Biosynthesis of zinc nanoparticles from the fungus Aspergillus niger and testing its effect with aqueous extract of sesban plant on bean plant treated with fungus *Macrophomina phaseolina*

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Abstract

he study included testing the effectiveness of biosynthetic zinc nanoparticles from the fungus A.niger and the aqueous extract of sisban blocks on plant height, number of branches and dry weight of beans infected with charcoal rot disease caused by the fungus *Macrophomina phaseolina*. The effect of nanoparticles and aqueous extract was tested at three concentrations (1000, 2000 and 4000 ppm).. The results showed that high concentrations of nanoparticles contribute to supporting plant growth and increased by a significant difference from the control treatment. When testing its effectiveness in increasing plant height, it was found that the highest height was when treated with nanoparticles loaded on the fungus A.niger (Z1), as it reached Plant length 27.7 As for its effect on dry weight, the best treatment was nanoparticles loaded on *A.niger* fungus mixed with sesban plant (Z1S0), as the weight reached 2.6 g. Also, the treatment Z1S0 gave the best result in increasing the number of branches, as it reached 5.2.

Introduction

Macrophomina. phaseolina is a common fungus spread all over the world, infecting nearly 500 species of plants belonging to more than 100 families and causing many diseases such as stem and root rot and charcoal rot (Ghosh, et al. 2018). Under unfavorable environmental conditions such as high temperatures (30-35%) and humidity less than 60%, this fungus can cause significant crop losses. Despite the efforts of many researchers to control the disease, integrated management strategies still represent a challenge. The infection of the plant occurs as a result of the interactions between the host, the pathogen, and the living and non-living environmental factors. One of the important crops that M. phaseolina parasitises is the bean plant, which is one of the economically important and most common legume plants for human consumption, because it contains carbohydrates, proteins, minerals, fibers, and fats. Therefore, it is a healthy food that provides the body with many benefits, as it lowers cholesterol levels, improves digestive system functions, and strengthens the immune system (Mustafa, 2010). The steady increase in the population and the rapid growth in urbanization and industry has led to an increase in pollution and environmental damage, in addition to the presence of great challenges facing countries in providing food for this huge population, which has led them to the

excessive use of fertilizers and chemical pesticides, and this in turn is reflected negatively on the soil and the beneficial neighborhoods that live in it. In addition to its pollution of water and air, because these substances are difficult to decompose, and their accumulation leads to a decrease in soil fertility, an increase in salinity in it, and a decrease in its ability to retain water (Savci, 2012). It also loses its effectiveness after a period of use. Therefore, several treatment methods have been proposed as alternatives to chemical methods, such as cultivation of resistant varieties, rotation of pest hosts, removal of root crop residues and the use of biocontrol agents (Bhau et al., 2016) and biofertilizers. One of the promising and commonly used methods as alternatives to chemical pesticides is the use of nanotechnology, which has revolutionized various sciences and is widely used in many fields such as medicine, pharmacy, food industry and agriculture. It is known that plant diseases caused by various factors are among the main factors limiting crop yields worldwide, so the use of modern technologies such as nanotechnology in various fields of agriculture will revolutionize them (Bhau et al., 2016).

Materials and methods:

Culture media for fungi:

Potato dextrose agar (PDA) The medium was prepared according to the manufacturer's instructions by dissolving 39 g of the powder in one liter of distilled water, and sterilized by autoclave at a temperature of 121 °C and a pressure of 1.5 bar for 20 minutes. At room temperature and kept in the refrigerator until use. This medium was used to grow Aspergillus niger and Macrophomina phaseolina.

liquid potato broth.

The media was prepared according to the manufacturer's instructions by dissolving 27 g of the powder in one liter of distilled water and sterilized in an autoclave at a temperature of 121 C and a pressure of 1.5 bar for 20 minutes. After cooling, the antibiotic streptomycen was added at a rate of 100 mg / L and kept in the refrigerator until use.

Preparation of A.niger fungus filtrate:

The PDB liquid culture media was prepared according to the manufacturer's instructions by dissolving 24 g of the culture medium in 24 g of the culture medium in 1000 ml of distilled water and distributed in a 500 ml beaker, then it was sterilized by the electric autoclave, then left to cool at room temperature and the antibiotic streptomycin was added and inoculated The decanters were placed in discs with a diameter of 0.5 of mushrooms and incubated at a temperature of 28 °C for 21 days.

Preparation of zinc nanoparticles from the fungus A.niger:

Zinc nanoparticles were prepared from the fungus filter A.niger by separating the biomass from the filter using Whatman.NO.1 filter paper, then passing it through 0.22 μm Millipore cell membranes, then preparing 1 M of aqueous zinc sulfate Znso4.H2O and 1 M of NaOH, then Add gradually and using a micropipette 100 ml of zinc sulfate solution to 100 ml of fungus filtrate with continuous stirring on the hot plate magnetic stirrer and after adding all the solution to the filtrate, drops of NaOH solution were added with continued addition and stirring until a precipitate is formed and the PH reaches To 5, then the

precipitate is placed in the incubator for 24 hours at a temperature of 28 ° C, then the separation was done by a centrifuge at a speed of 5000 revolutions per minute for half an hour, then the precipitate was poured into a filter paper and washed 3 times with distilled non-ionic water and dried by art at a temperature of 70 ° C (Kalpana et al. 2018, Al-Obaedi, et al.,2022)

Preparation of sesbanum extract

Weigh 50 gm of the plant and wash it 5 times with anionic distilled water, then the powder is placed in a 400 ml beaker, 300 ml of anionic distilled water is added to it, and the mixture is boiled with the lid covered for 40 minutes from the start of boiling until the water evaporates to obtain a little filter, then it is filtered with filter paper to obtain A clear solution and placed in the oven at a temperature of (90) for the purpose of drying and obtaining a powder (Kour, 2020

Preparation of treatment concentrations

Concentrations of 1000, 2000, and 4000 ppm were prepared by adding 0.1 g, 0.2 g, and 0.4 g of the prepared nanoparticles in 100 ml of distilled anionic water, then stirring them on a hot-plate magnetic stirrer, then adding them to plastic pots to test their effectiveness.

Soil preparation and sterilization

Mixed soil was used from one of the fields of Samarra district, as it was cleaned and sifted from the remains of plants and bushes. The soil was moistened and sterilized with formalin at a concentration of 5% of the commercial solution (37%), covered with polyethylene for 7 days, then ventilated for 3 days to get rid of formalin fumes, after that the soil was packed in 1 kg perforated plastic pots.

Preparation of fungal inoculum The seeds of local millet, Panicum miliaceum L., were used for the purpose of preparing fungal inoculants and loading the seeds with fungi to be used. The seeds were washed well with water several times to get rid of dust and impurities, and left immersed in water for the next day, where the excess water was removed using gauze cloth, then divided into 50 gm in each 250 ml beaker, and 15 ml of distilled water was added to it to moisturize it, then it was sterilized with an autoclave under a temperature 121 C and a pressure of 1 atmosphere for an hour, then the flasks were inoculated by putting (the diameter of each tablet 0.5 cm) from the fungus growing agar at the age of 7 days. Then the flasks were placed in an incubator at a temperature of 25 ± 2 C for a period of 10 days, taking into account that the flasks were shaken every 3 days to distribute the fungal inoculum to all the seeds and not to clump (Dewan, 1988).

Soil inoculation with fungi

50 gm of fungal inoculum loaded on millet seeds was added to 5 kg of soil, and it was mixed with sterilized soil. The soil contaminated with the fungus was divided into three replicates, so that 5 kg of soil contaminated with the fungus were placed in each pot. Thus, the process was repeated for all treatments, then the soil was slightly moistened and covered. The pots were opened with polyethylene nylon to preserve moisture and left for a week to ensure the spread of fungus in the soil

Plant cultivation

The bean seeds that were obtained from the local markets of the city of Samarra were sown on 20/8/2022, as they were distributed evenly in the soil, then 10 seeds were planted in each pot, and after germination, the number of plants was

reduced to avoid crowding between the plants, so that the number in each pot became 3 plants, and the treatments were added in concentrations mentioned after planting the seeds with the addition process repeated every two weeks.

characteristics of vegetative growth

Plant height (cm): Measure the plant height using a metric ruler, starting from the soil surface to the top of the plant

The number of plant branches (plant branch - 1). The number of branches for girls was calculated.

Dry weight of the shoot of the plant (gm plant-1) According to the dry weight of the plant after carefully uprooting it from the soil, washing it and cleaning it from the dust attached to it, and then placing the shoots of each plant in an electric oven at a temperature of 70 m for a period of 48-72 hours until the weight is confirmed, then weighed on a sensitive scale.

Results and discussion

plant height The results of Table (1) show the effect of the treatments on plant height with three different concentrations for each treatment. When Z0 treatment was added, an increase in plant height appeared with a significant difference from the control treatment, as the highest increase was 26.1 cm in the Z0C3 treatment, while the lowest increase was 24.8 cm in Z0C1 and it did not appear Significant differences between the heights when increasing the concentration. As for the treatment Z1, it gave a significant increase in the height of the plant compared with the control treatment. Focus Z1C1, reaching 28.5 cm. When the plant was treated with S0, it was observed that the treatment S0 C3 gave the highest plant length of 25.6 cm, with a significant difference from the treatment S0 C1 and S0 C2, while the lowest height was 23.6 cm at S0C1, but when the treatment Z1S0, the two treatments with concentrations Z1S0C2 and Z1S0C3 were significantly superior to both the control treatment and the treatment Z1S0C1 The highest plant length was 28.1 cm at Z1S0C3 and the lowest length was at Z1S0C1 concentration. The treatment Z0S0, the results did not show a significant difference between the three concentrations used when doubling them, but it gave a significant difference from the control treatment, and the highest plant length was 29.8 cm in Z0S0C3, while the lowest length was 28.1 in Z0S0C1 The results of the comparison between all treatments at 1000 ppm concentration showed that Z1 and Z1S0 were the best in increasing the plant height as it reached the highest height of 28.8 cm and the lowest length was in the S0 treatment as it reached 23.6. When doubling the concentration to ppm 2000, the highest plant length was for treatment Z1, as the plant length reached 30 cm, with a significant difference from the rest of the treatments. As for the lowest length, it was in treatment Z0 if the plant length reached 25 cm. When the treatment with ppm 4000 concentration, the highest plant length was 30.1. cm for the nano-zinc treatment Z1, while the least length was 25 cm for the treatment S0 When comparing the averages of overlap between concentrations for each treatment, the highest value was in treatment Z1 with a non-significant difference from Z1S0 with a value of 27.7 and 27.3, respectively. As for the comparison between the averages of concentrations of all treatments, C2 and C3 gave the highest value of 27.4 and 27.7, respectively, with a difference Significant

for treatment with concentration C1, reaching 26.4 Some studies indicate that the process of employing zinc nanoparticles to support plant growth is affected by many factors, including the type of plant, the nature of the soil, the pH value, the abundance of water, and the availability of other elements. Despite the important role of zinc in plant growth, its excess may lead to a toxic effect on plant cells. Its toxicity is based on the fact that it competes for binding sites for biologically active ions, which leads to a decrease in the absorption of iron Fe + 2 and Fe + 3, reducing plant biomass and inhibiting root growth and may lead to stunting. As supports for plant growth and inhibitors of plant and animal diseases alike, such as alkaloids, amides, cyclopeptides, pyranones, etc. In addition, the leaves of the sesban plant contain a good number of biologically important compounds such as flavonoids, tannins, and terpenes (Abdelaziz Amer, 2021). The results of Rizwan (2019) if they found that the addition of zinc nanoparticles led to an increase in the height of wheat plants compared to the addition of conventional zinc.

Table 1: Effect of treatment with different concentrations of zinc nanoparticles and sesbanum extract on the height of bean plant

Tretment	R	C1	C2	C3	Average
Z0	22.50B± 2.3	24.8 Ac ± 1.3	25.0Ac±1.3	26.1 Ac ±0.2	24,6±1.2c
Z1	22.50C± 2.3	28.5 B a ± 1.3	30.3Aa± 1	30.16A a ±0.7	27.7±1.3a
S0	22.50C± 2.3	23.6BC d ±0.7	24.1 B d± 0.76	25.66Ad ±0.5	23.9±1.1d
Z1S0	22.50B± 2.3	28.8 Aa ± 0.2	29.1 Aa± 1.	28.83A b ±1.0	27.3±1.1a
Z0S0	22.50C± 2.3	26.6 Bb ±1.0	26.8 ABb ±1.	28.16Ab ±0.7	26.2±1.2b
Average	22.5±2.3c	26.46±b	27.40±1a	27.78±0.6a	

plant dry weight The results appear in Table (2) the effect of the treatments on the dry weight of the bean plant. When Z0 treatment was added with three concentrations, significant differences were observed between C2 and C3 on the one hand, and C1 on the other hand, as the highest weight was 2.27 gm in the Z0C3 treatment and the lowest weight was 1.1 gm in Z0C1. No significant difference was observed between treatment R and treatment Z0C3. As for treatment Z1, significant differences were found between the added concentrations, as the highest plant weight was 2.7 gm at concentration Z1C3 and the lowest weight was 2.3 gm at Z1C1. In treatment S0, the highest weight was 2.2 g when adding S0C3 with a significant difference from the rest of the concentrations, while the lowest weight was 1.5 g when adding S0C1. When adding the treatment Z1S0, the treatments were almost equal in effect, as no significant difference was observed between the treatments. The highest weight was 2.56 gm when the treatment was Z1S0C3, and the lowest weight was 2.5 gm when the treatment Z1S0C1. As for Z0S0, the highest weight was at the Z0S0C3 concentration, reaching 2.5 g with a significant difference. For the two treatments Z0S0C3 and Z0S0C3 the lowest weight was 1.9 g. When comparing Z0, Z1, S0, Z0S0, and Z1S0 at a concentration of ppm 1000, the highest weight was 2.5 g in treatment Z1S0, with a significant difference from the rest of the treatments. The lowest weight was found in treatment Z0 and reached 1.1 g, and when

the concentration was increased to ppm 2000, the The highest weight was 2.6 gm in Z1S0 and the lowest weight was found in treatment Z0S0 as it reached 1.8 gm. When the plant was treated with a concentration of ppm 4000, the addition of treatment Z1 and Z1S0 had a significant superiority over the rest of the treatments, as the highest weight was 2.7 gm, while the lowest was 2.2 gm when the treatment S0. The results of the interaction between the concentrations for each treatment show that the highest value of dry weight was at treatment Z1 and Z1S0, which amounted to 2.5 and 2.6, respectively, with a significant difference from the rest of the treatments. A value of 2.5 g, with a significant difference from treatment C1, as it reached 1.9 The above results show that high concentrations of zinc nanoparticles give a significant increase in the dry weight of the plant compared to the high concentrations and the control treatment. 20ppm compared with the higher concentrations (80 ppm and 160 ppm), while it came in agreement with a study by Srivastv (2021) (2022) in that the addition of nano-zinc to wheat plants led to an increase in their ability to withstand salt stress and increased the building of plant biomass. The high concentrations ((800 and 1000 ppm) were better in increasing the dry weight of pea plants compared to the lower concentrations (100, 200, 100 and 600). Table 3 Effect of treatment with different concentrations of zinc nanoparticles and sesban plant extract on the dry weight of the plant

Table 2 Effect of treatment with different concentrations of zinc nanoparticles and sesban plant extract on the dry weight of the plant

Tretment	R	C1	C2	C3	Average
Z0	1.15Be±0.3	1.11Be ± 0.4	2.14Ac ± 0. 7	2.27Ab ± 0.3	1.8 ±0.4c
Z1	1.15D± 0.3	2.34Cb ±0.3	2.58Bca ± 0.13	2.78Aa ± 0. 9	2.5±0.4a
S0	1.15D± 0.3	1.54Cd ± 0.2	1.9Bc ± 0.24	2.25Ac ± 0.28	1.9±0.2c
Z1S0	1.15C ±0.3	2.50Aa ± 0.20	2.61Aa ± 0.26	2.65Aa ± 0.26	2.6±0.2a
Z0S0	1.15 C±0.3	2.07Bc ± 0.21	1.8Bc ± 0.11	2.53Aab ± 0.4	2.1±0.2b
Average	1.15±0.3C	1.91±0.2B	2.19±0.2B	2.50±0.4A	

The number of branches of the plant

The results show in Table (3) the effect of the treatments on the number of branches of the bean plant. When the plant was treated with Z0 and with three concentrations, it was observed that there were no significant differences between Z0C3 and treatment R, while the differences were observed between Z0C1 and Z0C2, and the highest number of branches was 4.3 at concentration Z0C3 and the lowest number was 3.3 at concentration Z0C3. The concentration Z0C3 and at treatment Z1 did not show significant differences between the concentration Z1C1 and the control treatment, while differences were found between Z1C3 and the control treatment, as the value of 5 branches at Z1C3 and the lowest value was 4.1 at Z1C1, but when adding treatment S0, the highest number of branches was 3.6 S0C3 with a significant difference from the rest Concentrations either the lowest number was 3.5 branches when adding focus S0C1 and S0C2. In

treatment Z1S0, the highest number of branches was at Z1S0C3 and the number was 5.6 with a significant difference from the rest of the treatments and the control treatment, and the lowest number of branches was found in treatment Z1S0C1 and it was 5. As for the Z0S0 treatment, the highest number of branches at Z0S0C3 was 4.6, and the lowest number was observed at Z0S0C1 and it was 4, and significant differences appeared only between each of the treatment R and Z0S0C1. When comparing all treatments with concentration ppm 1000, the highest number of branches was found in Z1S0, as it reached 5 branches, with a significant difference from the rest of the treatments. Less, it was found in treatment Z0, and reached 3.3 branches. When increasing the concentration, ppm 2000, the highest number of branches was in treatment Z1S0, as it reached 5. Branches and the least number was found in the transaction Z0S0, as it reached 3.8. When the plant was treated with 4000 ppm concentration, the highest number of branches was at Z1S0 and the number of branches was 5.6 with a significant difference for the rest of the treatments, while the lowest number was 3.6 when treatment S0. The results also showed when comparing the overlap between the concentrations for each treatment, that the highest number of branches was Z1S0, reaching 5.2, while the lowest value was when treating both S0 and z0, reaching 3.5 and 3.6, respectively. As for the average concentrations of all treatments, it showed that there were no significant differences between each of the two concentrations, C2 and C3, and the control treatment. The reason for the slight effect of the biosynthetic nano-zinc on the dry weight and the number of branches of the plant may be attributed to the fact that its absorption by the plant takes place through the roots either in the form of ions or in a form associated with organic matter, and then it is transmitted through the xylem to the aerial parts of the plant and that the fungus infects the roots The stems reduce the efficiency of zinc transfer from the soil solution to the parts of the plant, and the transfer process takes place through protein carriers specialized in transporting minerals that are stationed in the plasma membrane of the cells of the vascular bundles of the root (Hussain et al., 2004; AL-Samarraie, et al.,2021). Therefore, the damage caused by the penetration of the fungus into the plant tissues affects the process Absorption and utilization of zinc in building plant biomass.

Table 3 Effect of treatment with different concentrations of zinc nanoparticles and sesbanum extract on the number of branches

average overlaps	C3	C2	C1	R	experiments
3.6±0.35c	4.33A b ±0.5	3.9B b ±0.5	3.3 C b ±0.2	4.03AB±0.2	Z0
4.6±0.4b	5.0A a ±0.1	4.6AB a ±0.5	4.16Bb ±1.4	4.03B ±0.2	Z1
3.5±0.4c	3.6AB c ±0.5	3.5B b ±0.5	3.5Bb ±0.8	4.03A ± 0.2	S0
5.2±0.4a	5.67Aa ±0.5	5.0B a ±1	5.0B a ±0.5	4.03C ±0.2	Z1S0
4.3±0.4b	4.6A a ±0.5	4.3AB b ±0.5	4.0Bb ±0.5	4.03B±0.2	Z0S0

	4.72±0.4A	4.26±0.6A	3.99±0.6B	4.3±0.2A	average overlaps

References

1. **Al-Obaedi, A. I., Ahmed, N. M., AL-Samarraie, M. Q., & AL-Azzawie, A. F. (2022).** Genetic Diversity of Pathogenic Fungi Aspergillus flavus Isolates Using Random Amplified Polymorphic DNA-Polymerase Chain Reaction. **Journal of Drug Delivery Technology, 12(3), 1261-1265.**
2. **AL-Samarraie, M. Q., AL-Obaedi, A. I., Hamed, N. M., & AL-Azzawie, A. F. (2021).** Molecular Identification of Aspergillus nigar Using Randomly Amplified Polymorphic Deoxyribonucleic Acid Polymerase Chain Reaction Technique. **Journal of Drug Delivery Technology, 11(4), 1221-1224.**
3. **Abdelaziz Amer M. · Salem S. Salem · Ahmed M. A. Khalil · Deiaa A. El-Wakil Hossam M. Fouda · Amr H. Hashem.2022**. Potential of biosynthesized zinc oxide nanoparticles to control Fusarium wilt disease in eggplant (Solanum melongena) and promote plant growth. **Biometals Journal .** Springer **.35:601–616**
4. **Bhau, B., P. Phukon, R. Ahmed, B. Gogoi, B. Borah, J. Baruah, D. Sharma and S. Wann 2016** . A novel tool of nanotechnology: nanoparticle mediated control of nematode infection in plants. Microbial inoculants in sustainable agricultural productivity, Springer**:** 253-269.
5. **D. Hussain, M.J. Haydon, Y. Wang, E. Wong, S.M. Sherson, J. Young, J. Camakaris, 2004**. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis, Plant Cell, 16 1327-1339.
6. **Ghosh, T., Biswas, M. K., Guin, C., and Roy, P. 2018** . A review on characterization, therapeutic approaches and pathogenesis of Macrophomina Oxidative stress and cadmium concentration in wheat. Chemosphere 214, 269–277.
7. **Kalpana VN, Kataru BAS, Sravani N, Vigneshwari T,Panneerselvam A, Rajeswari VD. 2018**.Biosynthesis of zinc oxide nanoparticles using culture filtrates of*Aspergillus niger*: Antimicrobial textiles and dye degradation studies. *Open Nano.*;3:48-55.
8. **Mustafa,M.A.A.F. 2010.**Vegetables (food- prevention – Medication) knowledge Library grove, Egypt. Pp. 552
9. **Rizwan, M., Ali, S., Ali, B., Adrees, M., Arshad, M., Hussain, A., et al. 2019.**
10. **Savci, S., 2012**. An agricultural pollutant: chemical fertilizer. Int. J. Environ. Sci. Dev. 3, 77–80.
11. **Srivastav, A.; Ganjewala, D.; Singhal, R.K.; Rajput, V.D.; Minkina, T.; Voloshina, M.; Srivastava, S.; Shrivastava, M. 2021Effect of ZnO Nanoparticles on Growth and Biochemical Responses of Wheat and Maize.** *Plants***,** *10***, 2556.**