FIELD TEST OF CAPABILITY TO PREVENT CABBAGE CLUBROOT DISEASE CAUSED BY *Plasmodiophora brassicae* OF SILVER NANOPARTICLES SYNTHESIZED BY GAMMA RADIATION

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ABSTRACT: The effects of four dose rates 0.27; 0.90; 1.80 and 3.60 kGy/h on the solution of silver (Ag^+ 10^{-2} M, PVP 2%, ethylenglycol 6%) irradiated at 25 kGy were investigated. The results showed that as the dose rates increased, the absorption peak shifted to blue wavelengths and also the particles decreased in size. For field test, nano particles were prepared by irradiation of silver solution at 25 kGy with the dose rate of 3.60 kGy/h. The absorption peaks of the synthesized nanoparticles were obtained at wavelengths of 412 nm and the average diameter of particles were 14 nm. Using two concentrations of 15 and 20 ppm, silver nanoparticles had not affected the growth and development of cabbage but showed antifungal activity against *Plasmodiophora brassicae* cause club root in cabbage. Using nano particles, the clubroot disease index were 9-10% compared to 5% of nebijin (fungicide), and 12% of control. The yield of cabbage were 55 tons/ha, 63 tons/ha and 70 tons/ha for the control, nanosilver group, and nebijin group, respectively.

I. INTRODUCTION

Today, nanotechnology is topical question and interested by scientists. Various methods have been reported for the preparation of silver nanopaticles such as: mechanical grinding, co-precipitation, spraying, electrolysis sol-gel manufacture, irradiation,…These methods are disadvantageous because the size of the particles formed is difficult to control or high cost. On the other hand, γ-irradiation method is advantageous because size, shape and size distribution of particles are easily controlled and the particles may be prepared at the room temperature,..[1,2] First time, silver nanoparticles were produced for the antimicrobial aim of health care [3]. Nowadays, nanoparticles made of silver, have special optical properties that particularly harbour promising applications for medical technology [4]. In agriculture field, nanosilver have ben used for preservation and treatment of diseases. Nanosilver solution was used for controlling *Septoria* leaf blotch, yellow rust, *Fusarium*, and powdery mildew on wheat which showed that nanosilver controls wheat disease. [5]. *Plasmodiophora brassicae*-the casual agent of club root disease of crucifers. Plants infected have the low yield. This disease occurs all year in Dalat region of Lam Dong Province [6,7]. Many ways used to controll this disease include liming the soil, using chemical,…but the results are still restricted [8-10].

In 2009, the project "Radiation induced synthesis of colloidal silver nanoparticles for control of clubroot disease of cabbage caused by *Plasmodiophora brassicae*" was carried out and the results showed that silver nanoparticles prevent *Plasmodiophora brassicae* in cabbage in the laboratory. Based on the good results of the project, we continued to evalue capability to prevent cabbage clubroot disease of silver nanoparticles in field.

II. MATERIALS AND METHODS

Chemical:$AgNO_3$(PA): Merck, Germany

Facilities: Gamma Co-60 radiation source (Issledavachel-Russia), with the dose rate: 27 kGy/h and gamma Co-60 GC-5000 (BRIT,India), with the dose rate: 3.6 kGy/h.

Plant: Cabbage (Shogun)

Fungicide: Nebijin 0.3DP,

The size of the partocles wree determined by transmsstion electron Microscopy (TEM) and UV-vis spectrophotometer analysis. The solution of nano particles were tested to prevent cabbage clubroot disease caused by *Plasmodiophora brassicae* in the field.

III. RESULTS AND DISCUSSIONS

III.1. The effect of dose rate on silver nanoparticles

III.1.1. The UV absorbance of silver nanoparticles

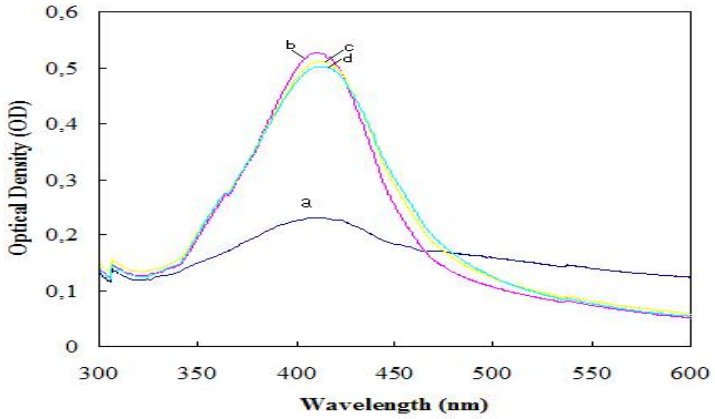
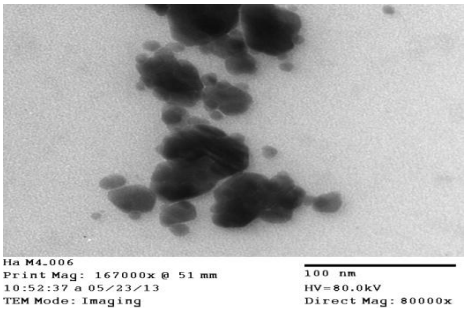


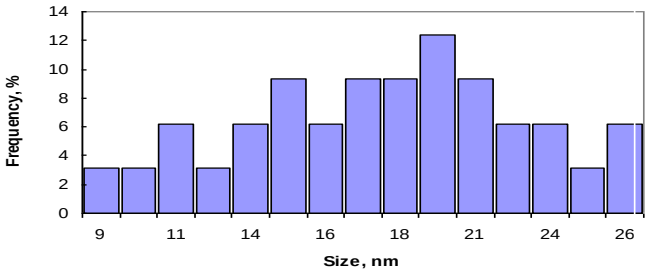
Figure 1: Uv-visible absorption spectra of silver nanoparticle solutions.

a) Dose rate: 0.27 kGy/h, λ_{max} = 418.0 nm
b) Dose rate: 0.90 kGy/h, λ_{max} = 411.5 nm
c) Dose rate: 1.80 kGy/h, λ_{max} = 411.5 nm
d) Dose rate: 3.60 kGy/h, λ_{max} = 411.0 nm

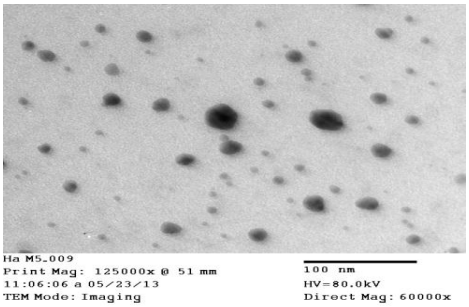
The results in fig.1 show that when the dose rates increased, absorption peak shifted to the blue wavelengths.

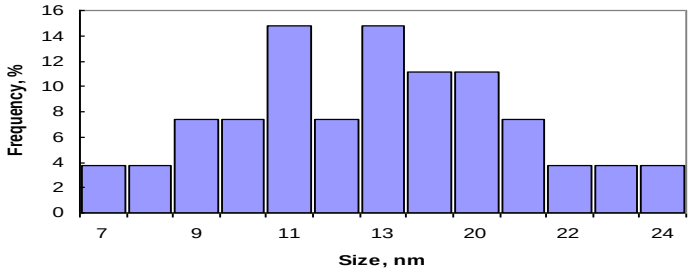
III.1.2. TEM image and silver nanopaticle size distribution



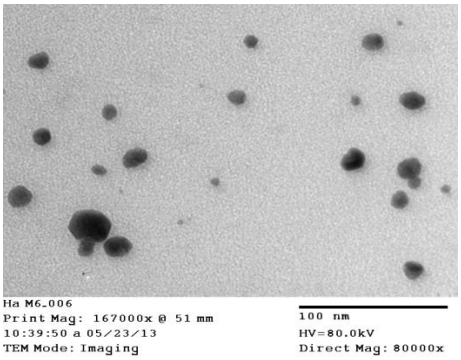


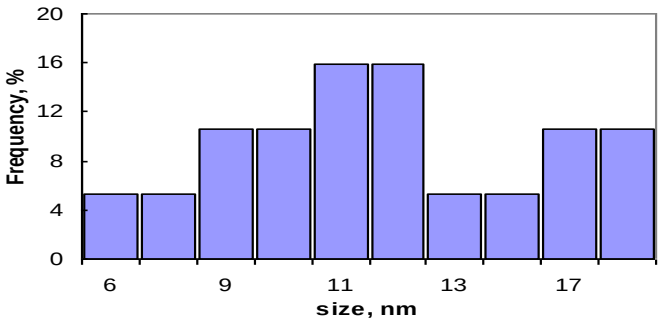
a) Dose rate: 0.27 kGy/h (d_a = 17.85 ± 2.05 nm).



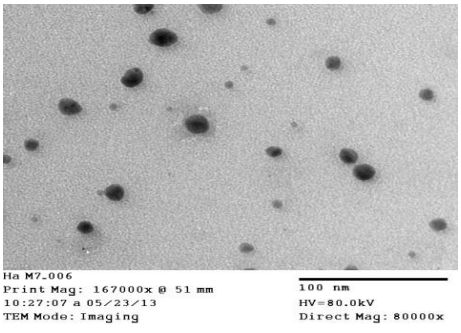


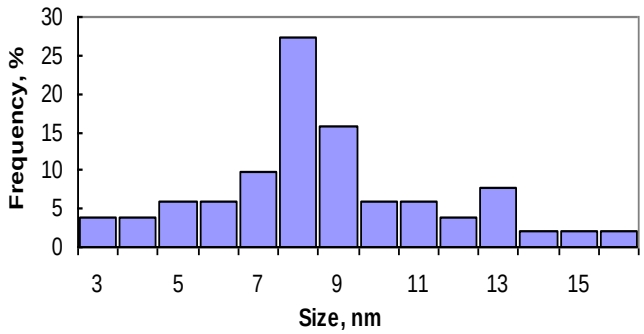
b) Dose rate: 0.90 kGy/h (d_a = 14.74 ± 2.72 nm).





c) Dose rate: 1.80 kGy/h (d_a = 12.21 ± 1.93 nm).





d) Dose rate: 3.60 kGy/h (d_a = 7.97 ± 1.85 nm).

Figure 2 (a-d): TEM image and silver nanopaticle size distribution with different dose rates.

The results showed that size of silver nanoparticles decreases with increasing dose rate.

III.2. Some characteristic of field test silver nanoparticle solution

III.2.1. UV absorbance of field test silver nanoparticle solution

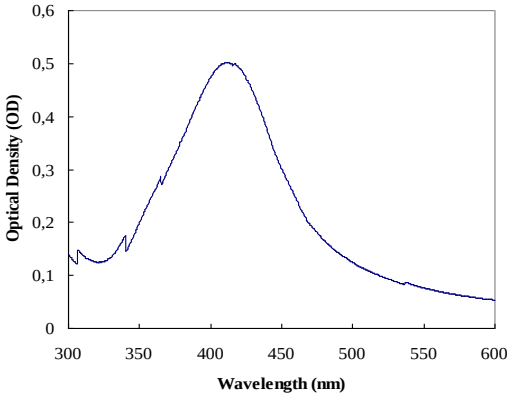


Figure 3: Uv-visible absorption spectrum of field test silver nanoparticle solution.

(λ_{max} = 412 nm, OD = 0.503)

The UV/Vis absorption spectrum of the silver nanoparticle solution is shown in Fig.3. The absorption peak is obtained at the wavelength of 412 nm.

III.2.2. TEM image and silver nanopaticle size distribution

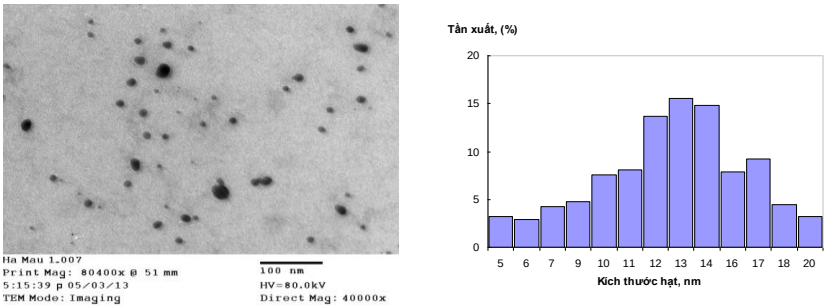


Figure 4: TEM image and silver nanopaticle size distribution.

(d_a = 13.24 ± 2.25 nm)

The average size of silver nanoparticles of field test solution is 13.24 ± 2.25 nm.

III.2.3. Stability of silver nanoparticles with storage time

The silver nanoparticle solution is observed for 3 months and it shows that the absorption peak shifted to red wavelengths at first 40 days. From the day of 41st, the λ_{max} kept at the same wavelength. This indicates that the prepared colloidal gold nanoparticles solution is fairly good stability in 3 months of storage .

III.3. Field testing results

III.3.1. Toxicity of silver nanoparticles on cabbage

Table 2: Toxicity of silver nanoparticle on cabbage.

Variants	Dose	Toxicity (level)		
		1(DAT)	*3(DAT)*	*7(DAT)*
1. Nano silver	15 ppm	1	1	1
2. Nano silver	20 ppm	1	1	1
3. Nebijin 0.3 DP	300 kg/ha	1	1	1

DAT: day after treatment.

Using two concentrations of 15 and 20 ppm, silver nanoparticles has not affected the growth and development of cabbage.

III.3.2. Effect of silver nanoparticles on percentage of infected cabbage

Table 3: Percentage of infected plants.

Variants	Dose	Percentage of infected plants (%)[1]						
		20	30	40	50	60	70	80
1. Nano silver	15 ppm	0.0a	0.0a	5.00a	6.67b	11.67b	15.00b	15.00b
2. Nano silver	20 ppm	0.0a	0.0a	3.33a	5.00b	10.00b	13.33b	14.33b
3. Nebijin	300 kg/ha	0.0a	0.0a	1.67a	1.67b	5.00b	6.67b	6.67b
4. Control	-	0.0a	1.67a	6.67a	16.67a	23.33a	25.00a	25.00a

[1]: day after treatment.

The results in table 3 showed that, silver nanoparticles had antifungal activity against *Plasmodiophora brassicae* cause club root in cabbage.

III.3.3. Effect of silver nanoparticles on the clubroot disease index

Table 4: Effect of silver nanoparticles on the clubroot disease index.

Variants	Dose	Disease index (%)
1. Nano silver	15 ppm	10.67 ± 4.04
2. Nano silver	20 ppm	9.00 ± 3.46
3. Nebijin 0.3 DP	300 kg/ha	4.67 ± 2.08
4. Control	-	21.33 ± 4.51

Using silver nano particles, the clubroot disease index were 9-10% compared to 5% of nebijin (fungicide), and 12% of control.

III.3.4. Effect of silver nanoparticles on yield of cabbage

Table 5: Effect of silver nanoparticles on yield of cabbage.

Variants	Dose	Yield (Ton/ha)
1. Nano silver	15 ppm	63.238
2. Nano silver	20 ppm	63.523
3. Nebijin 0.3DP	300 kg/ha	70.810
4. Control	-	55.619

The yield of cabbage were 55 tons/ha, 63 tons/ha and 70 tons/ha for the control, nanosilver group, and nebijin group, respectively.

IV. CONCLUSIONS

The effects of four dose rates 0.27; 0.90; 1.80 and 3.60 kGy/h on the solution of silver (Ag^+ 10^{-2}M, PVP 2%, ethylenglycol 6%) irradiated at 25 kGy were investigated.

The dose rates increased, the absorption peak shifted to blue wavelengths and also the particles decreased in size.

For field test, nano particles were prepared by irradiation of silver solution at 25 kGy with the dose rate of 3.60 kGy/h. The absorption peaks of the synthesized nanoparticles were obtained at wavelengths of 412 nm and the average diameter of particles were 14 nm.

Using two concentrations of 15 and 20 ppm, silver nanoparticles had not affected the growth and development of cabbage but showed antifungal activity against *Plasmodiophora brassicae* cause club root in cabbage.

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The yield of cabbage were 55 tons/ha, 63 tons/ha and 70 tons/ha for the control, nanosilver group, and nebijin group, respectively.

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