Preparation of Ag/SiO₂ nanocomposite and assessment of its antifungal effect on soybean plant (a Vietnamese species DT-26)

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Abstract

Soybean crop losses due to fungal diseases are considerable and directly depend on the severity of the disease. The objective of this study was to assess antifungal activity of silver/silica (Ag/SiO₂) nanocomposite against crop pathogenic fungi (Fusarium oxysporium and Rhizoctonia solani) in soybean farming. Firstly, silica particles with a size ranging from 20 to 30 nm were modified with 3-aminopropyl triethoxysilane (APTES) for 2 h. Then these amino acid-functionalized silica particles were exposed to silver ion solution followed by reduction of silver ions with sodium borohydride to form Ag/SiO₂ nanocomposite. The formation of the linkage between APTES and silica particles was confirmed by Fourier transform infrared (FTIR) spectroscopy. The surface plasmon absorption maximum at 400 nm confirmed the nano essence of the silver particles on silica particles. For the seed coating, bentonite from Lam Dong deposit, Vietnam, was used as an encapsulation substance, while carboxymethyl cellulose (CMC) was used as a binding agent. The assessment of fungicidal activity of the Ag/SiO₂ nanocomposite produced showed that this product is effective in inhibition of the pathogenic fungi in soybean plant.

Keywords: Ag/SiO₂ nanocomposite, antifungal activity, pathogenic, soybean seed

Classification numbers: 1.00, 2.03, 2.04, 4.02, 4.04

1. Introduction

Seed coating is a method to improve seed performance. The purpose of the seed coating is to apply substances such as growth promoting agents, fungicides, pesticides, safeners, micronutrients, and other compounds directly to the seed. Coating the seed with liquid-based polymeric adhesives improve its performance and physical properties. Coating is greatly different from pelleting since it represents only an increase of the seed weight of 1%–10% and the shape is still retained. Many formulations of the seed coating have been used in agriculture for promoting crop performance [1–9].

Recently, there have been some studies which prefer to use silver nanoparticles (AgNPs) as a fungicide for the plant growing. Silver has been known as the strongest natural bactericide which attracted researchers’ attention for decades. In spite of their efforts, the mechanism by which AgNPs destroy microorganisms is not yet completely understood, although several mechanisms have been discussed. These include interaction between silver ions and thiol groups (–SH) of cytoplasmic proteins, generation of reactive oxygen species (ROS) (for example HO⁺, O₂⁻, O₃⁻) and creation of cavities on the external cell membranes of microorganisms. Nevertheless, AgNPs have been successfully applied as a disinfectant in medicine, water treatment and other fields.
Some reports [10–13] showed that silver ions (Ag\(^{+}\)) are more active than bulk metal silver (Ag\(^{0}\)), but in the nano-sized form its bactericidal activity is much higher. Therefore for disinfection purposes using AgNPs the smaller NPs are used with a lesser quantity than the larger ones. However, practical experience showed that AgNPs with a diameter less than 20 nm tend to aggregate into larger clusters which worsen their antibacterial activity. To solve this problem, a wide range of materials such as TiO\(_2\), SiO\(_2\), Al\(_2\)O\(_3\), zeolites, activated carbon etc have been employed as a carrier that allows AgNPs to be evenly distributed on the carrier’s surface without aggregation [10, 14].

Many researches [10–12, 15, 16] presented the use of Ag/SiO\(_2\) nanocomposite for making powerful microbicides. In the current study, Ag/SiO\(_2\) nanocomposite was prepared in two steps: preparation of amino-functionalized silica particles (AFSPs) by using 3-aminopropyl triethoxysilane (APTES) and preparation of Ag/SiO\(_2\) nanocomposite, in which AFSPs come as a carrier for silver NPs [16, 17].

For preparation of the seed coatings bentonite was also used. Specific properties of this mineral clay make it to be able to encapsulate the seed so that on exposure to moisture it will swell, supporting the slow release of the coated components such as bactericides or fungicides.

This study aimed to assess the possible effects of Ag/SiO\(_2\) nanocomposite as a fungicide against some crop pathogenic fungi (Fusarium oxysporium and Rhizoctonia solani), which affect the germination process of soybean plant (a Vietnamese species DT-26).

2. Materials and methods

2.1. Preparation of Ag/SiO\(_2\) nanocomposites

Procedure for preparation of Ag/SiO\(_2\) nanocomposite was presented in figure 1. To prepare AFSPs, 100 g of silica powder (particle size 20–30 nm, Sigma-Aldrich) was mixed with 150 ml of 4% APTES (Sigma-Aldrich) solution in a 500 ml beaker. Due to the fact that the surface of silica particles as a rule contains a number of silanol groups (\(=\)Si –OH), the functionalization process on their surface takes place. APTES is hydrolyzed in an acidic water solution to form 3-aminopropyltriethoxysilane which then reacts with active OH groups on the surface of the silica substrate [15]:

\[
\begin{align*}
(C_3H_7O_2)\text{SiC}_2H_5\text{NH}_2 + \text{H}_2\text{O} + \text{H}^+ &\rightarrow (\text{HO})_3\text{SiC}_2\text{H}_5\text{NH}_2 + 3\text{C}_2\text{H}_5\text{OH} \\
(\text{SiO}_2)\text{h} &\equiv \text{Si} – \text{OH} + (\text{HO})_3\text{SiC}_2\text{H}_5\text{NH}_2 \\
&\rightarrow (\text{SiO}_2)\text{h} &\equiv \text{Si} – \text{O} – \text{Si(OH)}_2\text{C}_2\text{H}_5\text{NH}_2 + \text{H}_2\text{O}
\end{align*}
\]

This process finally tailors amino-functional groups on the surface of silica particles. The reaction mixture was aged at 80°C for 3 h and then removed APTES residue by filtration. Finally, the AFSPs were dried at 80°C for 20 h to obtain the product in a powder form.

To synthesize Ag/SiO\(_2\) nanocomposite (figure 1), 100 g of AFSPs were added to 200 ml of 0.5% AgNO\(_3\) (Merck) solution in a 500 ml beaker and stirred for 4 h followed by filtering and washing with distilled water to remove free silver ions adsorbed on the silica surface. Then the silica particles with attached Ag\(^{+}\) ions were dispersed into 500 ml distilled water in a 1000 ml beaker and stirred 7000 rpm for 15 min. Then, without disrupting stirring, 0.05 M NaBH\(_4\) (Cica) solution was added by drop to the mixture and the stirring was continued for another 15 min. The color of the solution slowly became yellow, indicating the formation of silver NPs immobilized on the surface of the silica particles. The APTES-functionalized Ag/SiO\(_2\) nanocomposite was washed with distilled water in order to remove free AgNPs and other components. Then sample was dried overnight at 50°C to obtain Ag/SiO\(_2\) nanocomposite in a powder form.

FTIR spectra were recorded on Perkin Elmer (model 783) IR spectrometer in KBr medium at room temperature in the region 4000–500 cm\(^{-1}\). Ultraviolet visible (UV-Vis) spectroscopy method (UV-Vis 2450 Shimazu) was applied to prove the existence of AgNPs decorated the surface of the silica particles. The wavelength scanning interval ranged from 300 nm to 800 nm. Particle size was determined by transmission electron microscopy (TEM, JEM1010 JEOL).

2.2. Coating soybean seeds

Soybean seeds of a Vietnamese species DT-26 were procured from Legumes Research and Development Center of the Field Crop Research Institute, Vietnam Academy of Agricultural Sciences. For coating soybean seeds a rotating drum was used and the following raw materials were needed: soybean seed of 25 g, bentonite clay with montmorillonite content of ≥50%
(1.4 g), carboxymethyl cellulose (CMC) 2% solution of 2 ml and APTES-functionalyzed Ag/SiO$_2$ nanocomposite of 2.6 g.

Bentonite clay was taken from Tam Bo deposit of Lam Dong province, processed and purified in Institute of Environmental Technology, Vietnam Academy of Science and Technology (VAST).

To prepare soybean seed pellets, 25 g of soybean seeds were put into a drum. As drum rotated, 2 ml of 2% CMC solution was sprayed onto the seeds followed by adding bentonite and Ag/SiO$_2$ nanocomposite. The pellets gradually formed during drum rotation and slightly increased in size with each turn of the drum. At the end of the pelleting process, the pelleted seeds were dried 1 h at 40°C [1].

2.3. Fungal inhibition test on the Ag/SiO$_2$ nanocomposite and soybean seed coating

2.3.1. Fungal inhibition test on the Ag/SiO$_2$ nanocomposite.

Fungal species Fusarium oxysporium and Rhizoctonia solani were obtained from the Research Institute of Plant Protection. The fungi were maintained on potato dextrose agar (PDA) medium and incubated for 7 days at 28°C and served as the testing fungi for antifungal activity assay.

An adequate quantity of Ag/SiO$_2$ nanocomposite (nanosilver concentration 10–200 ppm) was introduced to the molten PDA by mixing and 30 ml of the mixture was poured onto a petri dish. After solidification, one fungus-containing agar bit (0.5 cm) was placed in the center of the petri dish. Incubation period of 48–168 h at 28°C was maintained for determination of antifungal activity. The activity was evaluated by measuring the zone of fungal growth. The inhibition percentage of fungal growth was calculated by the following formula [18]:

\[
\text{Inhibition(\%)} = \frac{f_c - f_i}{f_c} \times 100,
\]

Figure 2. FTIR spectra of (a) silica particles and (b) APTES-incorporated silica particles.

Figure 3. UV-Vis spectrum of the Ag/SiO$_2$ nanocomposite.

Figure 4. TEM image of the Ag/SiO$_2$ nanocomposite.
Table 1. Inhibition effect of Ag/SiO$_2$ nanocomposite depending on nanosilver concentration.

| Ag/SiO$_2$ (Ag, ppm) | Rhizoctonia solani (2 days) | Rhizoctonia solani (7 days) | Fusarium oxysporium (7 days) |
|----------------------|---------------------------|---------------------------|-----------------------------|
| Control              | 8.77                      | 8.77                      | 53.59$^a$                   |
| 10                   | 4.07                      | 7.47                      | 57.81$^b$                   |
| 20                   | 3.70                      | 7.37                      | 60.89$^c$                   |
| 30                   | 3.43                      | 7.07                      | 71.15$^d$                   |
| 40                   | 2.53                      | 6.87                      | 79.13$^e$                   |
| 50                   | 1.83                      | 6.77                      | 83.69$^f$                   |
| 75                   | 1.43                      | —                         | 88.60$^g$                   |
| 100                  | 1.00                      | 5.53                      | 90.88$^{gh}$                |
| 125                  | 0.80                      | 4.03                      | 91.68$^{gh}$                |
| 150                  | 0.73                      | 3.83                      | 92.36$^{gh}$                |
| 175                  | 0.67                      | 3.57                      | 92.82$^a$                   |
| 200                  | 0.63                      | 2.17                      | 75.26$^a$                   |
| CV (%)               | 5.0                       | 2.0                       | 1.9                         |

The means followed by the same letters on each numeral column are not significantly different at $P < 5\%$ according to least significant different test. CV is coefficient of variation.
where \( f_c \) is diameter of fungal growth zone on the control sample and \( f_t \) is diameter of fungal growth zone on the tested sample.

### 2.3.2. Fungal inhibition test on the soybean seed coating

Using the growth rate method antifungal activity of the seed coating on PDA medium was evaluated in the presence of plant pathogens *Fusarium oxysporium* and *Rhizoctonia solani*. A first step, one milliliter of fungal suspension (500–1000 CFU/ml) was poured onto petri dish. Then the coated soybean seed was added and shaken with the contact time 5 and 15 min. For the next step, the coated seeds were cultivated in petri dishes (5 coated seeds per a dish) and erlenmeyer flasks containing mannitol salt agar (MSA) (10 seeds per a flask). These petri dishes and erlenmeyer flasks were placed into growth chamber and illuminated (1500 lux) 14 h per day at 28°C. Three replications were used for each treatment. Germination counts were carried out every day from the 2nd day. After 5 days of incubation the seedlings quality and antifungal activity of the samples were determined.

### 2.3.3. Field experiment

Field experiment included soybean seed coated with Ag/SiO\(_2\) nanocomposite and those without coating, which were sown in the spring using Ag/SiO\(_2\) nanocomposite. The experiment was conducted on an experimental plot at the Center for Legumes Research and Development of the Field Crop Research Institute in randomized complete block design mode and replicated three times. The plot with a size of 51 m\(^2\) was divided into 6 compartments (8.5 m\(^2\)/compartment, 5.0 × 1.7 m\(^2\)), containing 4 rows with 25 cm apart each other and a plant spacing of 35 cm. In total, 320 soybean seeds were shown on the plot. The fungal infection was estimated as follows:

\[
\text{Disease incidence (\%) = } \frac{\text{Number of infected plants}}{\text{Total number of plants tested}} \times 100.
\]

### 3. Results and discussion

#### 3.1. FTIR spectra of unmodified and APTES-modified silica particles

FTIR spectra of silica and APTES-attached silica particles were shown in figure 2. The absorption band at about 951.03 cm\(^{-1}\) proves the existence of Si–OH groups (curve a) which are essential for immobilization of APTES on the silica surface. But after immobilization of APTES (curve b) this band dramatically decreased, witnessing the formation of the linkage between APTES and silanol groups on the silica surface. The absorption band at 1182.15 cm\(^{-1}\) is caused by the stretching of Si–O–Si. These results suggested that APTES was successfully anchored on the silica surface [16, 17].

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Effect of the seed treatment mode on the inhibition of *Rhizoctonia solani* T: soybean seed without coating, without contact with fungus; TR: soybean seed without coating, contacted with fungus 5 min; TR*: soybean seed without coating, contacted with fungus 15 min; TS: soybean seed coated with nanocomposite, without contact with fungus; TSR: soybean seed coated with nanocomposite, contacted with fungus 5 min and TSR*: soybean seed coated with nanocomposite, contacted with fungus 15 min.

### Table 2. Effect of the seed coating mode on the germination of soybean.

| Variety | \( R. \) solani | \( F. \) oxysporium |
|---------|----------------|-------------------|
| T       | 100            | 100               |
| TR/TF   | 100            | 0                 |
| TR*/TF* | 98             | 0                 |
| TS      | 98             | 96                |
| TSR/TSF | 100            | 100               |
| TSR*/TSF* | 96         | 100               |

T: soybean seed without coating, without contact with fungus.  
TR/TF: soybean seed without coating, contacted with fungus 5 min.  
TR*/TF*: soybean seed without coating, contacted with fungus 15 min.  
TS: soybean seed coated with nanocomposite, without contact with fungus.  
TSR/TSF: soybean seed coated with nanocomposite, contacted with fungus 5 min.  
TSR*/TSF*: soybean seed coated with nanocomposite, contacted with fungus 15 min.
3.2. UV-Vis spectrum of Ag/SiO\textsubscript{2} nanocomposite

UV-Vis spectrum of Ag/SiO\textsubscript{2} nanocomposite presented in figure 3 showed that the broad peak at about 400 nm is attributed to the plasmon resonance absorption of silver nanoparticles. Appearance of this peak affirmed the presence of AgNPs in the nanocomposite [16].

3.3. TEM images of Ag/SiO\textsubscript{2} nanocomposite

Considering TEM image of Ag/SiO\textsubscript{2} nanocomposite presented in figure 4 one can distinguish clearly two phases of the nanocomposite: uncoated (white color) and silver-coated (black color) silica nanoparticles with an average size of 6–12 nm. This is in good agreement with the results from the study [14, 16, 17].
3.4. Fungal inhibition test on Ag/SiO₂ nanocomposite

Effects of Ag/SiO₂ nanocomposites on the fungal germination growth were shown in table 1 and demonstrated in figure 5. Briefly, freshly obtained sclerotia of *Rhizoctonia solani* were placed at the center of petri dish. At highest nanosilver concentration of the Ag/SiO₂ nanocomposite (200 ppm) sclerotial germination of *Rhizoctonia solani* was almost inhibited. After 2 days colony diameter was smallest (0.63 cm) compared to 8.77 cm of the control, equivalent to 92.82% of inhibition. Meanwhile at lower nanosilver concentration (10–50 ppm) the fungal inhibition still remained considerable (53%–79%). For the case of *Fusarium oxysporium*, after 7 days at nanosilver concentration 200 ppm the inhibition effect on sclerotial germination attained 75.26%, while at the lowest nanosilver concentration (10 ppm) the inhibition reached only 14.82%. These results demonstrated that inhibition effect of Ag/SiO₂ nanocomposite on *Rhizoctonia solani* is stronger than on *Fusarium oxysporium*, and depends on nanosilver concentration.

3.5. Fungal inhibition test on the seed treatment mode

Effects of the seed coating mode on the soybean germination were presented in table 2 and figures 6 and 7. The results indicated that coating soybean seed with Ag/SiO₂ nanocomposite increased its ability against the fungal infection. In this experiment, 100% of soybean seed without coating and contacted with fungi *Rhizoctonia solani* (R. solani) and *Fusarium oxysporium* (*F. oxysporium*) were infected depending on the exposition time. Using Ag/SiO₂ nanocomposite for coating soybean seed positively stimulated the seedling growth of soybean plant even in the presence of pathogenic fungi.

3.6. Field experiment

Results of the field experiment presented in table 3 and figure 8 indicated that coating soybean seed with nanocomposite affected the disease incidence on soybean plant at different growth stages. The experimental data showed that almost all the growth stages were affected by fungal diseases at different level, but in the presence of Ag/SiO₂ nano-composite this impact considerably decreased.

4. Conclusion

Ag/SiO₂ nanocomposite was prepared by chemical method using APTES as a silica-functionalizing agent and sodium borohydride as a reductant. The produced nanocomposite significantly stimulated the seedling growth of soybean. Soybean seeds coated with Ag/SiO₂ nanocomposite and bentonite (16%) were able to effectively fight against the crop pathogenic fungi *Fusarium oxysporium* and *Rhizoctonia solani*. These fungi were almost inhibited at 200 ppm concentration of nanosilver containing in the nanocomposite. Preliminary result of the field experiment showed that Ag/SiO₂ nanocomposite produced can be used to control the fungal disease incidence in soybean farming.

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