent. CS contains functional groups –OH and –NH$_2$ that can be complex with Zn$^{2+}$, Cu$^{2+}$, and Fe$^{2+}$ (Choudhary et al., 2017; Wang et al., 2005). The CS-Cu$^{2+}$ complex releases Cu$^{2+}$ ions in a controlled manner, thus reducing the ion’s toxicity (Akhtar et al., 2020).

Copper salt is also a poison to root-knot nematodes (Kim et al., 2022), but the effective dose of copper sulfate in the field is rather high, ranging from 500 to 750 kg/ha (Korthals et al., 1996). When copper salts were used in combination with organic acid, they showed a synergistic effect against root-knot nematodes (Kim et al., 2022).

Currently, several methods for synthesizing SNPs have been investigated including the acidification of sodium thiosulphate (Khairan et al., 2019; Rao and Paria, 2013) or polysulfide (Guo et al., 2006; Paralikar and Rai, 2018) in the presence of protective agents, ultrasonic treatment (Turganbay et al., 2013), mechanical dispersion (Rao and Paria, 2013), and sublimation (Xie et al., 2012) of sulfur powder. In this report, SNPs were synthesized by hydrolysis of sodium thiosulfate in lactic acid and stabilized in the CS-Cu$^{2+}$ complex solution. The obtained SNPs/CS-Cu$^{2+}$ complex has been studied for their characteristic properties and resistance ability to *M. incognita in vitro* on petri dishes and *in vivo* on coffee which is a crop commonly infected with fungal and nematode diseases, and has a large cultivation area in Vietnam (Clément et al., 2023), orienting to use as an agent against root-knot nematodes in agriculture.

**Materials and Methods**

Chemicals used in the experiment were of analytical grade, including Na$_2$S$_2$O$_3$·5H$_2$O 99%, Cu(NO$_3$)$_2$·3H$_2$O 99%, C$_2$H$_5$OH 99.7% (Xilong, Shantou, China), CS with molecular weight (Mw) ~90,000 g/mol and deacetylation degree (DD) ~92.6 (Suntze Chemical Co., Ltd, Can Tho, Vietnam), lactic acid 99% (Sigma-Aldrich, Darmstadt, Germany), and deionized water. The robusta coffee seeds are provided by EA KMAT Tay Nguyen Breeding Center, Vietnam.

**Preparation of solutions of CS, CS-Cu$^{2+}$ complex, and SNPs/CS-Cu$^{2+}$ complex.** Four grams CS was dissolved in 100 ml of lactic acid 4% to obtain CS solution 4% (w/v). CS-Cu$^{2+}$ complex solution was prepared with the –NH$_2$/Cu$^{2+}$ molar ratio of 2/1. The number of moles of the –NH$_2$ group in CS was calculated by the formula (1): $N_{NH_2} = m/MM$ (1), where m (g) is the amount of CS, MM (g/mol) is the average Mw of the monomer in CS, which was calculated by the formula (2): $MM = DD-MM_\text{glu} + (1 – DD)\cdot MM_{acetylglu}$ (2) (Gritsch et al., 2018). Based on the above calculation, the number of moles of –NH$_2$ and Cu$^{2+}$ were 0.022 and 0.011 mol, respectively. Thus, CS-Cu$^{2+}$ complex was prepared by dissolving 2.66 g of Cu(NO$_3$)$_2$·3H$_2$O in 100 ml of CS 4% (w/v), the obtained solution containing 4% CS and 0.7% Cu (w/v), equivalent to the –NH$_2$/Cu$^{2+}$ molar ratio of 2/1. SNPs/CS-Cu$^{2+}$ complex solution was synthesized by dissolving 2.66 g of Cu(NO$_3$)$_2$·3H$_2$O in 80 ml of CS 5% solution (w/v). Then dissolved 2.48 g of Na$_2$S$_2$O$_3$·5H$_2$O in 20 ml of deionized water, adding Na$_2$CO$_3$ solution drop by drop into the CS-Cu$^{2+}$ complex solution, stirring with a
magnetic stirrer until the Na$_2$S$_2$O$_3$ solution was completely added to obtain a solution containing 0.32% S, 4% CS, and 0.7% Cu (w/v).

**Characterization of materials.** SNPs sizes were determined by transmission electron microscopy (TEM) images on a TEM 1010 (JEOL, Tokyo, Japan). The particle size distribution was determined through dynamic light scattering (DLS) spectrum on a SZ-100 Horiba (Kyoto, Japan). The optical properties of the material were determined by ultraviolet-visible (UV-Vis) spectroscopy on a UV-Vis Jasco V630 (Tokyo, Japan). The powder samples of CS-Cu$^{2+}$ complex and SNPs/CS-Cu$^{2+}$ complex solutions were prepared by precipitation in C$_2$H$_5$OH 99.7% with the C$_2$H$_5$OH:solution sample volume ratio of 2:1. This mixture was filtered and dried at 60°C for X-ray diffraction (XRD) and Fourier transform infrared (FTIR) measurement. The crystal structure of the materials was determined by XRD pattern on an XRD D8 Advance (Bruker, Karlsruhe, Germany). The machine used Cu kα ($\lambda = 1.5406$ Å) radiation at 40 kV and 40 mA, 20 diffraction angle from 5° to 80°.

**Isolation and identification of the M. incognita.** The *M. incognita* was isolated following the method of Khan et al. (2022a). *M. incognita* was cultured on brinjal in a greenhouse, then separated and collected nematode eggs on egg-plant roots. These eggs were washed with distilled water and placed in a petri dish containing distilled water, which was lined with filter paper and a mesh of 25 μm pore size placed above the filter paper. The petri dishes were kept in the incubator. When the nematode eggs hatched, J2 nematodes passed through the strainer and sank to the bottom of the petri dish, while unhatched eggs were left on the sieve. J2 and eggs of nematodes were cultured for 5 days before being used for experiments. We used a Barska AY13180 20×, 50× stereo microscope of Barska (Pomona, CA, USA) to identify *M. incognita* species by the morphological features of adult females.

**In vitro inhibitory efficacy on M. incognita.** The experiments were carried out according to the method of Khan et al. (2022a).

**Mortality bioassay.** We added 1 ml of distilled water containing ~100 freshly hatched J2 nematodes into a petri dish, each containing 9 ml of the materials at a different concentration, with distilled water as the control sample. These Petri dishes were incubated at 28°C for 48 h. A stereo microscope was used to count the number of J2 nematodes alive which had movement and winding shape, nematodes are considered dead when not moving and straightening. We kept track of how many J2 nematodes were alive while repeating the experiment 5 times. Percent mortality (PM) of J2 nematodes was calculated using formula (3): PM (%) = (C$_0$ – T)/C$_0$ × 100 (3), where C$_0$ is the initial number of J2 nematodes (~100 J2 nematodes), and T$_0$ is the number of J2 nematodes alive.

**Hatching bioassay.** We selected six healthy egg masses and placed them in a petri dish, each containing 10 ml of the materials at a different concentration, with distilled water as the control sample. These Petri dishes were incubated at 28°C for 6 days. We used a stereo microscope to count the number of freshly hatched J2 nematodes while repeating the experiment 5 times. Percent inhibition (PI) of egg hatching was calculated using formula (4): PI (%) = (C – T)/C × 100 (4), where C is the number of freshly hatched J2 nematodes in the control sample, and T is the number of freshly hatched J2 nematodes in the material sample.

**In vivo inhibitory efficacy on M. incognita.** The experiment was carried out following the method of El-Ashry et al. (2022) and Özdemir et al. (2022) with several modifications. Robusta coffee seeds (*Coffeea canephora*) were sterilized by soaking it in NaClO solution for 20 min and washed with running tap water 3 times. Sterilized seeds of coffee were sown in pots (10 cm diameter × 20 cm height) that contained 2 kg of autoclaved soil and farmyard manure mixture in a 3:1 ratio. When the coffee plants had four pairs of leaves (5 months old), each coffee pot was inoculated with 10 ml of suspension containing 2,000 J2 into three holes around the root. After 2 days of inoculation, 30 ml of materials at different concentrations (100, 125, and 150 mg/l) were added into the soil around the root. The negative control was without nematodes whereas the positive control was inoculated with nematodes and treated with distilled water. These pots were placed in the greenhouse at 28-35°C and 70-80% humidity. After 60 days, roots and 100 g soil around the roots were collected to determine the number of nematodes using the Baermann funnel method (Hooper, 1990), the number of galls on roots, and egg masses on roots were counted as the mentioned above in vitro experiment and calculated by the formula (5): Reduction (%) = (C – T)/C × 100 (5), where C is the control sample and T is the treated sample. The in vivo experiment was carried out from May to November 2023.

**Statistical analysis.** All data were statistically processed...
using the IRRISTAT 5.0 software and presented as mean ± standard error. The means were compared using the least significant difference at 0.05 probability level (P ≤ 0.05). The regression equation demonstrated the dependence of PM, PI (y) on the concentration of the materials (x), from which the LC$_{50}$ and EC$_{50}$ values can be determined by replacing $y = 50$ and calculating $x$.

**Results**

**Synthesis and properties of materials.** The results of characterizing properties of the materials are shown in Figs. 1 and 2. The UV-Vis spectra of CS, the CS-Cu$^{2+}$ complex, and the SNPs/CS-Cu$^{2+}$ complex in Fig. 1A displayed a characteristic peak at 235 nm (Fig. 1Aa), 243 nm (Fig. 1Ab), and 245 nm (Fig. 1Ac), respectively. In addition, the UV-Vis spectrum of the CS-Cu$^{2+}$ complex and the SNPs/CS-Cu$^{2+}$ complex also appeared a new peak at 680 nm (Fig. 1Ab and 1Ac). Furthermore, the UV-Vis spectrum of the SNPs/CS-Cu$^{2+}$ complex (Fig. 1Ac) revealed a new peak at 270 nm assigned to SNPs.

Photographs of the material samples are presented in Fig. 1B. The solution of CS 4% was light yellow. The CS-Cu$^{2+}$ complex solution with the molar ratio of –NH$_2$/Cu$^{2+}$ of 2/1 had a green color, while the SNPs/CS-Cu$^{2+}$ complex solution was an opaque green colloidal solution.

The XRD patterns of powder samples of CS, the CS-Cu$^{2+}$ complex, and the SNPs/CS-Cu$^{2+}$ complex are presented in Fig. 1C. The XRD pattern of CS (Fig. 1Ca) showed two diffraction peaks at $2\theta \sim 10.18^\circ$ and $20.02^\circ$. The diffraction peak at $2\theta \sim 10.18^\circ$ disappeared and the peak at $2\theta \sim 20.02^\circ$ significantly reduced signal intensity (Fig. 1Cb). The dif-

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**Fig. 1.** The UV-Vis spectra (A), photographs (B), and XRD patterns (C) of CS (a), the CS-Cu$^{2+}$ complex (b) and the SNPs/CS-Cu$^{2+}$ complex (c). UV-Vis, ultraviolet-visible; XRD, X-ray diffraction; CS, chitosan; SNP, sulfur nanoparticle.
fraction curve of the SNPs/CS-Cu\textsuperscript{2+} complex in Fig. 1Cc appeared relatively many peaks, including the peaks at 20 \textapprox 22.01°, 23.14°, 27.76°, 31.45°, 42.81°, and 47.84°.

The TEM images in Fig. 2A showed that the SNPs stabilized in the CS-Cu\textsuperscript{2+} complex had an angular shape. The average size of SNPs measured through the TEM images was \~28 nm, and the particle size distribution was in the 16-35 nm range, as shown in the DLS spectrum (Fig. 2B).

Stereomicroscopic images of adult \textit{M. incognita} isolated from brinjal roots are shown in Fig. 3.

The effectiveness of SNPs/CS-Cu\textsuperscript{2+} complex against \textit{M. incognita} is presented in Table 1. The results in Table 1 showed that CS was effective against \textit{M. incognita} at relatively high concentrations. CS at a concentration of 550 and 700 mg/l completely inhibited egg hatching and killed J2 nematodes, respectively. The results in Table 1 also indicated that the nematicidal effect of materials was as the following order: SNPs/CS-Cu\textsuperscript{2+} complex > CS-Cu\textsuperscript{2+} complex > CS.

To calculate the LC\textsubscript{50} and EC\textsubscript{50} values, the results in Table 1 were converted to the chart in Figs. 4 and 5, respectively. The LC\textsubscript{50} values of CS, CS-Cu\textsuperscript{2+} complex, and SNPs/CS-Cu\textsuperscript{2+} complex were 251, 88, and 75 mg/l, respectively (Fig. 4). The EC\textsubscript{50} values of CS, CS-Cu\textsuperscript{2+} complex and SNPs/CS-Cu\textsuperscript{2+} complex were 119, 68 and 51 mg/l, respectively (Fig. 5).

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**Fig. 2.** Transmission electron microscopy images (A) and dynamic light scattering spectrum (B) of the SNPs/CS-Cu\textsuperscript{2+} complex. SNP, sulfur nanoparticle; CS, chitosan.

**Fig. 3.** Root-knot \textit{Meloidogyne incognita}: female (A), vulva and anus (B), egg masses (C), J2 isolated from brinjal root (D), and J2 dead after treated with materials (E).
Based on results from in vitro experiments, it could be seen that CS was effective against *M. incognita* only at high concentrations. Therefore, in the greenhouse experiment, we only investigated the effectiveness of the CS-Cu$^{2+}$ complex and the SNPs/CS-Cu$^{2+}$ complex against *M. incognita* on coffee pots at concentrations of 100, 125, and 150 mg/l.

The results in vivo test on the nematicidal effect of the CS-Cu$^{2+}$ complex and the SNPs/CS-Cu$^{2+}$ complex on coffee pots are presented in Table 2. The results in Table 2

![Graph](image)

**Fig. 4.** Effect of concentrations of materials on mortality of root-knot *Meloidogyne incognita*. PM, percent mortality; CS, chitosan; SNP, sulfur nanoparticle.
showed that the negative control treatment was not infected with nematodes and the nematicidal effect of materials increased with the increase of concentration used. The nematicidal effect against *M. incognita* of SNPs/CS-Cu$^{2+}$ complex reached 100% at a concentration of 150 mg/l. Notably, the treatment using the SNPs/CS-Cu$^{2+}$ complex at a concentration of 125 mg/l was more effective in controlling the number of nematodes in soil, galls, and egg masses in coffee root than using CS-Cu$^{2+}$ complex at a concentration of 150 mg/l.

**Discussion**

The UV-Vis of CS showed the peak at 235 nm represented the π-π$^*$ transition of the amino group (Bukola et al., 2023; Hao et al., 2021), this peak shifted to 243 nm for the CS-Cu$^{2+}$ complex. In addition, the UV-Vis spectrum of CS-Cu$^{2+}$ complex also appeared a new peak at 680 nm due to the d-d electron transition between the NH$_2$ group and Cu$^{2+}$ ion (Rhazi et al., 2002). Previous studies found that SNPs exhibited a maximum absorption peak in the ranges 200-280 nm (Tripathi et al., 2018) up to 292-296 nm (Paralikar and Rai, 2018). This absorption peak was in the 400-800 nm range, depending on the pH value, the ratio of Cu$^{2+}$/NH$_2$ and the nature of coordinated groups around the metal ions. Therefore, the peak at 270 nm in Fig. 1Ac was assigned to SNPs. This result was also consistent with the report of Anbinder et al. (2019).

The XRD pattern in Fig. 1Ca showed two diffraction peaks at 2θ ~10.18° and 20.02° that were characteristic of...
CS (Duy et al., 2011; Wang et al., 2004). For the CS-Cu\(^{2+}\) complex (Fig. 1Cb), the peak at 20 ~10.18° has disappeared and the peak at 20 ~20.02° significantly reduced signal intensity. Thus, the complexation between Cu\(^{2+}\) ions with amide and hydroxyl groups of CS reduced the crystalline properties of CS (Akhtar et al., 2020; Elmezyayen and Reicha, 2015). The characteristic peaks at 20 ~22.01°, 23.14°, 27.76°, 31.45°, 42.81°, and 47.84° in Fig. 1Cc corresponded to crystal planes (220), (222), (313), (044), (319), and (515), respectively. These peaks are assigned to S elemental according to the data of JCPDS No. 08247. This result was also consistent with other authors’ prior findings when studying the XRD spectrum of SNPs prepared by hydrolysis of Na\(\text{S}_2\text{O}_3\) in an acidic medium (Paralikar and Shankar et al., 2018). Notably, the concentrations of the CS-Cu\(^{2+}\) complex were relatively small, so using the SNPs/CS-Cu\(^{2+}\) in an acidic medium (Paralikar and Shankar et al., 2018) had a significant effect against M. incognita. The obtained results values of the SNPs/CS-Cu\(^{2+}\) complex over CS was 88 and 68 mg/l, respectively, lower than that of the CS-Cu\(^{2+}\) complex. Notably, the concentrations of the SNPs/CS-Cu\(^{2+}\) complex that reached 100% efficacy in killing J2 nematodes and inhibiting egg hatching were 125 and 100 mg/l (Table 1), respectively.

The key advantage of the CS-Cu\(^{2+}\) complex was its significantly reduced signal intensity. Thus, the complexation between Cu\(^{2+}\) ions with amide and hydroxyl groups of CS reduced the crystalline properties of CS (Akhtar et al., 2020; Elmezyayen and Reicha, 2015). The characteristic peaks at 20 ~22.01°, 23.14°, 27.76°, 31.45°, 42.81°, and 47.84° in Fig. 1Cc corresponded to crystal planes (220), (222), (313), (044), (319), and (515), respectively. These peaks are assigned to S elemental according to the data of JCPDS No. 08247. This result was also consistent with other authors’ prior findings when studying the XRD spectrum of SNPs prepared by hydrolysis of Na\(\text{S}_2\text{O}_3\) in an acidic medium (Paralikar and Shankar et al., 2018).

The TEM images in Fig. 2A showed that the SNPs have an angular shape with a narrow bell-shaped size distribution (Fig. 2B). This result also shows that the size of SNPs stabilized in the CS-Cu\(^{2+}\) complex was relatively small compared to that stabilized in PEG-200 (Xie et al., 2012).

The above research results demonstrated the successful preparation of a compound containing nano-sized sulfur stabilized in the CS-Cu\(^{2+}\) complex. Its characteristic properties were determined and compared with CS, and CS-Cu\(^{2+}\) complex. These materials were used to conduct in vitro testing of efficacy against M. incognita. Photographs of M. incognita in Fig. 3 showed the female M. incognita having a pear shape with an average length of about 670 µm. The perineal was characterized by a folded dorsal arch and smooth to slightly wavy margins. The ridges only form in the area from the back to the tail, no horizontal ridges appear between the vulva and anus. The above morphological features were typical for M. incognita (Jepson, 1987; Khan et al., 2022a).

For the in vitro effect of CS against nematodes, according to the results of Khalil and Badawy (2012), CS with low Mw \((2.27 \times 10^3 \text{ g/mol})\) and high DD (~89%) had a high effect against nematodes. In addition, Fan et al. (2020) reported that CS oligomer with Mw ~1,500 Da had an LC\(_{50}\) value against J2 nematodes of about 6,510 mg/l, which was significantly higher than the LC\(_{50}\) value (251 mg/l) in this study (Fig. 4). Thus, it can be deduced that CS had a greater effect against M. incognita than that of CS oligomer.

The key advantage of the CS-Cu\(^{2+}\) complex over CS was that the complexation between CS and Cu\(^{2+}\) had a high effect against M. incognita. Particularly, the results in Figs. 4 and 5 showed that LC\(_{50}\) and EC\(_{50}\) values of CS-Cu\(^{2+}\) complex was 88 and 68 mg/l, respectively, lower than that of CS (LC\(_{50}\): 251 mg/l and EC\(_{50}\): 119 mg/l). It is worth noting that the concentration of CS-Cu\(^{2+}\) complex that reached 100% efficacy in killing J2 nematodes and inhibiting egg hatching was 150 mg/l.

For the SNPs/CS-Cu\(^{2+}\) complex, the in vitro effect against M. incognita of all treatments using the same concentration as the CS-Cu\(^{2+}\) complex was statistically significantly higher than that of the CS-Cu\(^{2+}\) complex. Especially, the LC\(_{50}\) and EC\(_{50}\) values of the SNPs/CS-Cu\(^{2+}\) complex was 75 and 51 mg/l (Figs. 4 and 5), respectively, lower than that of the CS-Cu\(^{2+}\) complex. Notably, the concentrations of the SNPs/CS-Cu\(^{2+}\) complex that reached 100% efficacy in killing J2 nematodes and inhibiting egg hatching were 125 and 100 mg/l (Table 1), respectively.

The research results of Al Banna et al. (2020) using SNPs with size of 40 nm against M. javanica showed that the inhibitory effect on egg hatching reached 100% at a concentration of 30 ppm. In this study, the treatments using the SNPs/CS-Cu\(^{2+}\) complex at concentrations from 100-125 mg/l containing 6.4-8.0 mg/l S achieved 100% efficacy in killing J2 nematodes and inhibiting egg hatching. Therefore, the combination of SNPs with the CS-Cu\(^{2+}\) complex reduced the S concentration while still achieving effective resistance against root-knot nematodes.

In the in vivo experiment, the SNPs/CS-Cu\(^{2+}\) complex showed significantly higher effectiveness against M. incognita than the CS-Cu\(^{2+}\) complex. The obtained results might be due to the release of Cu\(^{2+}\) ions from the materials in the soil environment. According to Kumar et al. (2021), these ions are micronutrients that are directly consumed by plants. For the SNPs/CS-Cu\(^{2+}\) complex, plants can not directly consume sulfur element but consume oxidized sulfur that formed by a population of heterotrophic microorganisms capable of oxidizing sulfur into SO\(_{4}^{2-}\) (Germida and Janzen, 1993). The oxidation time of sulfur element by heterotrophic microorganisms takes place for quite a long time. The sulfur element was oxidized in the soil by 22.4% after 84 days at 30°C for sulfur concentration in soil of 0.2 g/kg (Zhi-Hui et al., 2010). Due to the slow oxidation of SNPs in soil, the nematicidal effect of the SNPs/CS-Cu\(^{2+}\) complex lasted in long-term, so it was more effective than that of the CS-Cu\(^{2+}\) complex after 60 days.

The above research results demonstrated the successful preparation of a compound containing nano-sized sulfur stabilized in the CS-Cu\(^{2+}\) complex. Its characteristic properties were determined and compared with CS, and CS-Cu\(^{2+}\) complex. These materials were used to conduct in vitro testing of efficacy against M. incognita. Photographs of M. incognita in Fig. 3 showed the female M. incognita having a pear shape with an average length of about 670 µm. The perineal was characterized by a folded dorsal arch and smooth to slightly wavy margins. The ridges only form in the area from the back to the tail, no horizontal ridges appear between the vulva and anus. The above morphological features were typical for M. incognita (Jepson, 1987; Khan et al., 2022a).
plants.

In conclusion, this study confirmed that the prepared SNPs/CS-Cu\textsuperscript{2+} complex was highly effective against nematodes (\textit{M. incognita}) due to the synergistic effect of components (CS, Cu\textsuperscript{2+}, and SNPs). Therefore, the SNPs/CS-Cu\textsuperscript{2+} complex has potential applications in plant disease control, particularly, in control of root-knot nematodes. In order to apply the SNPs/CS-Cu\textsuperscript{2+} complex against nematodes in practice, field experiments need to be further conducted.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

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**References**


Qiu, M., Wu, C., Ren, G., Liang, X., Wang, X. and Huang, J. 2014. Effect of chitosan and its derivatives as antifungal and preservative agents on postharvest green asparagus. Food...


