The enhanced antifungal effect against *Pyricularia oryzae* of Cu nanoparticles/chitosan supplemented with ammonium soap synthesised from palm oil

Tuan Nghiem Anh Le[1], Nghi Tran[2], Nhung Hong Nguyen[2], Tho Phuoc Tran[1], Giang Ngoc Doan[1], Ngoc Thanh Truong[1], Du Duy Bui[1*]

[1]*Institute of Advanced Technology, Vietnam Academy of Science and Technology, 1B TL29 Street, Thanh Loc Ward, District 12, Ho Chi Minh City, Vietnam*

[2]*PetroVietnam Fertilizer and Chemicals Corporation, 43 Mac Dinh Chi Street, Dakao Ward, District 1, Ho Chi Minh City, Vietnam*

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Abstract:

This study reports the preparation method of ammonium soap (AS) from palm oil and NH_4OH solution, in combination with chitosan-coated copper nanoparticles (CuNPs/CS), to enhance antifungal efficacy (AE) against *Pyricularia oryzae*, the causal agent of rice blast disease. The properties of AS, including the saponification reaction efficiency, pH, and its ability to reduce the surface tension of water, were also investigated. The CuNPs/CS nanocomposite was synthesised using N_2H_4 as a reducing agent for Cu^{2+} in solution. The resulting CuNPs exhibited a size of 17.9±3.4 nm, as determined by transmission electron microscopy (TEM) images. Ultraviolet-visible (UV-Vis), X-ray diffraction (XRD), and Fourier-transform infrared (FTIR) analyses confirmed the formation of CuNPs in the CS solution. The *in vitro* AE of CuNPs/CS against *in vitro* reached 100% at a Cu concentration of 30 mg/l. When 0.05% AS was added to the CuNPs/CS, the AE reached 100% at a Cu concentration of 25 mg/l. Notably, *in vivo* experiments demonstrated that spraying CuNPs/CS at a Cu concentration of 25 mg/l with 0.05% AS resulted in significantly higher rice blast control efficiency compared to spraying CuNPs/CS at 30 mg/l Cu concentration (86.91 vs 71.32%). AS supplementation was considered a promising approach to enhancing the activity of CuNPs/CS in controlling rice blast disease caused by *P. oryzae*.

Keywords: **ammonium soap, chitosan, copper nanoparticles, *Pyricularia oryzae*, rice blast disease.**

Classification numbers: **2.2, 3.1, 5.3**

1. Introduction

Surfactants are compounds that reduce the surface tension between two liquids or between a liquid and a solid [1]. In agriculture, surfactants are widely used to enhance the effectiveness of spray pesticides by improving adhesion and ensuring even spreading across surfaces [2, 3]. Additionally, some surfactants possess antibacterial, antifungal, and insect- or pest-repellent properties, such as ammonium soap (AS) [4, 5], saponin [6], *Citrullus lanatus* seed oil [7], sophorolipid, and rhamnolipid [8]. Therefore, the inclusion of AS can increase the biological efficacy of pesticides [5].

Soap is prepared through the hydrolysis of fats (triglycerides) or neutralisation of free fatty acids in oils using an alkaline solution, in a process known as saponification, which results in the formation of carboxylate salts of the original fatty acids [9, 10]. The bases commonly used for soap production include NaOH (solid soap) and KOH or NH_4OH (liquid soap) (Fig. 1) [9, 11]. Based on the raw materials used, soap is classified into two types: natural soap and synthetic soap. The production of natural soap often utilises vegetable oils or animal fats, thus avoiding the generation of harmful waste [12].

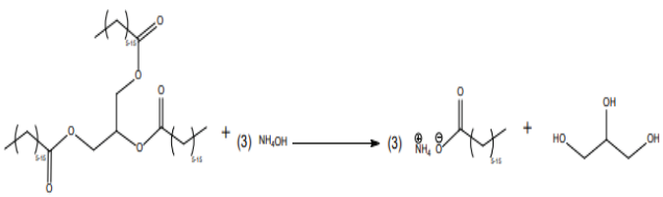

Fig. 1. The reaction between fat (triglyceride) and NH_4OH forming ammonium soap [13].

Palm oil is the most widely produced vegetable oil in the world and is cheaper than most other vegetable oils, making it a common ingredient in food, cosmetics, engine lubricants, and biofuels [14, 15]. Palm oil comprises 50% saturated fatty acids and 50% unsaturated fatty acids, with the main fatty acids being myristic, palmitic, stearic, oleic, and linoleic acids. These fatty acids are predominantly present in the form of triglycerides, 5-8% diglycerides, and a small amount of free fatty acids [16]. Yellow palm oil has a higher palmitic acid content and lower oleic acid content than red palm oil, making it suitable for soap and detergent production, whereas red palm oil is commonly used in food processing as a vegetable oil [17]. Ammonium soap can be prepared by reacting plant oil with an excess NH_4OH solution at

*Corresponding author: Email: vina9802@gmail.com

a high concentration (25%) [18]. Higher-order ammonium soaps are produced by reacting plant oil with alkanolamines (e.g., ethanolamine produces a quaternary ammonium soap) [19]. High solid-content ammonium soap is prepared by bubbling NH_3 gas into plant oil or by adding $(NH_4)_2CO_3$ to plant oil and then heating it to decompose the carbonate salt into NH_3 [19].

Ammonium soap serves as a surfactant with important roles in fields such as medicine and the chemical industry [20, 21]. In addition to its cleaning capabilities, AS has antibacterial and antifungal properties [22, 23]. The mechanism of pest control by AS has been studied by various authors [4, 5, 24-26]. AS penetrates the cell membranes of insects, causing cell damage and disrupting respiratory functions [5]. Furthermore, AS slowly releases NH_3, repelling larger animals such as deer and rabbits [4]. Soap and surfactants can kill pests and harmful insects, including members of the Hemiptera, Aleyrodidae, Aphidoidea, Coccidae, and Diaspididae families [24-26]. However, the drawback of using soap as a pesticide substitute in agriculture is the requirement for multiple applications at high concentrations, typically ten times higher than conventional pesticides (0.5-1%) [25]. For this reason, soap is often used as a co-adjuvant to enhance the efficacy of pesticides [27, 28]. The AS synthesised from palm oil and NH_4OH in this study is biodegradable, thus reducing its environmental impact compared to traditional alkali metal soaps. Consequently, one of the promising applications of AS is as a surfactant that enhances the efficacy of pesticides, allowing for lower dosages to be used.

One promising active ingredient for next-generation pesticides, due to its antibacterial and antifungal effects at low concentrations, is copper-based nanomaterials (CuO, Cu_2O, Cu) [29-31]. Copper is produced from inexpensive and widely available materials and is also a trace element essential for plant growth and development [32-34]. Thus, the use of copper nanoparticles (CuNPs) to control plant diseases leaves no harmful residues on agricultural products, as copper is absorbed by plants. Numerous studies have used CuNPs to control plant pathogens such as *Xanthomonas axonopodis*, *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium equiseti*, *Alternaria alternata*, *Curvularia lunata*, and *Phoma* destructiva [35-38]. The interaction between CuNPs and microorganism cells occurs due to copper's affinity for the carboxyl groups on the surface of the microorganism [39, 40]. Additionally, CuNPs stimulate the production of intracellular reactive oxygen species (ROS), which damage and alter the permeability of cell membranes, leading to enzyme inactivation and protein dysfunction in microorganisms, ultimately causing cell death [41, 42]. The antimicrobial activity of CuNPs depends on particle size and surface coating (stabiliser) [43, 44]. One commonly researched stabiliser for synthesising nanoparticles such as Ag, ZnO, CuCl, and Cu is chitosan (CS) [45-48]. Chitosan, a deacetylation product of chitin, is found in the shells of crustaceans (shrimp, crab, squid), fungi, and insects [49-51]. Chitosan is non-toxic, biocompatible, biodegradable, and has some special biological activities such as anticancer [52, 53], antibacterial [54, 55], and antifungal [56, 57]. In addition to its ability to kill microorganisms, CS is also considered a plant vaccine, helping plants enhance resistance and produce antibiotics when invaded by pathogens [58]. Additionally, CS is a highly flexible biopolymer due to its –OH and $–NH_2$ functional groups, which have unshared electron pairs that can form complexes with most metal ions such as Hg^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, etc. [36, 59] through electrostatic interactions with electron-rich metal nanoparticles and stabilising them [60]. In our opinion, the synthesis of CuNPs stabilised in CS creates a material capable of combining the antimicrobial properties of the metal phase and polymer.

Despite extensive studies on Cu-based nanomaterials against various plant pathogens [61, 62], there are still limited studies on their effectiveness against *P. oryzae*, which causes rice blast disease, a major threat to an essential food crop in Vietnam. This study reports the preparation of stable CuNPs in CS solution by reducing Cu^{2+} ($Cu(NO_3)_2$ salt) using the reducing agent N_2H_4. We investigated the antifungal activity of this composite against *P. oryzae*, the pathogen responsible for rice blast disease. Additionally, we explored the enhanced antifungal activity of CuNPs/CS when supplemented with AS synthesised from palm oil, with the aim of reducing the Cu concentration needed for disease control. This combination is expected to offer a safe and sustainable approach for managing plant diseases.

2. Materials and methods

2.1. Materials

The chemicals used in this experiment were of analytical grade and included lactic acid ($C_3H_6O_3$) 99%, hydrogen peroxide (H_2O_2) 30%, $Cu(NO_3)_2.3H_2O$, palm oil (Vietnam), ammonium hydroxide (NH_4OH) 25%, diethyl ether (Xilong, China), 0.5 M HCl standard solution (Samchun, Korea), hydrazine (N_2H_4) 80% (Merck, Germany), and chitosan (CS) with a molecular weight of approximately 90 kDa (Institute of Advanced Technology, Vietnam). The fungal strain *P. oryzae* was obtained from the Institute of Agricultural Science for Southern Vietnam. Deionised water was used throughout the experiment.

2.2. Methods

Determine the saponification value of palm oil [63]: 2 g of palm oil was placed in a 100 ml Erlenmeyer flask, to which 25 ml of 0.5 M KOH in ethanol and a small amount of zeolite were added. The flask was connected to a reflux condenser and heated slowly to the boiling point of the oil for 60 minutes. Following this, 0.5-1.0 ml of phenolphthalein indicator was added, and the solution was titrated with 0.5 M HCl standard solution. A blank sample underwent the same procedure, and the volume of HCl solution used to titrate until the pink colour disappeared was recorded. The experiment was repeated three times, with results presented as the mean ± standard error (SE). The saponification value (SN) was calculated using formula (1):

$$\text{SN (mg/g)} = \frac{(V_0 - V_1) \times c \times 56.1}{m} \quad (1)$$

where SN (mg KOH/g oil) is the saponification value, V_0 and V_1 (ml) are the volumes of standard HCl solution used for titration in

the blank and sample, respectively, c (M) is the concentration of the HCl solution, and m (g) is the mass of the oil.

Preparation of AS: 100 g of palm oil was heated in a 250 ml glass beaker to approximately 90°C. The amount of 25% NH_4OH solution, calculated based on the SN of palm oil, was slowly added to the hot palm oil, stirring the mixture until a homogeneous solution formed while maintaining the reaction temperature at approximately 90°C. Since NH_4OH is a weak and volatile base, a 20% excess of the 25% NH_4OH solution was used.

Determination of the efficiency of saponification reaction (SH): As ammonium salts of fatty acids are insoluble in esters [19], diethyl ether was used to extract the fat and determine SH. 5 g of AS was mixed with 10 ml of diethyl ether, covered, and shaken on a shaker at 170 rpm for 1 hour. The mixture was then transferred to an extraction flask and left to settle for 120 minutes. The AS at the bottom of the flask was collected and weighed to determine SH. The experiment was repeated three times, and the results are presented as the mean ± SE. SH was calculated using formula (2):

$$SH\,(\%)=100\times\frac{m}{m_0} \quad (2)$$

where m_0 (g) is the mass of the initial AS and m (g) is the mass of the remaining AS.

Determination of pH of AS [63]: 1 g of AS was dissolved in 50 ml of water, and the pH was measured using a pH meter.

Determination of the surface tension of AS solution: A 0.05% aqueous AS solution was used to measure surface tension on a digital surface tensiometer (DST-60, SEO, Korea) at 25°C. Water was also measured for comparison.

Preparation of CuNPs/CS: CuNPs/CS was prepared following the method of M.S. Usman, et al. (2012) [64], with some modifications. 5 g of CS was dissolved in 95 ml of lactic acid solution (5 ml lactic acid + 90 ml water), and the mixture was filtered through a steel mesh with a pore size of 100 µm to remove impurities. 1.89 g of $Cu(NO_3)_2.3H_2O$ was dissolved into the CS solution and heated to 80-90°C. Then, 3.5 ml of 8% N_2H_4 solution was added slowly while stirring, to reduce Cu^{2+} and obtain approximately 100 ml of CuNPs/CS solution containing 5% CS (w/v) and 5000 mg/l Cu.

Characterisation of CuNPs/CS: The size of CuNPs was determined from transmission electron microscopy (TEM) images, measured on a JEM 1010 (JEOL, Japan), and presented as mean±standard deviation (SD). The optical properties were analysed using ultraviolet-visible (UV-Vis) spectroscopy on a V630 spectrophotometer (Jasco, Japan). The crystalline properties were determined using X-ray diffraction (XRD) patterns measured on an XRD D8 Advance diffractometer (Bruker, Germany), with Cu kα (λ=1.5406 Å). Chemical bonds and functional groups in the material were identified using Fourier transform infrared (FTIR) spectroscopy on an FTIR 840S spectrometer (Shimadzu, Japan), in the wavenumber range of 4000-400 cm^{-1}.

Antifungal efficacy in vitro: The antifungal activity was evaluated according to the method of T.B.N. Doan, et al. (2020) [30], with slight modifications. Colonies of *P. oryzae* were placed in the centre of Petri dishes (90 mm) containing PDA medium diffused with CuNPs/CS at Cu concentrations of 15, 20, 25, and 30 mg/l, and 25 mg/l Cu+0.05% AS. The control treatment contained only PDA medium. After 7 days of incubation at 30±2°C in the dark, the diameter of the fungal colonies was measured, and antifungal efficacy (AE) was calculated using formula (3):

$$AE\,(\%)=100\times\frac{D-d}{D} \quad (3)$$

where D and d (mm) are the fungal colony diameters of the control and treated treatments, respectively.

Disease control efficacy in vivo: Disease control efficacy was assessed according to T.B.N. Doan, et al. (2020) [30], with some modifications. OM 5451 rice seeds were sterilised in NaClO solution for 20 minutes, and 6 sterilised seeds were placed in a plastic pot containing 5 kg of soil mixed with 3 g of NPK 16-16-8 fertiliser. The pots were placed in a greenhouse and watered daily. At 21 days after planting, a fungal suspension containing *P. oryzae* (10^5 cfu/ml) was sprayed on the rice plants. The CuNPs/CS solution was applied at a Cu concentration of 30 mg/l and 25 mg/l Cu+0.05% AS was applied on the third day after inoculation. Fourteen days after CuNPs/CS treatment, disease severity (DS, %) was determined using the Townsend-Heuberger formula (4) [65]:

$$DS\,(\%)=100\times\frac{\Sigma n_i\times\upsilon}{N\times v} \quad (4)$$

where n_i is the number of leaves with an i score, N is the number of total leaves observed, v is the highest scale of disease severity, and υ is the scale of disease severity (0-9).

Scale description:

0=no lesions;

1=small brown specks, pinhead size;

2=larger brown specks;

3=small, round to slightly elongated necrotic grey spots about 1-2 mm in diameter;

4=typical blast lesions, elliptical, 1-2 cm long, confined to the area of two main veins infecting <2% of the total leaf area;

5=typical blast lesions infecting <10% of the leaf area;

6=typical blast lesions infecting 10-25% of the leaf area;

7=typical blast lesions infecting 26-50% of the leaf area;

8=typical blast lesions infecting 51-75% of the leaf area;

9=all leaves dead.

The disease control efficacy (H) was calculated according to the formula of W.S. Abbott (1925) [66]:

$$H(\%)=100\times\left(1-\frac{Ta}{Ca}\right) \quad (5)$$

where *Ta* and *Ca* (%) are the DS of the positive control and treated treatments at the time of investigation, respectively.

Statistical analysis: The particle size and distribution were determined using ImageJ 1.54 g. Results from *in vitro* and *in vivo* experiments are presented as mean±SE. All data were statistically processed using IRRISTAT 5.0 software and Microsoft Excel 2013. Means were compared using the least significant difference at the 0.05 probability level ($LSD_{0.05}$). The inhibitory concentration 50% (IC50) of CuNPs/CS against *P. oryzae* was calculated from the regression equation by replacing y=50 and calculating x, where x is the Cu concentration (mg/l) and y is the AE (%).

3. Results and discussion

Preparation of ammonium soap and CuNPs/CS: The saponification value (SN) of palm oil was determined to be 198.75 mg KOH/g, which is consistent with previous research [67-69]. Therefore, approximately 60 g of 25% NH_4OH solution (20% excess) was required to saponify 100 g of palm oil. After 2 hours of saponification with 25% NH_4OH solution, an opaque white concentrated solution was formed. Photographs of palm oil and AS are shown in Fig. 2A.

The saponification reaction efficiency (H) was determined to be 95.5±0.7%, which is relatively high compared to T.L. Reiling (1962) [19] study on the saponification of plant oil using $(NH_4)_2CO_3$ (approximate efficiency of 92%). This higher efficiency may be due to the use of 20% excess NH_4OH in this study. The pH value of AS was determined to be 7.8-8.0, which is suitable for various applications, and NH_4OH produced less alkaline AS compared to NaOH [63]. At a concentration of 0.05% AS (equivalent to approximately 0.03% solid soap), the surface tension of water at 25°C decreased from 72.5 to 7.8 mN/m, which is similar to AS produced from tung oil as previously reported [18]. These results suggest that the obtained AS is an effective surfactant for use as a detergent or agricultural additive.

After reacting with N_2H_4 at 80-90°C, the CS-Cu^{2+} complex changed from dark green to the characteristic purple-brown colour of CuNPs, as shown in Fig. 2B.

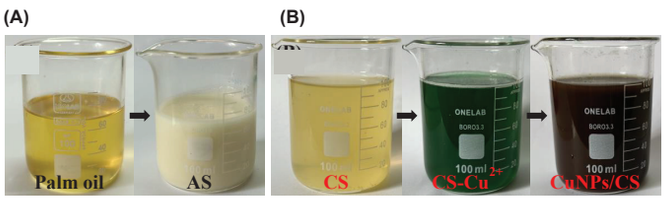

Fig. 2. Photographs of (A) palm oil, ammonium soap, and (B) chitosan, CS-Cu^{2+}, and CuNPs/CS.

The TEM image and size distribution in Fig. 3 indicate that the CuNPs are spherical, with an average size of 17.9±3.4 nm, and have a narrow distribution. The CuNPs in this study are smaller than those reported by M.S. Usman, et al. (2012) [64] (35-75 nm) and A. Manikandan, et al. (2015) [70] (88.2 nm), both of whom used CS as a stabiliser. This size difference may be because the CuNPs/CS in this study were prepared in solution rather than centrifuged and collected as a solid sample. Additionally, compared to our previous study, the CuNPs stabilised in CS solution are larger than those stabilised in alginate solution (4.1±1.4 nm) [30].

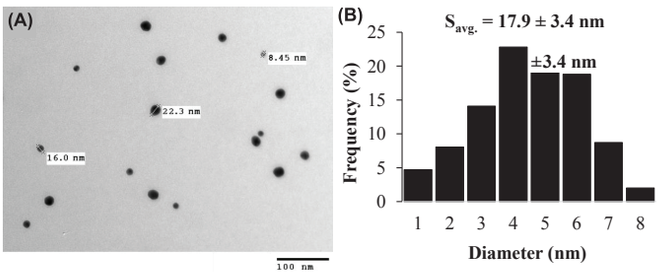

Fig. 3. (A) Transmission electron microscopy image and (B) particle size distribution of CuNPs/CS.

The UV-Vis spectrum of CS (Fig. 4A) shows an absorption peak at 238 nm, representing the n–π* transition of the amino group [71, 72]. This peak shifted to 249 nm for CuNPs/CS (Fig. 4A). Additionally, the UV-Vis spectrum of CuNPs/CS displayed a peak at 592 nm, which falls within the reported absorption range for CuNPs (500-600 nm) [73, 74], indicating the formation of CuNPs in the solution.

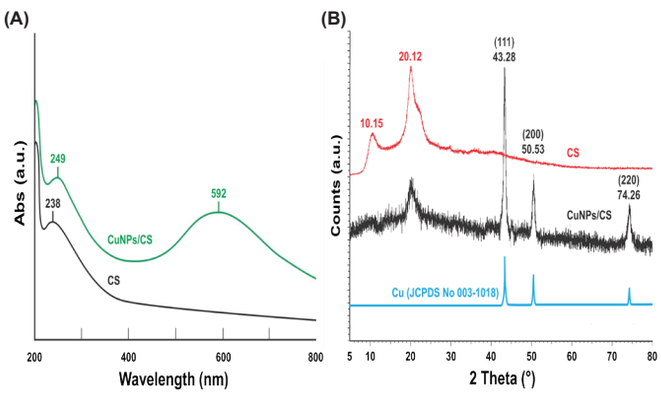

Fig. 4. (A) Ultraviolet-visible spectra and (B) X-ray diffraction patterns of chitosan and CuNPs/CS.

The XRD patterns of CS and CuNPs/CS are shown in Fig. 4B. The XRD pattern of CS exhibited two characteristic peaks at 2θ~10.15° and 20.12° [75, 76]. The XRD pattern of CuNPs/CS presented new peaks at 2θ~43.28°, 50.53°, and 74.26°, corresponding to the (111), (200), and (220) diffraction planes. These peaks are characteristic of the face-centred cubic (fcc) structure of Cu. The XRD results for CuNPs/CS align with the findings of M.S. Usman, et al. (2012) [64], A. Manikandan (2015) [70], and A. Bahari, et al. (2023) [31].

The FTIR spectra of CS and CuNPs/CS are presented in Fig. 5. The FTIR spectrum of CS showed characteristic vibration peaks, including 3449 cm^{-1} (stretching vibrations of O–H and N–H), 2930 and 2875 cm^{-1} (symmetric and asymmetric vibrations of $–CH_2$ groups), 1655 and 1591 cm^{-1} (bending vibrations of N–H in secondary amides), 1422 cm^{-1} (stretching vibration of $–CH_2$ groups), 1148 cm^{-1} (C–O–C stretching), 1012 and 1049 cm^{-1} (C–O stretching), and 877 cm^{-1} (C–H bending vibration) [76].

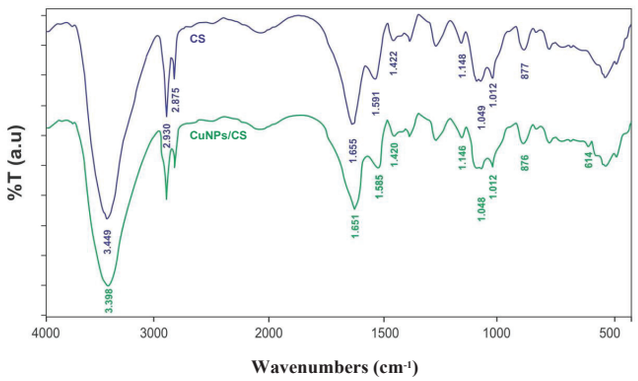

Fig. 5. Fourier-transform infrared spectra of chitosan and CuNPs/CS.

These results were similar to those from our previous study on preparing the SNPs/CS-Cu^{2+} complex [77]. The FTIR spectrum of CuNPs/CS also showed the characteristic peaks of CS, but they were shifted to lower wavelengths [64]. Specifically, the peak at 3449 cm^{-1} shifted to 3398 cm^{-1}, and the peaks at 1655 and 1591 cm^{-1} shifted to 1651 and 1585 cm^{-1}. Additionally, the FTIR spectrum of CuNPs/CS showed a new peak at 614 cm^{-1} [70, 78]. These results indicate the interaction of CuNPs with amino and hydroxyl groups and that the CuNPs were coated by the CS polymer.

The antifungal efficacy of CuNPs/CS against *P. oryzae* is shown in Table 1 and Fig. 6. The results in Table 1 indicate that antifungal activity increases with Cu concentration. After 7 days of incubation, *P. oryzae* in the control treatment had fully grown across the Petri dish, while treatments with CuNPs/CS at concentrations of 15, 20, 25, and 30 mg/l Cu achieved antifungal efficacy (AE) of 46.73, 61.94, 77.21 and 100%, respectively. Notably, treatment with CuNPs/CS at a concentration of 25 mg/l Cu and 0.05% AS also achieved 100% AE, whereas the same Cu concentration without AS supplementation resulted in only 77.21% efficacy. In our previous study, the *in vitro* antifungal activity of nano Cu-Cu_2O/alginate against *P. oryzae* also yielded similar results, with 100% AE at a Cu concentration of 30 mg/l [30].

Table 1. *In vitro* antifungal activity of CuNPs/chitosan against *P. oryzae*.

Treatments	Diameter (mm)	Antifungal efficacy (%)
Control	76.95^{a}±0.93	0.00
CuNPs/CS (15 mg/l Cu)	40.99^{b}±2.25	46.73
CuNPs/CS (20 mg/l Cu)	29.29^{c}±1.14	61.94
CuNPs/CS (25 mg/l Cu)	17.54^{d}±1.46	77.21
CuNPs/CS (30 mg/l Cu)	0.00^{e}±0.00	100.00
CuNPs/CS (25 mg/l Cu) + AS 0.05%	0.00^{e}±0.00	100.00
$LSD_{0.05}$	4.24	-

Different letters in the same row indicate significant differences at $p<0.05$.

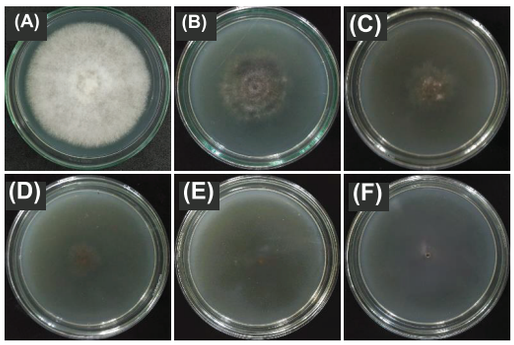

Fig. 6. *In vitro* antifungal activity of CuNPs/chitosan against *P. oryzae* after 7 days of incubation. (A) Control, **(B)** 15 mg/l Cu, **(C)** 20 mg/l Cu, **(D)** 25 mg/l Cu, **(E)** 30 mg/l Cu and **(F)** 25 mg/l Cu+0.05% AS.

The IC_{50} of CuNPs/CS against *P. oryzae* was calculated to be 16.37 mg/l Cu, based on the regression equation:

$$y=3.5015x-7.3151 \quad (6)$$

(R^2=0.9889) as seen in Fig. 7. This result suggests that CuNPs stabilised in CS have slightly higher efficacy against *P. oryzae* compared to those stabilised in alginate (IC_{50} is 17.80 mg/l Cu) [30]. This increased efficacy may be due to the synergistic effect of CuNPs and the CS polymer, both of which exhibit antifungal activity. Previous studies have also shown that Cu-based nanoparticles are effective against fungi that damage plants, such as *Fusarium oxysporum*, *Phomopsis vexans*, *Fusarium solani*, and *Rhizoctonia solani* [48, 79, 80]. The antifungal activity of CuNPs/CS was enhanced when supplemented with AS, as surfactants also exhibit antifungal properties. The antifungal mechanism of cationic surfactants (e.g., cetrimonium bromide) involves binding to negatively charged fungal surfaces, altering surface charge, and preventing fungal cell adhesion [22, 23].

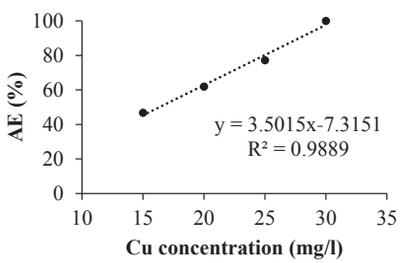

Fig. 7. Correlation between antifungal efficacy of CuNPs/chitosan against *P. oryzae* and Cu concentration.

Based on the results of the *in vitro* experiment, CuNPs/CS at concentrations of 30 and 25 mg/l Cu supplemented with 0.05% AS (both achieving 100% AE) were selected for the *in vivo* study to evaluate their effectiveness in controlling rice blast disease (Fig. 8). The results presented in Table 2 show that in the negative control treatment, rice plants remained healthy and exhibited robust growth (DS was 0%), while in the positive control treatment, DS reached 25.66% after 14 days. This demonstrates that the variables were well-controlled, and any changes in the experimental group were due to the tested variable, rather than other factors.

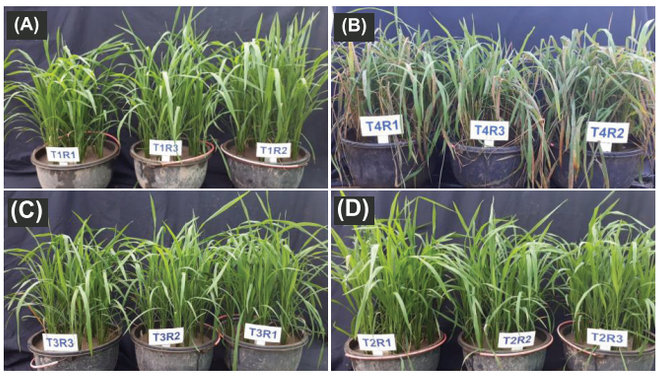

Fig. 8. *In vivo* antifungal activity of CuNPs/chitosan against *P. oryzae* causing rice blast disease. (A) Negative control; **(B)** Positive control; **(C)** CuNPs/CS 30 mg/l Cu; **(D)** CuNPs/CS 25 mg/l Cu + 0.05% AS.

The effectiveness of controlling rice blast disease in the treatments sprayed with CuNPs/CS at concentrations of 30 and 25 mg/l Cu supplemented with 0.05% AS were 71.32 and 86.91%, respectively (Table 2). In the treatment with CuNPs/CS at 30 mg/l Cu, a significant reduction in disease severity was observed, with DS at 7.36%, which is lower than the results reported by T.B.N. Doan, et al. (2020) [30], where DS was 10.05% after 15 days post-inoculation.

Table 2. *In vivo* effectiveness of CuNPs/chitosan in controlling rice blast disease.

Treatments	Disease severity (%)	The disease control efficacy (%)
Negative control	0.00^{a}±0.00	-
Positive control	25.66^{b}±0.56	-
CuNPs/CS (30 mg/l Cu)	7.36^{c}±1.04	71.32
CuNPs/CS (25 mg/l Cu + 0.05% AS)	3.36^{d}±0.34	86.91
$LSD_{0.05}$	2.20	-

Different letters in the same row indicate significant differences at $p<0.05$.

Y. Chen, et al. (2022) [81] concluded that cultivating rice in Yoshida nutrient solution supplemented with 50 mg/l nano CuO significantly reduced the severity of rice blast disease compared to the control. Similarly, a previous study by M.O. Jibrin, et al. (2021) [82] showed that the addition of the commercial surfactant Cohere could enhance the anti-pathogen efficacy of pesticides on plants. When 0.05% Cohere was added, the powdery mildew index on squash caused by *Podosphaera xanthii* was reduced from 15.5 to 11.5% when using 1 mM Carvacrol. Similarly, the bacterial spot index on tomatoes caused by *Xanthomonas perforans* was significantly reduced from 13.75 to 2.5% when using 2.1 g/l Kocide 3000 combined with 0.05% Cohere [82]. Our findings, which show enhanced antifungal activity of CuNPs/CS with AS supplementation, are consistent with M.O. Jibrin, et al. (2021) [82], who also reported improvements in plant disease control by adding surfactants to agricultural active ingredients.

The above results demonstrate that CuNPs/CS is highly effective in controlling rice blast disease, and the addition of AS significantly enhances the plant disease control efficacy of the nanomaterial. The mechanism for this enhancement is attributed to the synergistic effect of both materials, which possess the ability to inhibit microorganisms. Moreover, surfactants reduce surface tension, improve the dissolution or dispersion of active ingredients, and enhance their adhesion and penetration into the cell walls of microorganisms and the plant epidermis, particularly for spray pesticides [25, 83]. Surfactants are commonly added to pesticides to reduce the loss of pesticides that do not adhere to the leaf surface, thus enhancing the effectiveness of microbiological insecticides [84].

Additionally, AS, produced from inexpensive and environmentally safe raw materials, is considered a sustainable pest management tool, particularly for Integrated Pest Management (IPM) programmes and organic production [25]. Therefore, the addition of AS to CuNPs/CS not only reduces the required Cu concentration but also improves the adhesion of the sprayed material. Beyond combating plant pathogens, CuNPs readily convert into Cu^{2+} ions under environmental conditions, which plants can absorb as micronutrients, leaving no harmful residue when used at low concentrations. Furthermore, both CS and AS are of natural origin and biodegradable, making them environmentally friendly and safe for human use.

4. Conclusions

The properties of AS were studied, demonstrating its ability to reduce the surface tension of solutions. Additionally, the addition of 0.05% AS to CuNPs/CS prepared by reducing $Cu(NO_3)_2$ with N_2H_4 at a Cu concentration of 25 mg/l increased the AE against *P. oryzae* from 77.21 to 100% in the *in vitro* experiment. In the *in vivo* experiment, the rice blast resistance achieved with CuNPs/CS at 25 mg/l Cu combined with 0.05% AS was significantly higher than that with CuNPs/CS alone at 30 mg/l Cu (86.91 vs. 71.32%). Furthermore, both AS and CuNPs provide copper micronutrients and nitrogen, contributing to improved plant growth. The integration of AS with CuNPs/CS shows significant potential for practical applications in sustainable and safe agricultural practices, providing both disease control and nutritional benefits.

CRediT author statement

Tuan Nghiem Anh Le: Data analysis, Methodology; Nghi Tran: Investigation and Editing support; Nhung Hong Nguyen: Investigation and Editing support; Tho Phuoc Tran: Writing the original draft and Performing experiments; Giang Ngoc Doan: Performing experiments; Ngoc Thanh Truong: Performing experiments; Du Duy Bui: Methodology, Conceptualisation, and Editing support.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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