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Green Synthesis of Some Nanoparticles (Gold, Copper) and Their Effect on Seed Growth and Plant Physiology Anise (Pimpinella Anisum)

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ABSTRACT

This experiment was conducted out in Al- Suwaira farms in Wasit during the winter season of 2021-2022.To examine the effects of nano gold at concentrations of (0, 25, 50, 75, and 100) ppm and nano copper at concentrations of (0, 10, 20, 30, and 40) mg/L on germination percentage, vigor index I VI (I), 1000 seeds weight, carotenoids, volatile oil, anise-aldehyde, anise on growth single and two way interaction. Just one application of the previously listed compounds was shown to have an important impact on anise seed development and plant physiology, particularly at high concentrations. The effective use of two-way interactions resulted in a variety of consequences. In a completely randomized design, treatments are developed as a perfectly randomized factorial experiment (5×5×3), with three replicates. The aim of this research is to determine anise with (gold and copper) nanoparticles effects on seed growth and plant physiology.

Keywords: Green synthesis, Nanoparticles, gold, copper, anise, Pimpinella anisum

1. Introduction

P anisum (anise) is an annual grass of the Apiaceae family. It grows to a height of 30-70 cm, has very little white blooms, and small greyish-green to greyish-brown seeds, and can be discovered in the Eastern Mediterranean Region, Egypt, Spain, West Asia, the Middle East, and Mexico. There are 23 species in this genus, three of which are particular to Iraq. It is one among the world's oldest and most useful medicinal herbs. Its active ingredients are used in a variety of pharmaceutical and food industries (Abdulhalem etal., 2019).It is mainly grown in southern Europe and Southeast Asia. Anise fruits, also known as seeds, are edible parts of the plant. They include 2-6% essential oils, phenolic acids, eugenol, estragole, and trans-anethole, a potent phytoestrogen and the oil's primary component (80-95%). Anise has been used for its antioxidant, antimicrobial, antibacterial, antipyretic, and antifungal characteristics for years. (Al-Shammari et al., 2017).Nanotechnology is one of the significant factors increasing anise production. Many researchers throughout the world have recently shown an interest in developing nanoparticle-free products to ensure safety and quality. Green synthesis makes use of a variety of species such as algae, sea cucumbers, marine creatures, plants, and microbes. Because of their essential abilities, biological approaches make it easier to convert dissolved metal ions to a zero-valence state and manufacture the appropriate nanoparticles. Plant-based NPs synthesis is preferable to microorganism-based NPs synthesis because it eliminates the complex method of maintaining cellular cultures and can be easily scaled up.Considering the significance and benefits of green synthesis (Kausar etal.,2022).

Green synthesized metal nanoparticles are made from different parts of plants, and these processes are also eco-friendly, non-toxic, and cost-effective. Green synthesized nanoparticles outperformed typical physical and chemical approaches in terms of active performance in removing dyes, antibiotics, and metal ions. Green synthesis is the ideal approach for producing nanoparticles since it helps to minimize toxicity, enhance stability, is eco-friendly, and is cost-effective (Vijayaram etal., 2011). Gold is one of several noble metals, and its historical significance cannot be overstated. Gold nanoparticles are also important in chemistry, physics, and biology (Tsi, 2016). Because of the presence of phytochemicals from the bio-extract, AuNPs synthesized using the green synthesis approach have been shown to have antibacterial, antifungal, anticancer, and anti-inflammatory effects, as well as antioxidant and catalytic activity. All of these variables have made the green synthesis methodology more profitable than traditional approaches (Santhosh etal., 2022). Copper nanoparticles (Cu NPs) have attracted the interest of researchers in recent years due to its uses in industries, health, and agriculture. Plant extract may be used to produce a large amount of copper nanoparticles. In comparison to chemical methods, it may be used in large quantities with no problems (Alshammari etal., 2023).

2. Materials and Methods

In the winter of 2021-2022, in Al- Suwaira farms in Wasit. The impacts of nano gold at concentrations of (0,25,50,75, and 100) ppm and nano copper at concentrations of (0,10,20,30, and 40) mg/L treated by nanoparticles have been examined in the field, where they were planted one on row at 65 cm and 25 cm in between. Each replication had a plot area of $2m^2$ and ten plants. After the complete germination, the seedlings were thinned to 2 plants per hill 30 days later of emergence .The synthesis method of Au-NPs was adapted from the (Ji etal., 2019). Gold and copper nanoparticles (CuNPs) may be synthesized using plant extracts, which is considered one of the safest ways in green chemistry according to method (Shende etal., 2016)

After 30 days of seed coating,and spraying flower on the plant the average :
1-germination percentage (G) was calculated in accordance with ISTA (2011).
G (%) = (number of germinated seeds / total number of seeds) × 100
2-Vashisth and Nagarajan (2010) were used to determine 2-Seedling vigours.
Vigor index I VI (I) = Germination% × Seedling length

1000 seeds weight. Anise seedlings and mature plants were mixed in acetone using a MagNALyser (Vilvoorde, Belgium) at 7000 rpm for 1 minute, then centrifuged for 20 minutes (4 C, 14,000× g). After that, the supernatant was filtered (Acrodisc GHP filter, 0.45 µm 13 mm). Following that, the resulting solution had been analyzed by HPLC (Shimadzu SIL10-ADvp, reversed-phase, at 4 C). Carotenoids were separated using two solvents on a silica-based C18 column (Waters Spherisorb, 5 m ODS1, 4.6× 250 mm, Agilent Technologies, Santa Clara, CA, USA). while (A) acetonitrile: methanol: water (81:9:10) and (B) methanol: ethyl acetate (68:32) were used (Almuhayawi etal.,2020). The percentage of volatile oil was calculated using the British Pharmacopoeia (1963). The method that was supplied (Zheljazkov et al., 2008) was used for analysing anise-aldehyde and linalool.

Statistical Analysis: To investigate how statistically various treatments differed, the Randomized Complete Block Design (RCBD) analysis of variance test was utilized. To discover discrepancies between means, analysis of variance and least significant difference tests were used. The level of statistical significance was set at P 0.05% 1980 (Steel and Torrie).

3. Results and Discussion

Average Germination percentage (G): According to Table 1, Nano gold has a considerable influence on average germination %. The mean germination percentage increased from 106.87 to 120.73% when the nano gold concentration was increased from 25 ppm to 100 ppm. The plants that were treated with 100 ppm nano gold grew the fastest. The nano copper had a significant influence on the average germination percentage as well. Similarly, increasing the concentration of nano copper enhanced the average germination percentage. In comparison to 10 mg/L (107.67%) and the control (100.00%), nano copper at 40 mg/L exhibited the highest germination percentage (121.87%). Nano gold and nano copper have a significant interaction influence (see Table 1).Germination % increase in plants treated with 100 ppm nano gold and 40 mg/L nano-copper. grew germination percentage.

Table 1 Nano gold and Nano copper and their two-way interactions on average Germination percentage (G) %

Nano gold ppm	**Nano copper mg/L**					**Average Nano gold**
	0	**10**	**20**	**30**	**40**	
0	**83.33**	**96.33**	**101.33**	**104.67**	**108.33**	**98.80**
25	**97.00**	**103.00**	**106.33**	**111.33**	**116.67**	**106.87**
50	**101.67**	**108.67**	**115.67**	**122.33**	**127.67**	**115.20**
75	**109.00**	**115.00**	**117.00**	**121.00**	**125.33**	**117.47**
100	**109.00**	**115.33**	**121.00**	**127.00**	**131.33**	**120.73**
Average Nano copper	**100.00**	**107.67**	**112.27**	**117.27**	**121.87**	

LSD (P=0.05) Nano copper	**0.856**	**LSD (P=0.05) Nano gold**
		0.856
Two-way interaction LSD (P=0.05)	**1.915**	

Average Vigor index I VI (I): According to Table 2, Nano gold significantly affects the average Vigour index. The mean vigour index constantly increased from 447.40 to 479.71cm as the nanogold concentration increased from 25 ppm to 100 ppm. The fastest-growing plants received treatment with 100 ppm nanogold. The average vigour index was significantly impacted by the nano copper as well. Similar to this, when nano levels of copper grew, so did the average Vigour index. The greatest Vigour index was created by 40 mg/L of nano copper (463.37cm) as compared to 10 mg/L (451.45cm) and the control (445.39cm).The interaction between nanogold and nanocopper had a significant influence (see Table 2).Plants treated with 40 mg/L of nanocopper and 100 ppm of nanogold rose in vigour index.

Table 2 Nano gold and Nano copper and their two-way interactions on average Vigor Index I VI (I)

Nano gold ppm	**Nano copper mg/L**					**Average Nano gold**
	0	**10**	**20**	**30**	**40**	
0	**438.47**	**441.13**	**445.93**	**448.11**	**451.65**	**445.06**
25	**440.43**	**444.48**	**448.88**	**450.14**	**453.10**	**447.40**
50	**445.19**	**447.36**	**449.25**	**452.10**	**455.14**	**449.81**
75	**447.72**	**453.25**	**457.98**	**459.87**	**463.36**	**456.44**
100	**455.17**	**471.02**	**485.88**	**492.84**	**493.62**	**479.71**
Average Nano copper	**445.39**	**451.45**	**457.58**	**460.61**	**463.37**	
LSD (P=0.05) Nano copper	**1.389**					**LSD (P=0.05) Nano gold**
						1.389
Two-way interaction LSD (P=0.05)	**3.106**					

Average 1000 seeds weight (g): According to Table 3, Nano gold significantly affects the weight of 1000 seeds on average. The mean 1000 seed weight continuously increased from 9.617 to 16.251 g as the nano gold concentration was increased from 25 ppm to 100 ppm. The fastest-growing plants received treatment with 100 ppm nanogold. The average weight of 1000 seeds was significantly impacted by the nano copper as well. Similar to this, the average weight of 1000 seeds grew as nano copper concentration did. The biggest 1000 seed weight was obtained by 40 mg/L of nano copper (15.891g), compared to 10 mg/L (9.881g) and the control (8.576g).Nanogold and nanocopper had a significant interaction effect (see Table 3).Plants treated with 40 mg/L of nanocopper and 100 ppm of nanogold grew 1000 seeds weight.

Table 3 Nano gold and Nano copper and their two-way interactions on average 1000 Seeds weight

Nano gold ppm	**Nano copper mg/L**	**Average Nano gold**

	0	10	20	30	40	
0	**5.657**	**7.440**	**9.063**	**10.213**	**11.103**	**8.695**
25	**7.110**	**8.287**	**9.027**	**10.733**	**12.930**	**9.617**
50	**8.767**	**9.397**	**10.613**	**13.767**	**16.243**	**11.757**
75	**10.247**	**10.917**	**13.880**	**17.100**	**18.330**	**14.095**
100	**11.100**	**13.367**	**16.513**	**19.427**	**20.847**	**16.251**
Average Nano copper	**8.576**	**9.881**	**11.819**	**14.248**	**15.891**	
LSD (P=0.05) Nano copper	**0.3604**					**LSD (P=0.05) Nano gold**
						0.3604
Two-way interaction LSD (P=0.05)	**0.8059**					

Average Carotenoids: According to Table 4, Nano gold significantly affects average carotenoids. The mean carotenoids continuously increased from 11.376 to 19.145 when the concentration of nanogold was increased from 25 ppm to 100 ppm. The fastest-growing plants received treatment with 100 ppm nanogold. The average carotenoids were significantly impacted by the nano copper as well. Similar to this, the average carotenoids increased as the concentration of nano copper did. The biggest carotenoids (18.531) were formed by nano copper at 40 mg/L when compared to 10 mg/L (11.329) and the control (9.883).The interaction between nanogold and nanocopper had a significant influence (see Table 4).Plants treated with 40 mg/L of nanocopper and 100 ppm of nanogold produced carotenoids..

Table 4 Nano gold and Nano copper and their two-way interactions on average Carotenoids

Nano gold ppm	**Nano copper mg/L**					**Average Nano gold**
	0	**10**	**20**	**30**	**40**	
0	**7.390**	**8.413**	**10.203**	**11.947**	**12.587**	**10.108**
25	**8.540**	**9.513**	**11.063**	**13.367**	**14.397**	**11.376**
50	**9.570**	**10.290**	**10.997**	**13.763**	**15.540**	**12.032**
75	**10.733**	**13.627**	**16.890**	**21.470**	**24.483**	**17.441**
100	**13.180**	**14.803**	**19.537**	**22.553**	**25.650**	**19.145**
Average Nano copper	**9.883**	**11.329**	**13.738**	**16.620**	**18.531**	
LSD (P=0.05) Nano copper	**0.4424**					**LSD (P=0.05) Nano gold**
						0.4424
Two-way interaction LSD (P=0.05)	**0.9893**					

Average Volatile oil %: Table 5 shows that nano gold significantly affects average volatile oil. When the concentration of nano gold was raised from 25 ppm to 100 ppm, the mean volatile oil consistently ranged from 3.047 to 9.407%. Plants treated with 100 ppm nano gold grew the fastest. Nano copper also significantly affected average volatile oil. Similarly, when the concentration of nano copper increased, it also increased the average volatile oil. The greatest percentage of volatile oil was created by nano copper at 40 mg/L (6.811%), compared to 10 mg/L (3.682%) and the control (2.983%).Nanogold and nanocopper had a significant interaction effect (see Table 5).Plants treated with 40 mg/L of nanocopper and 100 ppm of nanogold produced volatile oil.

Table 5 Nano gold and Nano copper and their two-way interactions on average Volatile oil

Nano gold ppm	Nano copper mg/L					Average Nano gold
	0	10	20	30	40	
0	**1.567**	**2.060**	**2.730**	**2.917**	**3.473**	**2.549**
25	**2.283**	**2.700**	**2.930**	**3.440**	**3.883**	**3.047**
50	**2.910**	**3.330**	**3.503**	**4.367**	**5.503**	**3.923**
75	**3.097**	**3.590**	**4.800**	**6.243**	**7.280**	**5.002**
100	**5.057**	**6.730**	**9.613**	**11.723**	**13.913**	**9.407**
Average Nano copper	**2.983**	**3.682**	**4.715**	**5.738**	**6.811**	
LSD (P=0.05) Nano copper	**0.2834**					**LSD (P=0.05) Nano gold** **0.2834**
Two-way interaction LSD (P=0.05)	**0.6337**					

Average Anise-aldehyde: According to Table 6, Nano gold significantly affects average anise-aldehyde. The mean anise-aldehyde continuously varied from 3.255 to 7.617 when the concentration of nanogold was increased from 25 ppm to 100 ppm. The fastest-growing plants received treatment with 100 ppm nanogold. The average anise-aldehyde was significantly impacted by the nano copper as well. Similar to this, the average anise-aldehyde level grew as nano copper concentration did. The greatest amount of anise-aldehyde (6.613) was formed by 40 mg/L of nano copper in comparison to 10 mg/L (3.386) and the control (2.653).

Table 6 Nano gold and Nano copper and their two-way interactions on average Anise-aldehyde

Nano gold ppm	Nano copper mg/L					Average Nano gold
	0	10	20	30	40	
0	**1.620**	**1.807**	**2.097**	**2.817**	**3.810**	**2.430**
25	**1.960**	**2.447**	**3.107**	**4.030**	**4.733**	**3.255**
50	**2.650**	**2.840**	**3.723**	**5.180**	**5.887**	**4.056**
75	**3.000**	**4.033**	**4.180**	**6.143**	**8.057**	**5.083**
100	**4.033**	**5.803**	**7.797**	**9.873**	**10.580**	**7.617**
Average Nano copper	**2.653**	**3.386**	**4.181**	**5.609**	**6.613**	
LSD (P=0.05) Nano copper	**0.3280**					**LSD (P=0.05) Nano gold** **0.3280**
Two-way interaction LSD (P=0.05)	**0.7335**					

The interaction between Nano gold and Nano copper had a significant influence (see Table 6). Anise-aldehyde was produced by plants exposed to 100 ppm Nano gold and 40 mg/L Nano copper.

Average Linalool: According to Table 7, Nano gold significantly affects average linalool. The mean linalool continuously varied from 4.133 to 6.565 when the Nano gold concentration was increased from 25 ppm to 100 ppm. The fastest-growing plants received treatment with 100 ppm Nano gold. The average linalool was significantly impacted by the Nano copper as well. Similarly, as the Nano copper concentration rose, the average linalool level rose as well. The highest amount of linalool (7.141) was created by 40 mg/L of Nano copper, compared to 10 mg/L (3.871) and the control (3.093).The interaction between Nano gold and Nano copper had a significant influence (see Table 7).Plants treated with 40 mg/L of Nano copper and 100 ppm of Nano gold produced linalool..

Table 7 Nano gold and Nano copper and their two-way interactions on average Linalool

Nano gold ppm	**Nano copper mg/L**					**Average Nano gold**
	0	**10**	**20**	**30**	**40**	
0	**1.773**	**1.920**	**2.553**	**3.067**	**3.380**	**2.539**
25	**2.467**	**3.250**	**3.960**	**5.140**	**5.850**	**4.133**
50	**3.467**	**4.137**	**5.403**	**6.657**	**7.320**	**5.397**
75	**4.137**	**5.360**	**6.960**	**8.137**	**9.097**	**6.738**
100	**3.623**	**4.690**	**5.687**	**8.770**	**10.057**	**6.565**
Average Nano copper	**3.093**	**3.871**	**4.913**	**6.354**	**7.141**	
LSD (P=0.05) Nano copper	**0.2964**					**LSD (P=0.05) Nano gold** **0.2964**
Two-way interaction LSD (P=0.05)	**0.6629**					

Foliar spray with (gold and copper) nanoparticles changed seed development parameters positively (Tables 1, 2, 3). AuNPs enhance seed germination. AuNPs increase the number of leaves, leaf area, plant height, chlorophyll content, and sugar content, all of which contribute to increased production from agriculture (Imtiaz etal,2022).According to studies, gold nanoparticle exposure improves free radical scavenging capability and antioxidant enzymatic activities, as well as changes microRNA expression, which regulates several morphological, physiological, and metabolic processes in plants. Plant development and yields are improved as a result of these modulations (Siddiqi and Husen., 2016). Plants exposed to moderate concentrations of AuNPs grew faster, with longer primary roots, more and longer lateral roots, and greater rosette diameter, as well as decreased oxidative stress responses.Positive impacts on growth, likely due to their protective influence on oxidative stress responses. Analysis of differential transcriptomics and proteomics indicated that oxidative stress responses are downregulated while growth-promoting genes/proteins are increased (Ferrari etal., 2021). Higher chlorophyll concentrations in the responded to seedlings are attributed to the suggested inhibition of ethylene activity by gold nanoparticles. Absorption with the use of chlorophyll molecules enhanced the photochemical reaction. This results in increased availability of lowering power (NADPH2) and strength (ATP) for CO2 fixation (Abdel-aziz and Al-othman, 2019). Cu NPs have been shown to modulate auxin-related genes, which play an important role in the formation of apical meristems (Essa etal., 2021) The foliar spray with (gold and copper) nanoparticles showed a favourable effect on active chemicals (Tables 4,5,6,7). Many studies have demonstrated that various plant types may collect gold to differing degrees. Exposure to gold nanoparticles, for example, improved seed germination in cucumber and lettuce. Plant development enhancement is attributed to an increase in free radical pressure and antioxidant enzymes. Treatment with gold nanoparticles reduces the time necessary for seed germination; this might be due to improved permeability, which allows for the insertion of water and dioxygen into the cells, which speeds up metabolism and germination. The treatment with nanoparticles increased the number of leaves per plant, which is controlled by an intricate interaction of several genes whose expression is influenced by growth hormones. Inhibiting ethylene mobility causes an increase in the number of leaves, which minimises the occurrence of abscission. As a result, gold nanoparticles may reduce the influence of ethylene, resulting in an increase in leaf length.The gold nanoparticle treatment inhibits the activity of endogenous plant hormones and causes alterations in seedling growth characteristics. It is also possible that a modest dosage activates hormone function (Abdel-aziz and Al-othman, 2019).The usage of nano copper offers several advantages. It increases nutrient utilisation, reduces soil toxicity, and reduces the detrimental consequences of over-fertilization. The time and pace of nutrient release are matched with the nutritional requirements of the plant using nanocopper. As a result, the plant can absorb the maximum quantity of nutrients, decreasing element leaching and improving dry matter produce (Lafmejani etal., 2018). Copper nanoparticles most likely contributed to a rise in plant adaptation capacity, which resulted in the stabilisation of growth processes and higher lodging resistance (Seregina etal., 2020).

4. Conclusion

Green synthesis of some nanoparticles (gold and copper) and their effect on seed growth and plant physiology anise (**Pimpinella anisum**) to the plant significantly increased all traits, regardless of whether the seed growth or plant physiology achieved the highest concentrations used the highest effects, whether alone or in combination with two other compounds, according to the results of the previously mentioned study.

Competing Interests: Authors have declared that no competing interests exist.

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