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Full Length Research Paper

Application of silver nano-particles for protection of seeds in different soils

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To investigate the possibility of using silver nano-particles for protection of a living organism, wheat seeds were nano-coated and planted. Effect of nano-treatment on seed germination and protection against fungi was compared with that of a conventional preplanting fungicide (Carboxitiram). Influence of soil conditions on the effectiveness of nano-seed treatment was also investigated. Soil conditions included different levels of nutrients, pH and moisture content. Results showed that soil conditions do not affect seed protection provided by silver nano-particles against fungi, and this effect is completely independent of soil conditions. Data analysis indicated that silver nano-particles do not reduce seed germinability. Moreover, seed protection afforded by silver nano-particles and Carboxitiram was not significantly different. Thus, nano-coating may be considered as potential preplanting fungicide.

Key words: Silver nano-particles, seed treatment, preplanting fungicide.

INTRODUCTION

Nanotechnology has the potential to revolutionize the scientific world by allowing scientists to manipulate matter at atomic or molecular scale using physics, engineering, chemistry and biology (Roco et al., 1999). Nanotechnology is a broad and interdisciplinary area of research and development activity that has been growing at a rapid pace worldwide in the past few years (Kulzer and Orrit, 2004).

In agriculture, using many monitoring and control systems already in place, nanotechnology devices for CEA[1] providing "scouting" capabilities could tremendously improve the grower's ability to determine the best time of harvest for the crop, crop vitality, and food security issues, such as microbial or chemical contamination (US Department of Agriculture, 2003).

Nanotechnology is being used in various fields such as precision farming, smart delivery systems, packaging, food safety and food processing (ETC group, 2004; Syngenta, 2004 and Dunn, 2004).

Nanotechnology has been utilized in various areas from the production of artificial cell walls (Cybulska et al., 2010) to photo-catalysis. The latter is a reaction in which chemical compounds react in the presence of light and itself not being completely consumed in the reaction. In the presence of UV light the valance electrons and positive holes are strong oxidizers. When harmful substances (pesticides) adhere to positive holes, they are disintegrated into harmless compounds. The excited electrons are also injected in bacteria in contact with nano-particles and hence act as a disinfectant (Blake, 1997; Herrmann, 1999; Peral et al., 1997).

Thermal vision and NIR imaging had made it possible to detect pathogens (Hellebrand et al., 2005). This can be done using nanotechnology today as well (Warad et al.,

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Abbreviations: RS, Number of seedlings remaining; **TP,** total number of seeds planted; **S%,** “survival" percentage.

[1]Controlled Environment Agriculture

2003). Ultra miniaturized identification tags and nano-barcodes (Dejneka et al., 2003; Nicewarner-Pena et al., 2001) are other applications of nano-particles. Today nano-silver provides protection against fungi and bacteria; therefore, it is used to produce safer foods. New nano packages containing silver nano-particles keep foods fresh, healthy and safe. Also, these particles are opening their way into our homes now. Recently, refrigerators, freezers, washing machines and vacuum cleaners use these particles.

The main challenge now is to utilize these new useful particles to treat a living organism. Can seeds be protected against harmful organisms such as fungi in agricultural practice? Is this treatment harmless for the organism? What are the effects of ambient conditions on the effectiveness of seed protection?

This study was designed to answer the above questions. To achieve this goal, wheat seeds were coated with silver nano-particles. Soils having various pH, moisture content and nutrient levels were infected with fungus and then planted in soil. Seed emergence and anti-fungus protection effect of silver nano-particles were measured and data were statistically analyzed using "PASW Statistics18" software.

MATERIALS AND METHODS

In order to achieve the objective of this study, three experiments were designed in which wheat seeds coated with silver nano-particles were used. Then, wheat seeds were coated with silver nano-particles using biogenic silver nano-particles with average size of about 50 nm. The nano-particles were procured from the Biotechnology Research Center; Tehran University of Medical Sciences (Tehran, Iran) produced using culture supernatants of enterobacteria (Shahverdi et al., 2007). Soils having various pH, moisture content and nutrient levels were infected with fungus and the treated seeds were planted in the soil. Seed germination and the protecting effect of silver nano-particles were measured and the obtained data were statistically analyzed using "PASW Statistics18" software. Comparison of the means was undertaken using LSD (Least Significant Difference).

Experiment 1: Effect of nano-coating on seed viability

Percent germination was utilized as the indicator for investigating the effect of nano-coating on seed viability. To compare this index among the treatments including the control (non treated seeds), seeds treated with Carboxitiram (a chemical fungicide), and those treated with silver nano-particles, some seeds were kept non-treated while a batch of seeds was covered with Carboxitiram and another batch was coated with silver nano-particles. To treat seeds with Carboxitiram, a paper bag containing seeds and a conventional fungicide powder, Vitavax (including 200 g/L Carboxin and 200 g/L Thiram) was well shaken to achieve coating. To coat the seeds with silver nano-particles, a 100 ppm nano-silver solution was sprayed on them and left to dry. This was repeated twice to obtain three replications.

Fifteen Petri dishes in 3 sets were provided and lined with wet filter paper. Each set consisted of 5 Petri dishes. According to a completely randomized design with 5 replications and 5 observat-ions per replication, each batch of treated seeds as indicated above was planted in 5 Petri dishes. Each dish having 5 seeds. Thus, 25 non-treated seeds, 25 nano treated seeds and 25 seeds treated with Carboxitiram were used in this part of the experiment. The number of germinated seeds in each dish was counted after 15 days and germination percentage was calculated using Equation 1:

$$\text{Germination (\%)} = \left(\frac{\text{Number of Germinated seeds}}{\text{Number of Planted seeds}}\right) * 100 \quad (1)$$

Experiment 2: Effect of silver nano-particles on seed protection

To determine the effect of silver nano-particles on seed protection, another completely randomized design was implemented using, 10 soil filled pots. Disinfected soil was inoculated with fungus and pots were filled with this soil. Then 5 seeds were planted in each pot. Survival percentage, percentage of seedlings remaining alive after 2 weeks, shown by %S was defined as shown in Equation 2 for comparison:

$$S\ (\%) = \left(\frac{RS}{TP}\right) * 100 \quad (2)$$

Where S% is "Survival" percentage, while RS and TP indicate the number of seedlings remaining and the total number of seeds planted, respectively.

Experiment 3: Effects of soil

Effect of soil nutrients

In this experiment, To investigate the effect of soil nutrient level on seed protection against fungus using silver nano-particles, two seed treatments-Carboxitiram and silver nano-particles, as well as five levels of soil nutrients were provided. These included soil samples containing 100% (the recommended level of nutrients for wheat seeds), 75, 50, 25, and 0% (no nutrients). This experiment was conducted according to a factorial design with 30 plots including 10 treatments (at 2 levels) and 3 replications. Thirty pots were filled with soil samples as described above. So each 6 (set of) pots called "a block of pots" were filled with one of the soil types. Half of each block (3 pots) was planted with seeds treated with Carboxitiram and the other half was planted with nano-treated seeds. Since the experimental plan was designed with 5 observations per treatment, 5 seeds were planted in each box. The effect of soil nutrient level on seed protection was studied by calculating the S index using Equation 2.

Effect of soil pH

In the fourth part of study, Effects of soil pH and soil moisture content on seed protection were investigated using a factorial experimental design. In this experiment, soil pH at 3 levels (Acidic, Normal and basic) and seed treatment at two levels (Carboxitiram and nano-silver treated) were studied in 3 replications. Thus, 18 pots were provided forming three blocks of 6 pots each. Pots of each block were filled with one of the soil treatments. Since the experimental plan was designed with 5 observations per plot, each pot in half of the block (3 pots) was planted with 5 seeds treated with Carboxitiram and each pot in the other half was planted with 5 seeds treated with silver nano-particles.

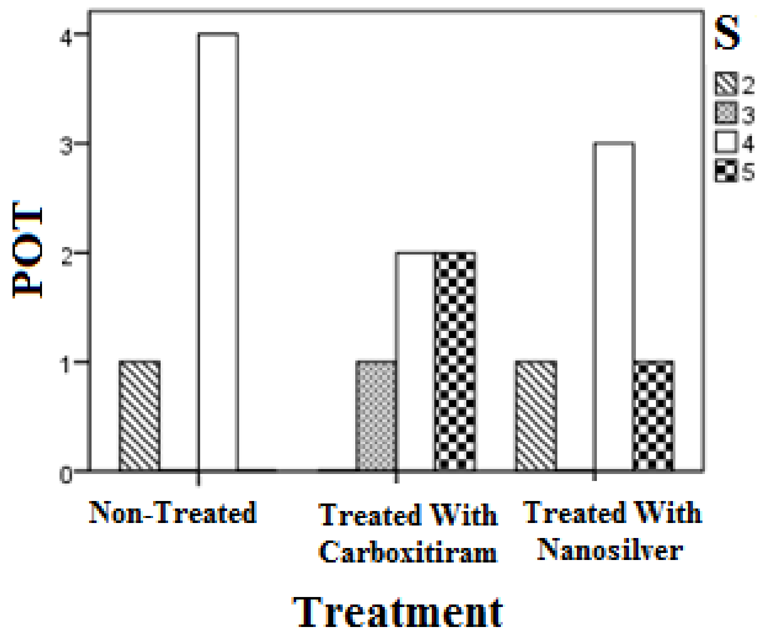

Figure 1. Effect of seed treatment on wheat seed germination (viability). In this Figure, "S" denotes the number of seeds germinated in each pot originally planted with 5 seeds. The label "pot" indicates the numbers of pots with the same value of S. Five pots were used for each treatment. For example, the results of non-treated (control) pots indicate that there were 4 pots with 4 germinated seeds (out of 5 planted) and 1 pot with one germinated seed. This is a common method for extracting the results from the following figures too.

Effect of soil moisture content

The experimental design to investigate the effect of soil moisture content was the same as that applied to study the effect of soil pH. But this time each block of pots was filled with soils having different levels of moisture content including 5, 20 and 40% of soil's dry weight. The pots were planted in the same manner as that indicated for pH.

RESULTS

Results of germination test are shown in Figure 1[2]. According to the statistical analysis of the data based on LSD and with 1% probable error, there was no significant difference between treatments. Based on these results, although silver nano-particles have been known as a kind of antibiotic, wheat seeds treated with these particles not only germinate like seeds treated with the fungicide Carboxitiram, but also they do as well as untreated seeds. Therefore, it is concluded that use of silver nano-coating does not adversely affect the ability of seeds to germinate.

Figure 2 shows the results of protection effect test. Statistical analysis of these data with certainty of 99% confirms that there is no significant difference between treatments in this test as well. Thus, silver nano-particles and the well know fungicide Carboxitiram can protect the seeds against fungi to the same extent. The possibility of using silver nano-particles instead of common chemical fungicides such as Carboxitiram is the most important outcome of this test.

Results of the effect of soil moisture content, pH and nutrient level are presented in Figures 3 to 5, respectively. According to the output of the LSD test, the effects of all levels of nutrients on seed protection afforded by the treatments were the same. In other words, soil nutrient level does not affect seed protection significantly.

Although, basic (alkaline) soils and soils with less moisture content (5%) were different than other treatments significantly (Appendix A), this was not due to the effect of silver nano-particles. This issue is discussed in the discussion section.

These recent results show that the protective effect by silver nano-particles is independent of the soil environment. Based on this new discovered information, it is not necessary to gather additional information about seed growth under field conditions before treating them with nano-silver.

DISCUSSION

Attention to the following challenges is necessary to

[2]For a better understanding, attention to the explanation below the Figure 1 is recommended.

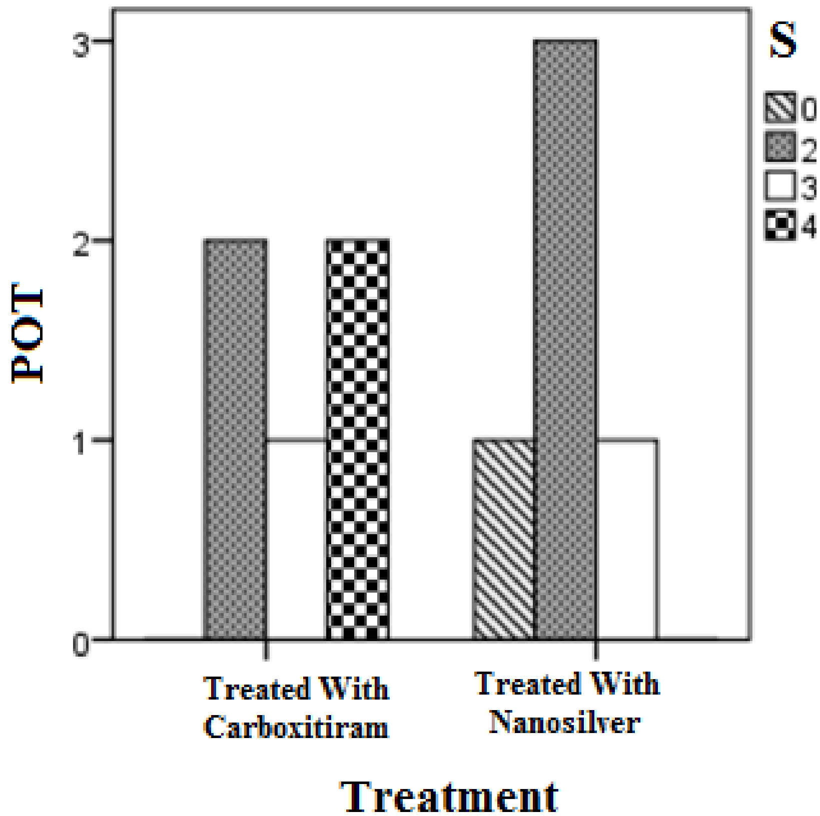

Figure 2. Effect of silver nano-particles on seed protection.

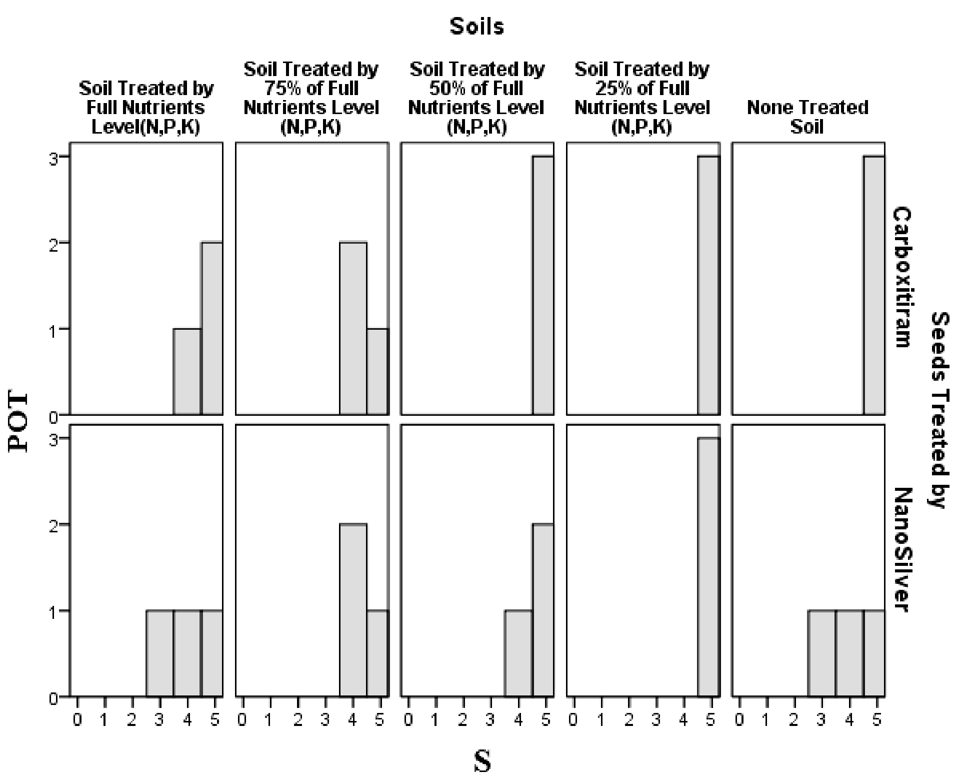

Figure 3. Effect of soil nutrient level on seed protection.

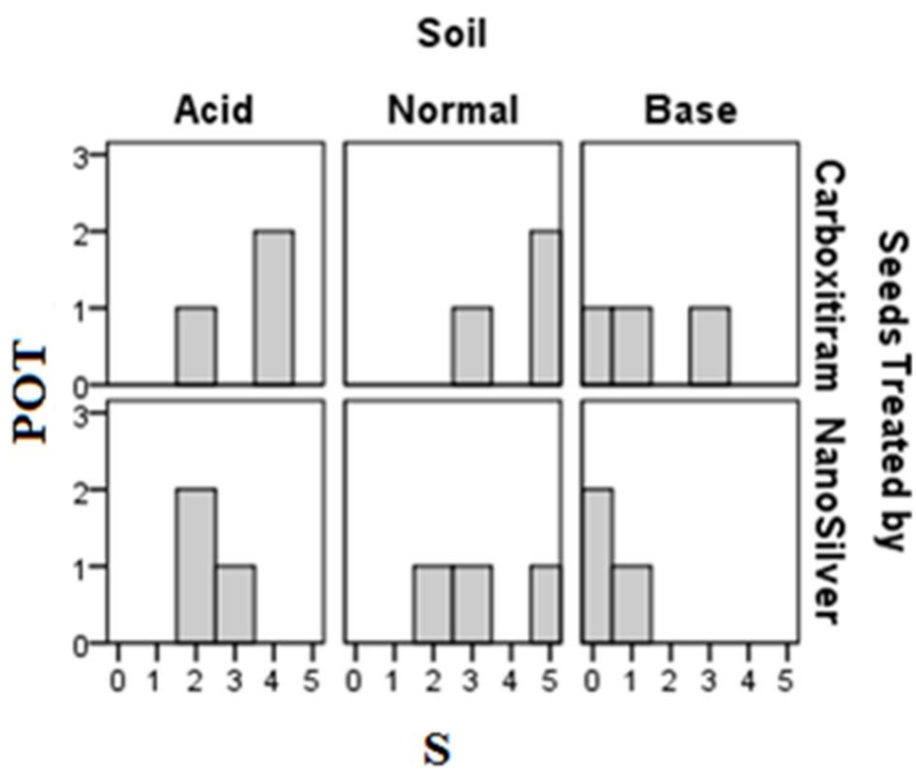

Figure 4. Effect of soil pH on seed protection.

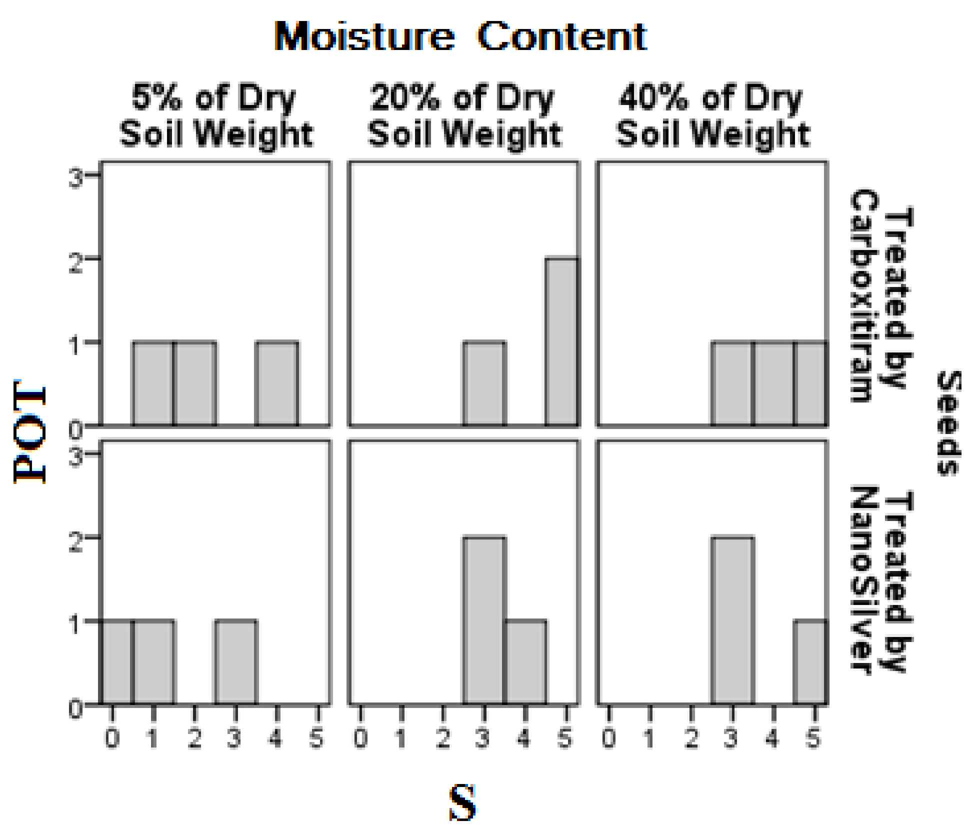

Figure 5. Effect of soil moisture content on seed protection.

utilize seeds treated with sliver nano-particles instead of seeds treated with conventional fungicide, Carboxitiram.

1. Silver nano-particles should not adversely affect the seeds ability to germinate.
2. The protective effect of these particles against fungi should be more than or at least equal to that of the fungicide utilized conventionally.
3. These particles must be safe for human consumption.

Since results showed that treating seeds with silver nano-particles does not reduce germination, it is possible to use this treatment in agricultural practice. In other words, silver nano-particles did not adversely affect the seed living process. Thus, the answer to the first question posed in the introduction is clear: Wheat seed as a living organism can survive treatment with silver nano-particles.

Results of protection test indicate that silver nano-particles may be an alternative to conventional fungicides for protecting seeds against fungi because no significant difference for this factor was observed. Thus, nano-treated seeds can be used in precision planters to lower the environmental impacts of fungicides and reduce the cost of agricultural production. The nano-particles used in the experiments were biogenic silver produced by a biotechnology research institute in the college of pharmacy indicating that it has pharmaceutical applications. Therefore, it is not unsafe for human consumption.

Based on the results, it is clear that soil nutrients level does not significantly affect seed protection afforded by nano-particles. At the first glance, alkaline soils and soils having less than 5% moisture content seam to decrease the positive effect of silver nano-particles (Appendix, B).

However, the similar effect of nano-particles and Carboxitiram fungicide on seed protection indicates that low germination is due to low soil moisture content and high pH and not due to the nano-treatment of seeds. Hence, it is concluded that the protective effect of nano-particles is independent of soil conditions.

Conclusions

Silver nano-particles do not adversely affect the seed living process. Silver nano-particles can protect seeds against fungi as well as the conventional fungicide, Carboxitiram and seed protection afforded by silver nano-particles is independent of soil conditions including nutrient level, pH and moisture content.

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Appendix A. Statistical analysis of soil pH.

(I) Soil	(J) Soil	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
Acid	Normal	-1.00	.667	.159	-2.45	.45
	Base	2.00*	.667	.011	.55	3.45
Normal	Acid	1.00	.667	.159	-.45	2.45
	Base	3.00*	.667	.001	1.55	4.45
Base	Acid	-2.00*	.667	.011	-3.45	-.55
	Normal	-3.00*	.667	.001	-4.45	-1.55

Based on observed means. The error term is Mean Square (Error) = 1.333. *. The mean difference is significant at the 0.05 level.

Appendix B. Statistical analysis of soil moisture content.

(I) Moisture	(J) Moisture	Mean difference (I-J)	Std. error	Sig.	95% Confidence Interval	
					Lower bound	Lower bound
5% of dry soil weight	20% of dry soil weight	-2.00*	.694	.014	-3.51	-.49
	40% of dry soil weight	-2.00*	.694	.014	-3.51	-.49
20% of dry soil weight	5% of dry soil weight	2.00*	.694	.014	.49	3.51
	40% of dry soil weight	.00	.694	1.000	-1.51	1.51
40% of dry soil weight	5% of dry soil weight	2.00*	.694	.014	.49	3.51
	20% of dry soil weight	.00	.694	1.000	-1.51	1.51

Based on observed means. The error term is Mean Square (Error) = 1.444. *. The mean difference is significant at the 0.05 level.