Effect of spraying nano zinc on the growth of two cultivars of broad beans infected with alternarial spote

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Abstract:

The results of the research showed that nano zinc are highly effective in inhibiting the growth of the fungus *Alternaria alternata*, which causes alternarial leave spot in beans. It was found that the concentration of 20 micrograms/ml was the best, as the inhibition rate was 100% for the fungal isolate used when treated with ZnON-Ps compared to the control treatment. which reached 0% . The field experiment was carried out to study effect of adding nano-zinc oxide on broad bean growth indicators. The results of the field experiment showed the superiority of spraying with nano-zinc oxide in all treatments, as the lowest percentage of infection with the fungus *A. alternata* in nano-zinc oxide treatment was 28.33%.Compared to the fungi treatment alone, which amounted to 88.33%, the infection severity was also lower when using nano-zinc oxide by 6.75%. When compared to the fungi treatment alone, which amounted to 70.91%, the results also showed effect of nano-zinc oxide on the studied vegetative growth traits. This study showed that nano zinc (ZnO-NPs) were the best, as they gave the highest results in recording the highest average chlorophyll content of 54.01. The average dry weight of vegetative growth, which amounted to 65.1 g.plant^{-1}, the average dry weight of the root system, which was 5.92 g.plant^{-1}, and the plant height, where effect of adding nano-zinc oxide on plant height was 47.56 cm in the treatment of the healthy plant to which ZnO-NPs were added.

Keywords: ZnO-NPs, zinc oxide nanoparticles, Alternaria spot in broad beans, Alternaria alternata

İntroduction:

Broad beans (*Vicia faba* L.) are an important leguminous crop in most countries of the world, especially Asia, Africa, and Latin America, where they are a major source of protein and energy because the seeds contain a high percentage of essential amino acids such as lysine, leucine, and arginine, in addition to their high content of carbohydrates (54-48%), mineral elements, fiber, and vitamins (11)Broad beans are grown in different regions of the world, and China produces approximately 50% of global production. Broad beans plants belong to Fabaceae family. This crop represents an important component of the global food production system as it is one of the most consumed vegetable crops during its season due to its multiple uses, whether as green pods, green seeds, or dry seeds. Plants also have the ability to fix atmospheric nitrogen through symbiotic bacteria present in the

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roots, and thus the plant works to improve the physical and chemical properties of the soil.

Due to the importance of this crop, it is exposed to many agricultural pests, and alternarial spote, caused by the fungus Alternaria alternate, is one of the diseases spread in most bean fields in Iraq .It is found where beans are grown in different regions of the world. It is a sac fungus that belongs to the Pleasporaceae family, which includes fungal species with conidia and opaque spores. This fungus is one of the most widespread pathogens and is found in the root zone of many plants, and many of them cause diseases to plants. High-end foods such as barley, corn, and rice (2)Recently, attention has turned towards green nanotechnology and it has been widely used in combating plant diseases due to its effective and unique properties, as well as the ease of manufacturing and use (3)Metal oxide nanoparticles are used, which have interesting strength and distinctive physical and chemical properties. They are also used as an antimicrobial agent due to the large surface area that these particles have compared to used antibiotics. In addition, zinc oxide nanoparticles are one of five zinc compounds currently registered and recognized as a safe substance by the World Health Organization (14). This study aimed to combat leaf spot disease in the broad bean plant using bio-synthesized nano-zinc oxide and to know its effect in inhibiting the growth of the fungus Alternaria alternata, as well as its effect on the growth of the plant. The remaining ones.

Materials and methods

A laboratory experiment and a pot experiment were coundected in the laboratories of the College of Agriculture/Field Crops Department of Al-Qasim Green University.

Laboratory experiment

Preparation of pathogenic fungal vaccine

Isolates of the pathogenic fungus were grown on the medium of Panicum miliaceum L. millet seeds. They were inoculated with the inoculum of the pathogenic fungus isolate individually, at the rate of five discs with a diameter of 0.5 cm. The flasks were placed in the incubator at a temperature of 25 ± 2°C for 10 days, taking into account shaking the flasks every 3 days. - 2 days to ensure that the fungal vaccine is distributed and does not clump (5).

Nano zinc oxide ZNO

Zinc oxide nanoparticles prepared from honey solution with the addition of zinc acetate were used, and nano zinc were obtained, which were previously prepared in the laboratories of the College of Agriculture, University of Kufa.(Doctoral dissertation by the researcher).

The chemical fungicide used in the experiment

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Use the chemical fungicide Swift, produced in Spain, according to the recommended concentration (0.1 ml/100 ml culture medium).

Evaluating effectiveness of nano zinc (ZnO) in inhibiting the radial growth of the fungus Alternaria alternata. Prepare the P.D.A culture medium, placing 20 ml of the medium in each petri dish with a diameter (9 cm), three dishes for each treatment of the nanomaterial test treatment. 5, 10, and 20 micrograms/ Pour ml of the nanomaterial into the dishes before the medium hardens, using a rotary stirrer to ensure that the nanomaterial is distributed over the culture medium. All dishes were inoculated with discs of the fungus A. alternata with a diameter of 5 mm from the fungi colony growing on P.D.A medium at five days old. Three dishes were used for each treatment, in addition to the control treatment, which contained culture medium only. The dishes were incubated at a temperature of 28±2°C, and after arriving Growth in the control treatment to the edge of the dish. The percentage of fungal growth inhibition was calculated according to the following equation (1).

$$\text{Inhibition }\% = \frac{Growth\ diameter\ rate\ in\ control - Growth\ diameter\ rate\ in\ the\ treatment}{Diameter\ growth\ average\ \ in\ control} \times 100$$

Experiment with pots

This experiment was conducted, beginning by sterilizing the soil prepared for planting using a 37% commercial formalin solution, which was diluted to 5%. The soil was covered with a tight polyethylene cover for seven days, then aeration was conducted to evaporate the formalin. After that, it was packed in plastic pots with a capacity of 9 kg. Then they were moistened and left for another three days. The pots were planted with seeds of the two types of beans (local/Spanish) after sterilizing them superficially with a 1% sodium hypochlorite solution for 2-3 minutes, at a rate of ten seeds per pot. The pots were watered carefully and those pots were divided into two groups and three replicates.

Estimating the rate and severity of plant injury

The percentage of infection at the flowering stage was estimated using the following equation:

$$\text{infection }\% = \frac{Number\ of\ infected\ plants}{The\ total\ number\ of\ plants\ tested} \times 100$$

The severity of infection on the leaves was calculated, and then ten leaves were selected. The degree of infection was determined based on the degree of injury to the leaves, according to the disease index consisting of six grades, from zero to 5 (15) for the leaves, as follows: 0 = no infection, 1 = The appearance of spots on the leaves at a rate of 1-5% of the leaf surface, 2=The appearance of spots on the leaves at a rate of

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6-20% of the surface of the leaf, 3=The appearance of spots on the leaves at a rate of 21-40% of the surface of the leaf, 4=The appearance of spots on the leaves at a rate of 41-70% of the leaf surface, 5 = spots appear on leaves greater than 70% of the leaf surface.

From the degree of infection, the severity of infection on the leaves was calculated

For each treatments on the papers, it was calculated according to the McKinney equation (16) as follows:

$$\text{Severity of infection}=\frac{Sum\ (number\ of\ leaves\ in\ degree\ 0\ x\ 0\ +\ldots number\ of\ leaves\ in\ degree\ 5\ x\ 5)}{The\ total\ number\ of\ leaves\ tested\ x\ the\ highest\ degree\ in\ the\ pathological\ index}\times 100$$

Studied traits

Vegetative growth indicators: The following measurements were made during the flowering stage of the plant

Measurement of chlorophyll content in leaves (SPAD):

The chlorophyll content in the plant leaves was estimated after 50% of flowering using a SPAD device, type Chlorophyll meter Spad-502 plus, by taking readings for three plants randomly from each anvil and taking the average.

Dry vegetative weight (g.plant^{-1})

The dry weight of vegetative growth was calculated by washing vegetative growth to get rid of the soil stuck to it, then drying the plant parts in an electric oven at a temperature of 60°C until the weight was stable, then extracting the average dry weight of vegetative growth (g) using a sensitive balance.

Dry root weight (g.plant^{-1})

The dry weight of the root system is calculated by washing the root system to get rid of the soil stuck in it, then drying it in an electric oven at a temperature of 60°C until the weight is stable, then extracting the average dry weight of the root system (g) using a sensitive balance.

Plant height (cm)

Plant height was calculated from the location of the stem's contact with the soil to the highest peak of the plant using a tape measure, and the reading was recorded as an average for three plants taken randomly from each experimental unit.

11.3 Statistical analysis

Statistical analysis of the data was performed using the Genstat program according to the randomized complete block design (RCBD), and the averages were compared according to the least significant difference (LSD) test at a probability level of 0.05 (20).

Results and discussion

Results of testing the efficiency of bio-synthesized nano zinc . The results of Figure (1) showed that all concentrations of materials used in the study, 5, 10, and 20 micrograms/ml, inhibited the mycelial

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growth of the pathogenic fungus, and the concentration of 20 micrograms/ml had the most impact on the fungal isolate used. The average percentage of inhibition of the fungus A. alternata reached 100%. The explanation for the high antagonistic efficiency of nano zinc , ZnO-NP, is due to their high ability to inhibit fungi due to the large surface area of the nanoparticle and the sharp composition of the particle's surface features. Zinc oxide also has a powerful effect. Endurance and increased selectivity (performs specific functions in specific environments) (17).The use of ZnO-NPs reduced the growth of fungi P. expansum by 61-91%, as it causes a systemic disruption of cellular function within the pathogen, leading to hyphal deformation and fungal death (10), where Wani and Shah, ((21) found a significant rate of inhibition in the germination rate of fungal spores from Alternaria, F. oxysporum , Rhizopus stolonifer, and Mucor plumbeus upon exposure to ZnO-NP.

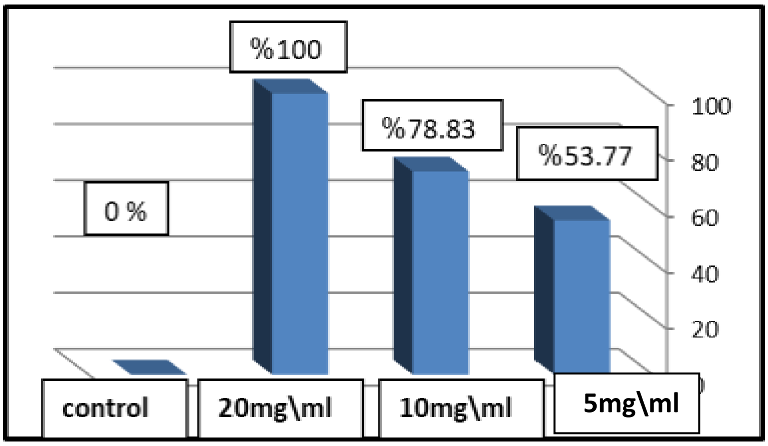

Figure (1) Effectiveness of nano zinc in inhibiting the radial growth of the fungus A. alternata in P.D.A medium at 28±2 °C.

Effect of nano-zinc oxide (ZnO) on the percentage and severity of infection in two types of broad beans under conditions of infection with the pathogenic fungus A. alternata.

The results shown in Table (1) showed effect of nano-zinc oxide ZnO on the infection rate % of two cultivars of broad beans under conditions of infection with the pathogenic fungus A. alternata. The local and the Spanish cultivar, respectively, compared to the treatment of the healthy plant to which nano-zinc oxide (ZnO) was added, which had a infection rate of 0%. The reason may be that the nano zinc affect the cell walls and plasma membranes of the pathogens and prevent the movement of protons through the cell membrane, as this leads to inhibit the growth and killing of the pathogen (18), the small size of the NPs also facilitates easy

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entry into the bacterial cell membrane and allows the inhibition of mechanisms occurring inside the cell. ZnO-NPs generate hydrogen peroxides that chemically react with membrane proteins and lipid bilayers (4).

Table (1) effect of nano-zinc oxide ZnO on the infection rate% of two types of broad bean under conditions of infection with the pathogenic fungus A. alternate

treatments average	cultivars		treatments
	Spanish	local	
0.00	0.00	0.00	Healthy plant (control)
88.33	83.33	93.33	The fungus A.alternata
33.33	26.67	40.00	The pathogenic fungus A. alternata + full concentration of the chemical fungicide Swift
28.33	23.33	33.33	Pathogenic fungus A. alternata + nano-zinc oxide ZnO
0.00	0.00	0.00	Healthy plant + nano zinc oxide ZnO
0.00	0.00	0.00	Healthy plant + full concentration of Swift chemical fungicide
	22.22	27.77	cultivar average
=interaction 6.424	=cultivars 4.543	=treatments 2.623	L.S.D (0.05)

As for Table (2), it shows effect of nano-zinc oxide ZnO on the infection severity % for two types of beans under conditions infected with the pathogenic fungus A. alternata. We notice an increase in the severity of the disease in the treatment of the pathogenic fungus and for the two studied cultivars, as it reached 73.40% and 68.43%, respectively, followed by the fungicide treatment. Chemical Swift and the pathogenic fungus A. alternata, which amounted to 25.27% and 16.67% for the local and Spanish cultivar,

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respectively, then 8.50% and 5.00 in the treatment of the pathogenic fungus A. alternata and nano-zinc oxide ZnO for the two cultivars used, compared to the treatment of the healthy plant, the nano-zinc oxide ZnO, and the plant treatment. The healthy plant, the chemical fungicide Swift, and only the healthy plant that had an infection severity of 0% were treated.

Table (2) Effect of nano-zinc oxide ZnO on the severity of infection % for two types of beans under conditions of infection with the pathogenic fungus A. alternate

treatments average	cultivars		treatments
	Spanish	local	
0.00	0.00	0.00	(Healthy plant (control
70.91	68.43	73.40	The fungus A.alternata
20.97	16.67	25.27	The pathogenic fungus A. alternata + full concentration of the chemical fungicide Swift
6.75	5.00	8.50	Pathogenic fungus A. alternata + nano-zinc oxide ZnO
0.00	0.00	0.00	Healthy plant + nano zinc oxide ZnO
0.00	0.00	0.00	Healthy plant + full concentration of Swift chemical fungicide
	15.01	17.86	cultivar average
=interaction 6.389	=cultivars 4.517	=treatments 2.608	L.S.D (0.05)

Studied traits:

Measurement of chlorophyll content in leaves (SPAD))

The data in Table (3) indicate that the two study factors had a significant impact on the chlorophyll percentage, where nano-zinc oxide was the most influential in recording the highest chlorophyll content, reaching 55.20 for the local cultivar and 52.83 for the Spanish cultivar. It is noted that the fungus affected the chlorophyll percentage, so it was 35.10 and 33.96 for the two cultivars used.

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respectively .The significant increase in chlorophyll levels in the leaves when using nano-zinc oxide is due to the fact that zinc is considered a catalyst for the oxidation process in plant cells. The importance of this process comes in regulating sugar consumption and increasing the energy needed to produce the chlorophyll pigment. It may also be due to the fact that zinc contributes in an insignificant way. Direct in the process of manufacturing chlorophyll, through its direct influence on the formation of carbohydrates, energy compounds, and amino acids. Zinc is also a catalyst for the work of the carbonic anhydrase enzyme, which plays a regulating role in the pH inside the chloroplast, which leads to protecting proteins from losing their nature and vitality, which contributes to Increased chlorophyll (6).

Table (3) Effect of nano-zinc oxide ZnO and the chemical fungicide Swift on two types of broad bean under conditions of infection with the pathogenic fungus A. alternata in measuring chlorophyll content in leaves (SPAD).

treatments average	cultivars		treatments
	Spanish	local	
50.98	49.83	52.13	(Healthy plant (control
34.53	35.96	33.10	The fungus A.alternata
42.38	43.16	41.60	The pathogenic fungus A. alternata + full concentration of the chemical fungicide Swift
42.03	42.10	41.96	Pathogenic fungus A. alternata + nano-zinc oxide ZnO
54.01	52.83	55.20	Healthy plant + nano zinc oxide ZnO
47.48	47.20	47.76	Healthy plant + full concentration of Swift chemical fungicide
	45.18	45.29	cultivar average
0.8278 =interaction	=cultivars 0.5853	=treatments 0.3379	L.S.D (0.05)

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Dry weight of vegetative growth and roots (g.plant^{-1})

Table (4) shows the excelled of plants treated with nano-zinc oxide in recording the highest dry weight of vegetative growth, which reached 64.7 and 65.5 g.plant^{-1} for the local and Spanish cultivars, respectively, compared to the fungi treatment alone, which reached 25.9 and 29.7 g.plant^{-1} for the two cultivars used, where it is noted that The fungus has had a direct effect on the dry weight of vegetative growth. The reason may be due to the stimulating effect of the induction agent, nano-zinc oxide, ZnO, which was proven by a decrease in the severity of the infection, which was reflected in the vegetative growth indicators and productivity, where it is noted that the superiority of the vegetative growth indicators is observed, and this may be attributed to effect of zinc oxide. Nano-ZnO in inhibiting pathogenic fungi through higher values of these indicators in the treatment of healthy plants and nano-zinc oxide ZnO compared to the lowest values in the treatment of pathogenic fungi only, due to the pathogenic fungus possessing digestive enzymes that decompose plant tissue and mycotoxins that inhibit many of the plant's vital activities.

Table (4) Effect of nano-zinc oxide ZnO and the chemical fungicide Swift on two types of broad bean under conditions of infection with the pathogenic fungus A. alternata on the dry weight of vegetative growth (g. plant^{-1})

treatments average	cultivars		treatments
	Spanish	local	
55.5	61.2	49.8	(Healthy plant (control
27.8	29.7	25.9	The fungus A.alternata
37.9	40.7	35.2	The pathogenic fungus A. alternata + full concentration of the chemical fungicide Swift
52.3	57.2	47.5	Pathogenic fungus A. alternata + nano-zinc oxide ZnO
65.1	65.5	64.7	Healthy plant + nano zinc oxide ZnO
64.9	66.6	63.2	Healthy plant + full concentration of Swift chemical fungicide
	53.4	47.7	cultivar average
=interaction 0.4262	=cultivars 0.3013	=treatments 0.1740	L.S.D (0.05)

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As for the dry weight of the root system (Table (5), the treatment of the healthy plant to which nano-zinc oxide was added was the best, reaching 5.61 and 6.23 g.plant^{-1} for the two cultivars, respectively, compared to the treatment of the fungus alone, which reached the lowest dry weight of the root system, 1.92 and 2.79 g.plant^{-1} for the two types used respectively. The reason may be due to the fact that nanoparticles have unique properties, as they are characterized by the small size of zinc oxide nanoparticles, thus increasing plant absorption of them, in addition to the ability of these nanoparticles to penetrate the cell membrane, where they work to improve the growth and productivity of plants (12)

Table (5) Effect of nano-zinc oxide ZnO and the chemical fungicide Swift on two types of broad bean under conditions of infection with the pathogenic fungus A. alternata on the dry weight of the root system (g.plant^{-1})

average treatments	cultivars		treatments
	Spanish	local	
4.88	5.23	4.53	Healthy plant (control(
2.35	2.79	1.92	The fungus A.alternata
3.51	4.01	3.01	The pathogenic fungus A. alternata + full concentration of the chemical fungicide Swift
5.40	5.69	5.12	Pathogenic fungus A. alternata + nano-zinc oxide ZnO
5.92	6.23	5.61	Healthy plant + nano zinc oxide ZnO
5.05	5.44	4.67	Healthy plant + full concentration of Swift chemical fungicide
4.51	4.89	4.14	cultivar average
interaction= 0.7990	cultivars= 0.5650	treatments= 0.3262	L.S.D (0.05)

Plant height (cm):-

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The results in Table (6) indicated that adding nano-zinc oxide had a significant effect on the plant height, and the highest average plant height was recorded, reaching 50.90 and 44.23 cm for the two cultivars, respectively, compared to the fungi treatment alone, which amounted to 32.83 and 29.00 cm, respectively. We note that the fungi had an effect on Significantly on plant height. The reason for the significant increase in plant height when using nano-zinc oxide is due to the significant effect that zinc plays in the formation of the amino acid tryptophane, which is responsible for the formation of indol acetic acid (9), whose concentration increases in plants treated with nano-zinc oxide (ZnO), which has an effective role. In many physiological functions in plants, it works to increase the growth in the stem plant length, and increasing the growth in the stem length occurs in two ways: either through cell division by mitosis, which will increase new cells, or through the expansion or expansion of new cells in the meristem. The apical part, which will be distinguished into a bud, a leaf, the origin of the flower, and various tissues that form the stem. Also, zinc has a role in the process of cell division and elongation, and in increasing the process of photosynthesis, which increases the height of plants. Likewise, the role of zinc in the formation of the basic compounds for plant growth, including growth regulators such as auxins and gibberellins. (7). The effect may also be due to the fact that nanosized zinc oxide can penetrate through the surface of the leaf (19). Moreover, the movement of nanoparticles is very high, which ensures that nutrients reach all parts of plants (8). Also, the small size of the particles and their lack of aggregation leads to easy They are absorbed by the plant, and thus the ability of these molecules to penetrate the cell wall is faster and easier, in addition to the fact that nanobodies can pass freely through the Casper strip in the cells of the inner cortex when their sizes are less than 36 nanometers (13).

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Table (6) The effect of nano-zinc oxide ZnO and the chemical fungicide Swift on two cultivars of broad bean under conditions of infection with the pathogenic fungus A. alternata on plant height (cm)

treatments average	cultivars		treatments
	Spanish	local	
50.57	47.37	53.77	(Healthy plant (control
30.91	29.00	32.83	The fungus A.alternata
34.00	31.67	36.33	The pathogenic fungus A. alternata + full concentration of the chemical fungicide Swift
31.51	30.63	32.40	Pathogenic fungus A. alternata + nano-zinc oxide ZnO
47.56	44.23	50.90	Healthy plant + nano zinc oxide ZnO
42.45	38.97	45.93	Healthy plant + full concentration of Swift chemical fungicide
	36.97	42.02	cultivar average
=interaction 1.913	=cultivars 1.353	=treatments 0.781	L.S.D (0.05)

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