EFFECT OF BIOSYNTHESIZED SILVER NANOPARTICLES ON MORPHOPHYSIOLOGY OF HOST

PRANJAL KR KAMAN, SARODEE BORUAH, HIMADRI KAUSHIK & PRANAB DUTTA

Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam, India

ABSTRACT

Phytotoxicity is an important aspects of nanoparticles in relation to the environment and plants. Here, we report on the effects of biologically synthesized silver nanoparticles (Ag NPs) from Trichoderma asperellum on the development of Mustard, Chili, and French bean. Four toxicity indicators (seed germination, root and shoot length Chlorophyll content and Lipid peroxidation) were quantified following exposure to each nanoparticle at a concentration of 1000ppm, 500ppm, 100ppm, 50ppm, 20ppm, 10ppm, 7ppm, 5ppm, 3ppm, 1ppm and carbendazim @0.3%. Results showed that silver nanoparticles at a concentration of 100 ppm and 50ppm effective in increasing all the growth parameters of the crops as compared to other concentration and carbendazim whereas it showed detrimental effects at a concentration of 1000ppm.

KEYWORDS: Phytotoxicity, Nanoparticle & Growth Parameters

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INTRODUCTION

Recent applications of nanoparticles have concerns over the phytotoxic effect on plant health. Various reports said about the positive and negative aspects of nanoparticles on plant growth. Among positive effects, soybean germination and growth were promoted by a mixture of nano-sized silicon dioxide (nSiO₂) and nano-titanium dioxide (nTiO₂) at low concentrations, which increased nitrate reductase activity, enhanced the ability to absorb water and fertilizer, and stimulated the antioxidant system (Lu *et al.*, 2002). The addition of nTiO₂ of 2.5 to 40 g/kg of soil promoted the growth of spinach, likely by protecting the chloroplasts from ageing during long-term illumination (Hong *et al.*, 2005; Zheng *et al.*, 2005). Similarly, nSiO₂ enhanced the growth of Changbai larch (*Larix olgensis*), and the enhancement, increased with the nSiO₂ concentration up to 500 mg/L (Lin *et al.*, 2004). In contrast, root growth inhibition by 2,000 mg/L nano-aluminum oxide (nAl₂O₃) was reported for five plant species: corn, cucumber, soybean, cabbage, and carrot (Yang and Watts 2005). But still, there is a gap in the phytotoxic effect of nanoparticles on morphophysiology effect on the plant. Here in this experiment, an effort was made to study the effect of biologically synthesized Ag NPs on the plant growth parameters.

MATERIALS AND METHODS

Source of Silver Nanoparticles

Biologically synthesized Ag NPs was collected from Deptt of Plant Pathology size 27.64nm, charge -1.34 MV and crystalline cubic in nature

Source of Seeds

Seeds of Mustard, Chili, and French bean were collected from the local market.

Seed Treatment

Seeds were treated at a concentration of 1000ppm, 500ppm, 100ppm, 50ppm, 20ppm, 10ppm, 7ppm, 5ppm, 3ppm, 1ppm of Ag NPs and carbendazim @0.3% in a beaker and kept for 1hrs.

Plant Growth Conditions

The treated seeds were grown in plastic trays by using a cocopit and kept in a greenhouse condition for 25 days.

Chlorophyll Content

Chlorophyll content was determined by the acetone method. The following calculations will be made to ascertain sample chlorophyll concentrations.

Chlorophyll a [milligrams/milliliter (mg/ml)] = $12.7 (A)_{663} - 2.69 (A)_{645}$

Chlorophyll b (mg/ml) = $22.9 (A)_{645} - 4.68 (A)_{663}$

Where: (A) 645 = absorbance at a wavelength of 645 nm; (A) 663 = absorbance at a wavelength of 663 nm.

Lipid Peroxidation

0.5 g leaf sample was homogenized in 10 ml of trichloroacetic acid (TCA) and centrifuged at 15000g for 5min. From this 2ml of the supernatant and 4ml of 0.5% tribarbutric acid (TBA) in 20% of TCA was mixed. The mixture was heated at 95° C for 30 min and cool at ice bath. Again, it was centrifuged at 1000g for 5min. Record the absorbance at 532nm and 600 nm.

Measurements of Toxic Effects

Germination percentage, root and shoot length, chlorophyll content and lipid peroxidation was measured after 25 days of planting.

RESULTS AND DISCUSSIONS

Seed Germination

Seed germination was found to be increased with the decreased in the concentration of silver nanoparticles. Seed germination percentage was found to be highest at a concentration of 100 ppm (100%) and carbendazim (43.25) at the recommended dose in all the crops. (Figure.1)

Figure 1 (a): Seed Treatment of a French Bean with Ag NPs

Figure 1(b): Seed Treatment of Mustard with Ag NPs

Figure 1(c): Seed Treatment of Chili with Ag NPs

Root Length and Shoot Length

A similar trend was observed in case of root and shoot length that is with decreased in the concentration of Ag NPs the root length and shoot length increases as compared to the carbendazim and control. Root length was found to be 10cm in case of French bean, 5.2 cm in Mustard and 6.2 cm in Chili and shoot length of 20cm in French bean, in Mustard 9cm and in Chili 6.4cm at a concentration of 100 ppm as compared to control (6.0, 3.3 and 2.8cm) and in carbendazim (7.9, 3.4 and 4.2 cm) root length and shoot length (6.0cm, 5.4cm and 3cm) and in carbendazim (8.6cm, 7.2cm and 4.0cm) whereas at 1000ppm concentration it was found to be decreased in both the root and shoot length. The mechanism responsible for enhanced root and elongation is unclear. Thus we can report the minimal toxicity of silver nanoparticles on the tested plants, this is a good evidence for demonstrating response of Ag NPs in Mustard, Chili, and French bean in a limited range (100ppm), above which toxic levels are reached causing subsequent declines in growth. These results agree with Mahajan *et al.* (2011); Mihaela and Dorina (2007) and Seif *et al.* (2011)

Chlorophyll Content

Chlorophyll content was found to be highest at a concentration of 100 ppm, followed by control and carbendazim treatment. Chlorophyll 'a' content in French bean was found to be 0.118mg/l at 100 ppm concentration of Ag NPs and in control 0.107mg/l and in carbendazim 0.037mg/l. Similarly, in case mustard and chili Chlorophyll 'a' content was 0.018mg/l and 0.123mg/l at a 1000ppm concentration of Ag NPs and in control 0.004mg/l and 0.006mg/l and in carbendazim 0.03mg/l and 0.07mg/l.

Chlorophyll 'b' content was found to be 0.183 mg/l in French bean, 0.081mg/l in the mustard and 0.551 in chili.

The increase in the chlorophyll content might be due to the deposition of Ag NPs in the plant cell. The findings are similar to the results obtained from other studies (Karthick and Chitrakala, 2011), they demonstrated that chlorophyll A content was significantly increased by silver nanoparticles in green gram and sorghum. In other studies, response of corn (*Zea mays*) seedlings to the administration of 20, 40, 60, 80 and 100 µl/l of Fe₃O₄ nanoparticles suspension concentration resulted in the diminution of chlorophyll ratio, while *Cucurbita pepo* seedlings exhibited a slight increase of chlorophyll contents (Racuciu *et al.*, 2009). Studies also demonstrated that TiO₂ nanoparticles promoted photosynthesis and nitrogen metabolism, and thus the greatly improved growth of spinach at a concentration as low as 20 mg/l (Hong *et al.*, 2005 and Zheng *et al.*, 2005)

Lipid Peroxidation

In case of French bean, lipid peroxidation was found to be 185.80 mol/cm, in mustard 128.9 mol/cm and chili 332.12 mol/cm at a concentration of 100ppm as compared to control 122.16 mol/cm, 9.2 mol/cm and 141.67 mol/cm. The exact mechanism how Ag NPs increased or decreased in the lipid peroxidation is not known.

CONCLUSIONS

The present study demonstrated the effect of silver nanoparticles on crop plant species Mustard, Chili, and French bean. The presence of AgNPs affects the growth of Mustard, Chili, and French bean at different concentrations. The maximum effect was found at 100 ppm for the three crop plants. Beyond this concentration, the growth was inhibited (1000ppm). The effective growth at certain optimum concentration and inhibited growth beyond this concentration may be attributed to the accumulation and uptake of AgNPs by the roots. It was found that the accumulation and uptake of nanoparticles were dependent on the exposure concentration

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