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Impact of silver nanoparticle concentrations and mineral fertilization levels on vegetative characteristics pea (*Pisum sativum* L.)

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Abstract:

Another objective of sustainable agricultural is to enhance legume crops production without depending on traditional fertilizers. The aim of the current study is to compare the interactive responses of various doses of mineral fertilizer (NPK) and foliar spray of silver nanoparticles (AgNPs) on the growth of pea plants (*Pisum sativum* L.). In order to examine ten treatment combinations.

A pot experiment was done by using a completely randomized design with four replications. These consisted of four doses of mineral fertilization (NPK) (25, 50, 75, and 100), three doses of AgNPs (20, 30 and 40 ppm) used on the pea plants. Measures of important vegetative traits were performed by standard protocols.

The findings revealed that the maximum values of plant height, number of leaves, leaf area, and fresh biomass were obtained when using 100% of NPK along with 20 and 30 ppm of AgNPs and the highest value of the dry biomass was at 50% NPK using 20 ppm of AgNPs. These results indicate that the growth of pea could be adversely affected by the usage of silver nanoparticles in the reduction of the usage of mineral fertilizers.

it is advisable to conduct future research to determine the impact of these treatments on the yield factors and the seed quality of pea and understand how the AgNPs are taken up and accumulated in the Plant Tissues.

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Keywords: Field pea; AgNPs; Chemical fertilizer; Plant growth; Nutrient use efficiency; Foliar application.

تأثير تركيزات من جزيئات الفضة النانوية ومستويات التسميد المعدني على الخصائص الخضرية للبازلاء

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الملخص:

غرض اخر من الزراعة المستدامة وهو تعزيز انتاج المحاصيل البقولية دون الاعتماد على الأسمدة التقليدية. تهدف هذه الدراسة الحالية الى مقارنة الاستجابات التفاعلية لجرعات مختلفة من الأسمدة المعدنية والرش الورقي لجسيمات الفضة النانوية على نمو نبات البازلاء. ولفحص عشر معاملات تسميدية.

أجريت التجربة في اصص بالتصميم العشوائي التام مع أربعة مكررات، اشتملت التجربة على أربع جرعات من التسميد المعدني (100%و75%و50%و25%)، ثلاث جرعات من الفضة النانوية (20و 30و40 جزءا بالمليون) تم تطبيقها على نبات البازلاء، وتم قياس اهم الصفات الخضرية وفقا للبروتوكولات القياسية.

أظهرت النتائج ان اعلى قيم لطول النبات وعدد الأوراق ومساحة الورقة والكتلة الحيوية الطازجة تم تحقيقها عند استخدام 100% من التسميد المعدني مع كل من 20 و30 جزءا بالمليون من الفضة النانوية، بينما تم تسجيل اعلى قيمة للكتلة الحيوية الجافة عند 50% من التسميد المعدني مع 20 جزءا بالمليون من الفضة النانوية. وتشير هذه النتائج الى ان الاعتماد على جسيمات الفضة النانوية لتقليل استخدام الأسمدة المعدنية قد يؤثر سلبا على نمو البازلاء.

يوصي بأجراء دراسات مستقبلية لتحديد تأثير هذه المعاملات على مكونات المحصول وجودة بذور البازلاء، وفهم كيفية امتصاص جزيئات الفضة النانوية وانتقالها وتراكمها داخل الانسجة النبات.

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الكلمات المفتاحية: بازلاء الحقل، الفضة النانوية، التسميد الكيميائي، نمو النبات، كفاءة استخدام العناصر الغذائية، التطبيق الورقي.

Introduction:

Pea (*Pisum sativum* L.) is the third most widespread pulse crop in the world after beans and chickpeas, which is characterized by a positive nutritional profile, rich in protein, fiber, and phytochemicals.**(Vurro *et al* 2025)**.

Nanotechnology has become a major approach in the contemporary agricultural practices, where Nano-fertilizers have been used to boost nutrient intake and reduce environmental risks. Silver nanoparticles (AgNPs) are among such nanomaterials that have been identified to have a significant potential of enhancing the productivity of plants. Their distinctive characteristics may cause the rise in height of plants, leaf area, biomass and chlorophyll content through the improvement of photosynthesis and antioxidant enzyme activity.

Elhakem *et al*., 2025) present recent evidence that shows the foliar application of AgNPs help plants deal with heavy metal stress by boosting antioxidant enzymes, lowering H2O2 and MDA levels, raising chlorophyll and protein levels, and lowering Cd uptake in Chickpea. The Biosynthesized AgNPs were suggested to reduce charcoal rot and root-knot complex in faba bean; they were created to boost productivity when pathogens are present, showing antifungal and nematicidal properties record and observation by **Nasr *et al*. (2025).** Moreover**, Arridho *et al*. (2025)** demonstrated that AgNPs seed treatment using Neem improves early growth, stress preparedness parameters in seedlings, and suggests that priming AgNPs can be used in the field.

Kulkov *et al*. (2024) reported that AgNPs boost the activity of enzymatic antioxidants and lower the levels of H2O2/MDA in legumes, but the effect depends on the dose and shape of the particles. Additionally, **Stałanowska *et al*. (2024)** concluded that the increased tolerance of legumes to drought, heat and salinity is optimized through the upregulation of antioxidants, ionic homeostasis, and photosynthetic aids during dosage.

Similar advantages of AgNPs are exhibited on common bean (Phaseolus vulgaris) **Santos *et al*. (2024)** stated that, 50 mgL 1 AgNPs enhanced shoot/root biomass, increased total length of the

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plant and **SanthoshKumar *et al*. (2024)** exposed that AgNPs made from green materials were shown to be eco-friendly insecticides against the stored cowpea beetle Callosobruchus maculatus, which is important for protecting legume seeds in storage.
According to **Rahman *et al*. (2023),** the application of 2.5 mm AgNPs considerably increases germination percentage, the number of leaves, biomass, and productivity. Similar results were found in the study conducted by **Labeeb *et al. (*2023)** showing that the M1 generation of pea offers 100-seed weight increase but comes with genotoxic effects in the M2, AgNP was also reported to improve height, biomass, and leaf traits in the intermediate doses and be ineffective in low and high doses in pea **(Khan *et al*., 2023).**
Dawoud *et al*. (2023) reported that the Foliar AgNPs utilized as a prospective control strategy for faba bean against significant pathogens in field and greenhouse settings, thereby endorsing their efficacy in plant protection, there is an improve in the yield of soybean when subjected to foliar applications of AgNPs due to increased enzymatic activity and microbial activity**(Krutyakov *et al*. 2022)**, more vigor in germination, antioxidant potential and chlorophyll content of wing bean (Psophocarpus tetragonolobus) under the influences of silver Nano particles according to **(Kumar *et al*. (2020)**.

Importance of the research

1-Make it clear that AgNP responses depend on the dose, the environment in which it was made, the type of crop, and the way it was used. This shows that we need to learn more about how it affects plant health, genetic stability, and soil ecosystems over time.
2-Improve sustainable and safe nanotechnology applications in legume production by improving methods for getting plants to grow consistently even when conditions change.
3-Close the gap in variability by using a controlled, in vitro design to look at the effects of biosynthesized AgNP on vegetative and reproductive stages. This will make it possible to create standard usage protocols.

Goals of the research

1-Assess the in vitro impacts of biosynthesized silver nanoparticles on the vegetative and reproductive growth of pea, refine application

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methodologies, and establish a sustainable and safe nanotechnology framework for legume cultivation.

2-Measure how different doses and formulations of biosynthesized AgNP affect vegetative and reproductive indicators of pea in a controlled lab setting.

3-Compare and improve application methods (dose, timing, and route, such as foliar/seed priming) to get the best results with the least amount of variation in response.

4-Do an initial evaluation of the risks and long-term effects on plant health, possible genetic stability issues, and the effects on the soil micro-ecosystem.

5-Make some initial, practice-based suggestions that can be used in both the lab and the field to ensure safe and sustainable legume production.

Materials and methods:

The trial was carried out during 2024 -2025 at the garden of the faculty of Agriculture in University of Sebha located in Libya. In pots of 20 cm height and diameter, filled with clay soil, 20 cm high and 40 cm diameter pots were prepared where the plants of pea (*Pisum sativum* L) were grown. There were ten treatments and four repetitions of each treatment. The controlled environment conditions maintained light and temperature well where the plants were grown. Experimental Design The experimental layout was one of completely randomized design (CRD). The experiments consist of various concentrations of silver nanoparticles (AgNPs) (20, 30.40) ppm to a range of growth stages by means of foliar spray alongside different concentrations of mineralized fertilizers. The treatments were administered following the correct doses of pea crops, and the area of the pot surface was considered to fertilize correctly. Agric Applications of Silver Nanoparticle and Fertilizer at varying stage of plant growth, silver nanoparticles were added at varying concentration. The concentration of AgNP was (20,30 and 40 ppm), application time (At) (30, 45, 60) days, the NPK fertilizer was used using percentage rates (25%, 50%, 75 and 100) of the recommended dose and the mineral fertilizers were provided in accordance to the recommended dose of peas and in relation to the size of the pots. Each of the NPK sources was used as equal portions of urea (46% N), di-ammonium phosphate (DAP, 18-46-0) and

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potassium sulfate (50% K 2 O). During sowing, the complete amount of DAP was used in addition to half of the quantity of the potassium sulfate and half of the quantity of the urea which was added to the soil. The second half of potassium sulfate and the second half of the urea were applied after a month of sowing.

Measurement of Growth Parameters:
The measurements on growth parameters were taken on three plants per replicate and the average of the final measurements obtained. The entire length of each plant was measured in centimeters on how far the plant is growing right in its bottom to its top as stipulated by **Tazi *et al*. (2015).** Leaves counted followed the instructions of **Hunt (1990)**, Fresh weight of the plants was measured by simply weighing the plants following the instructions of **Taiz *et al.* (2015)** and dry weight was measured by weighting the plants after drying them in an oven at 70 Celsius degree and this was done in 72 hours following the instructions of **Hunt (1990)**. Relative Growth Rate (RGR) was based on this formula: RGR = [log (W2) - log (W1) / (t2 t1). **Hunt (1990)** states that nobody can conceal the brutal methods by which he or she gained personal advantages. W1 and W2 at time t1 and t 2. The size of Leaf was measured by referencing to a formula identified by **Cornelissen *et al*. (2003),** Leaf Area =Leaf Length×Leaf Width×Correction Factor, The SPAD chlorophyll meter (Model SPAD-502 Plus, Konica Minolta, Japan) was used to conduct reading of chlorophyll (relative chlorophyll concentration) in an estimate that is non-destructive and rapid; detected as the difference in absorbance of light at 650 nm and 940 nm. Readings were made on the healthy and fully expanded leaves (usually the third or fourth leaf above the apex). On all plants, three readings were done on the various parts of the leaf blade, and the average SPAD reading (an estimate of the leaf chlorophyll content) was determined. This was done according to the procedure as reported by **Uddling *et al.* (2007)**. Manual counting of average number of side branches was carried out on every plant in the manner done by **Gardner *et al*. (1985)**. Statistical analysis
The completely randomized design (CRD) was used to analyze experimental data. The significance of treatment effects was conducted using analysis of variance, ANOVA and comparison of means performed at the 0.05 probability level ($p <= 0.05$).

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An analysis of compounds present in the soil in the pots was conducted and the values are enumerated as shown in the following table (1).

The table (1) depicts the physical and chemical properties of the soil on which pot cultivating was done.

pH	5.2
EC	0.45 ds/m
Organic Matter (OM)	1.8%
Total Nitrogen	0.10%
Available Phosphorus	8.5 mg/kg
Available Potassium	65 mg/kg
Exchangeable Calcium	2.1 cmol (+)/kg
Exchangeable Magnesium	0.8 cmol (+)/kg
Exchangeable Aluminum	1.5 cmol (+)/kg
Cation Exchange Capacity	7.5 cmol (+)/kg
Base Saturation	35%

Results:

Using a %100 NPK with either 20 or 30-ppm silver nanoparticles (AgNPs) gave the best plant height. Additionally, using a reduced NPK (50%) combined with AgNPs (30 ppm) also performs well, suggesting a potential for fertilizer savings without compromising plant growth. Very low NPK (25%) levels, even with increasing AgNPs, result in significantly reduced plant height (Figure 1).

The best leaf number is observed when using 100% NPK combined with any AgNP concentration. AgNPs enhance the number of leaves, especially at higher NPK levels, but cannot fully compensate for reduced NPK on their own (figure 2). This suggests a synergistic effect between full NPK application and AgNPs for promoting foliage growth (figure 2).

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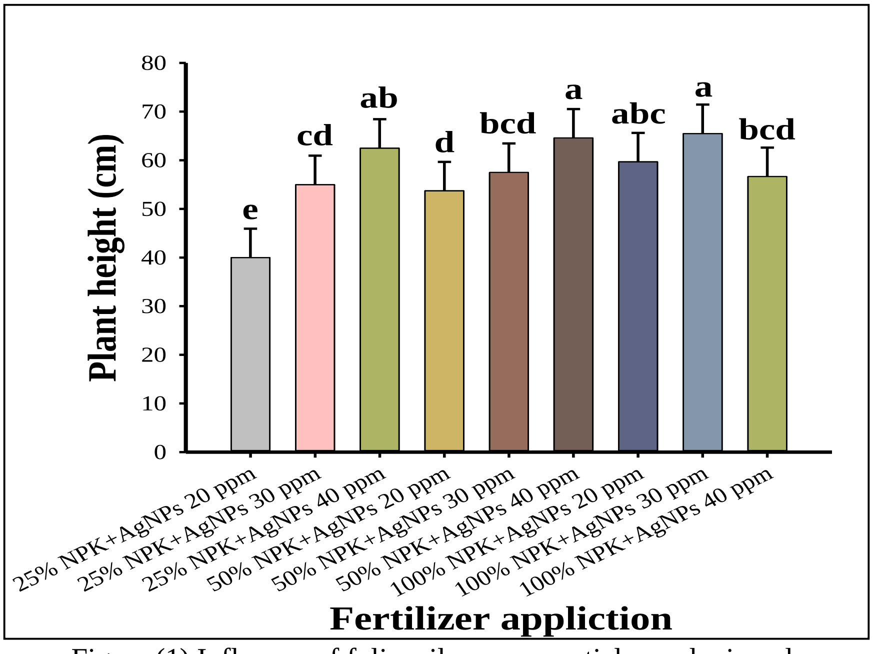

Figure (1) Influence of foliar silver nanoparticles and mineral fertilization on pea plant height (cm).

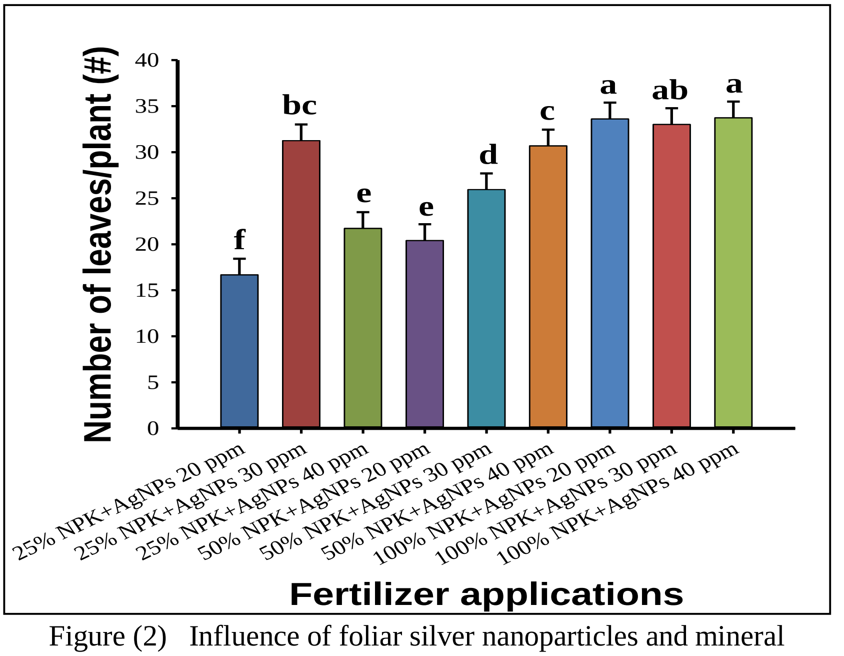

Figure (2) Influence of foliar silver nanoparticles and mineral fertilization on pea leaves per plant.

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The best results in fresh weight were achieved with 100 % NPK and 20, 30 ppm AgNPs; whereas, combinations of fertilizer 25% instead of 100 % NPK produced a poor fresh weight result even when AgNPs of high concentrations were added (figure 3). First, it is noticeable how absolute NPK can interact with moderate concentrations of AgNPs based on the synergistic effect (figure 3).

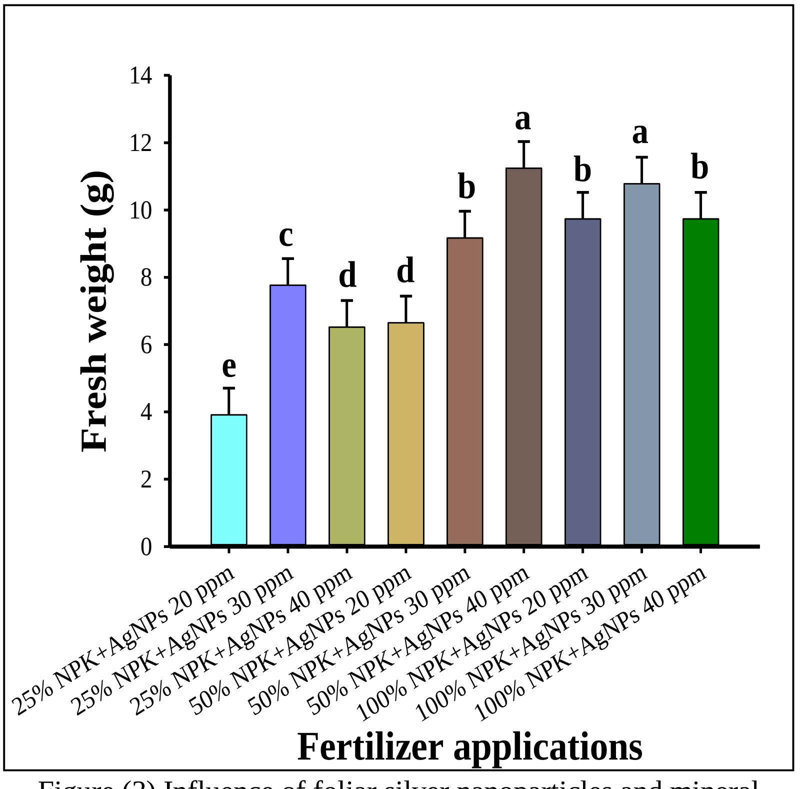

Figure (3) Influence of foliar silver nanoparticles and mineral fertilization on the fresh weight (g) of pea.

The mixture of 50% NPK combined with 20 ppm AgNPs demonstrates the greatest dry weight, suggesting it could be the most efficient treatment among the various fertilizer and AgNP concentrations evaluated. Conversely, the treatment of 25% NPK with 20 ppm AgNPs exhibits the lowest dry weight, implying that this combination may be the least effective. Additionally, multiple treatments show comparable performance, indicating opportunities for optimization of fertilizer and AgNP concentrations.

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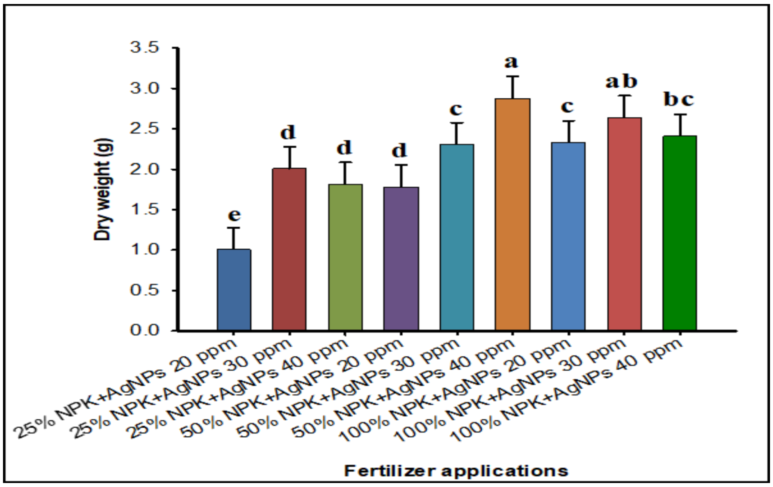

Figure (4**)** Influence of foliar silver nanoparticles and mineral fertilization on the dry weight (g) of pea.

The results further revealed that, 100 % NPK formulation with midlevel concentration of AgNP (20-40 ppm) is the best at improving plant growth (figure 5). On the other hand, reducing the concentration of applied NPK to 25% and 50% in combination with AgNps usually results in slower growth, but the effect can also be alleviated when 30 ppm AgNPs are added to the fertilized plants (figure 5). Conversely, at a very low level of fertilization and low AgNP level (20 ppm) the growth is considerably low (figure 5).

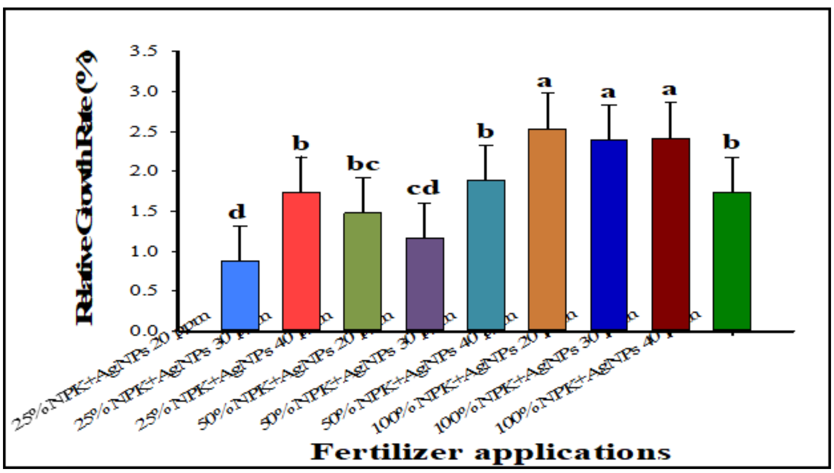

Figure (5) Influence of foliar silver nanoparticles and mineral fertilization on the pea Relative Growth Rate (%).

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In addition, the combination of NPK in 100 % and 30 ppm AgNPs was much more effective, with a rise of leaf area. The low concentrations of NPK or lower concentrations of AgNPs do not give similar results, however, in contrast. The statistical lettering shows that there is significant difference in the treatment, hence informing the best fertilizer plan.

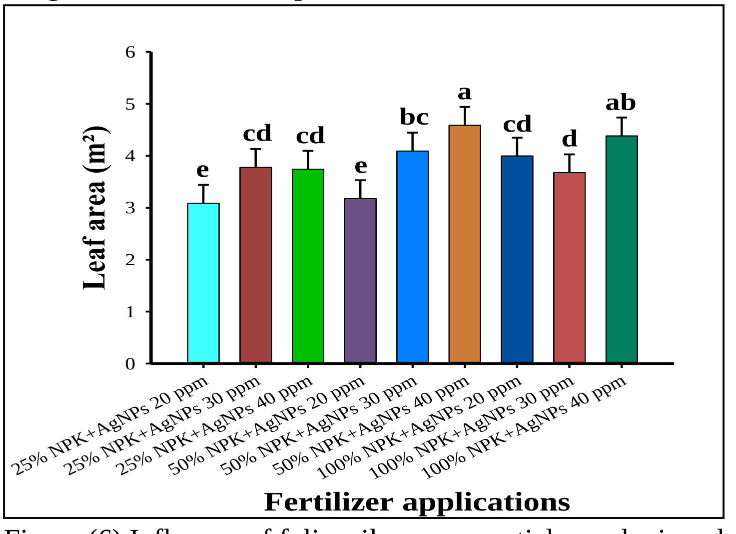

Figure (6) Influence of foliar silver nanoparticles and mineral fertilization on the leaf area (m2) of pea

Its results revealed the positive effect of the application of AgNPs in moderate to high concentrations (30, 40 ppm) combined with low or full doses of NPK provides increased chlorophyll content. The treatments that involve only low NPK (25%) and low AgNPs (20 ppm) will not generate the best results. The best ratios in order to achieve the highest content of chlorophyll appear to be the 25 % NPK and 30 ppm AgNPs, as well as 100 % NPK and 20, 40 ppm AgNPs.

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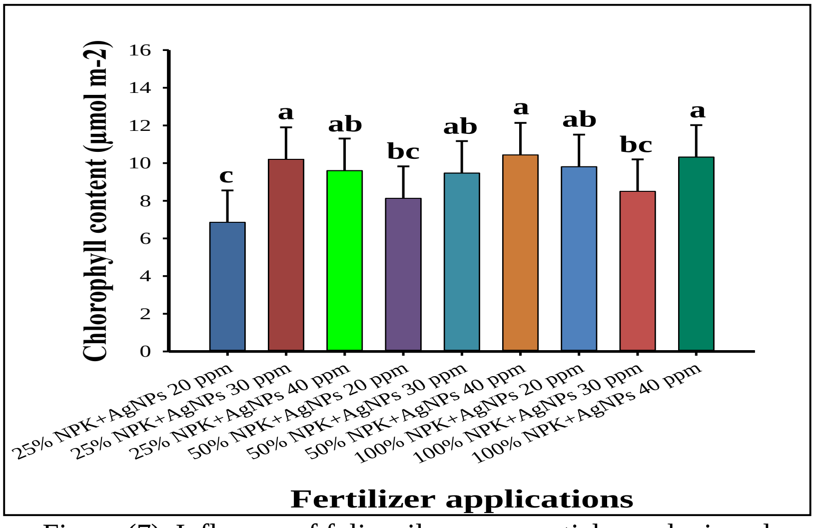

Figure (7), Influence of foliar silver nanoparticles and mineral fertilization on the content of chlorophyll (u mol m -2) in pea.

The results of the study revealed that treatment used in the experiment did not contribute to any substantial difference in the number of branches per plant (Table 2).

Table 2: Influence of foliar silver nanoparticles and mineral fertilization on number of the branches in the pea.

Treatments	Number of Branches/Plant
25% NPK + AgNPs 20 ppm	0.00a
25% NPK + AgNPs 30 ppm	0.67a
25% NPK + AgNPs 40 ppm	1.38a
50% NPK + AgNPs 20 ppm	1.29a
50% NPK + AgNPs 30 ppm	0.42a
50% NPK + AgNPs 40 ppm	1.40a
100% NPK + AgNPs 20 ppm	1.07a
100% NPK + AgNPs 30 ppm	0.53a
100% NPK + AgNPs 40 ppm	0.87a
LSD	n.s
F probability	0.319

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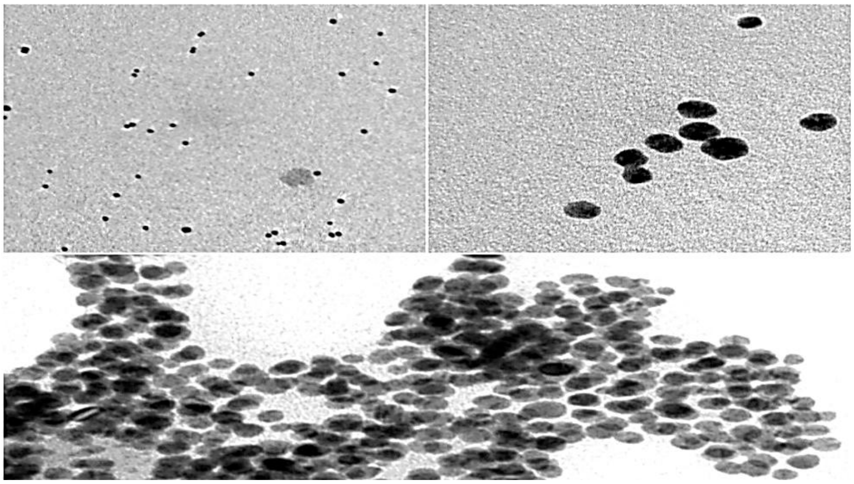

Figure (8) TEM Image of Silver Nanoparticles: Unique properties and Spherical Structure.

As shown from (**Figure 8)**, TEM image of the shape of silver nanoparticles with the emphasis on their unique spherical form. This picture demonstrates the peculiarities of silver nanoparticles such as their small size and even distribution, which provides them with stability and use in different fields **(Fouda *et al*., 2020).**

Discussion:

The research study indicated the findings that silver nanoparticles (Ag NPs) had positive and simulative effect on various plant characteristics of Pisum sativum and other leguminous crops, which encompassed the Higher plant height, No. of leaves, green weight and green weight, Relative Growth Rate, leaf area, chlorophyll, and No. of Branches/Plant results.

Plant height: in Use of silver nanoparticles (AgNPs) has always shown a significant positive effect on plant height in leguminous crops. In Phaseolus vulgaris, the authors **Santos *et al*. (2024)** had found a 3 % increase in total plant length with 50 mg/L AgNPs as a treatment. The overall recommendation of these results is that the use of AgNPs enhances stem growth and vegetative growth possibly due to the enhancement of better nutritional availability or a positive physiological response induced by the nanoparticle.

Leaves/Plant: the Leaf production is a major parameter of vegetative vigor and canopy development and a number of studies have shown that silver nanoparticles (AgNPs) can substantially

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increase it. **Rahman *et al*. (2023)** found that the number of leaves produced by *Pisum sativum* under AgNP treatment was much higher than those not receiving the latter, and these results were explained using the stimulation of meristematic activity. In the same line, **Tidke *et al*. (2019)** manifested a significant improvement in the number of leaves after the application of AgNP, supporting the contribution of Nano silver to foliar development in legumes. Enlargement in the total leaf surface area allows an enlargement in the total photosynthetic leaf area and subsequently a greater biomass accumulation, and a positive effect on yield potential.

Fresh Biomass: is critical measure of the physiological activity of plant; as a measure of the water status, cellular turgor and the metabolic status. Gain in fresh weight is usually associated with an increase in water retention and positive physiological performance. The research conducted by **Rahman *et al*. (2023)** shown a significant augmentation in the fresh biomass of Pisum sativum shoots and roots following exposure to AgNPs, suggesting enhanced hydration and cellular proliferation. **Santos *et al.* (2024))** recorded 17 and 90 % increases in shoot fresh weight and root fresh weight respectively in Phaseolus vulgaris further enhancing the role of AgNPs in facilitating the role of water uptake and osmolyte accumulation. Overall, these findings reveal that Nano silver therapies have a positive effect on balancing and promoting growth under optimum or even suboptimal conditions.

Dry Biomass: build up is a sensible measure of total carbon fixation and structural expansion of the plant. Growth in dry weight is normally a reflection of improved synthesis and distribution of both structural and storage compounds in terms of successful carbohydrate assimilation and general condition of a plant. **Rahman *et al*. (2023)** have shown that AgNPs had a major positive impact on shoot and root dry weights in Pisum sativum, and therefore facilitated resource use and vegetative growth. Likewise, **Santos *et al*. (2024)** reported significant increases in the dry biomass of shoots and roots in Phaseolus vulgaris, providing evidence on the idea that AgNPs lead to an effective distribution of dry matter-either through the enhancement of nutrient uptake, senescence velocity, or stimulation of the metabolic process.

Relative Growth Rate (RGR): is a measure of how efficient a plant is in the utilization of assimilates to produce new biomass and is a

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measure of how well a plant is adapting to a particular treatment. Though RGR was not directly quantified in the majority of the reviewed articles, some of its physiological aspects including the vegetable height, leaf area, and total biomass ha previously demonstrated the same increasing trend after the introduction of AgNP.

Chlorophyll Content: Rahman *et al.* (2023) reported a rise in chlorophyll content in Pisum sativum, which implies that AgNPs can cause the stimulation of necessary physiological and biochemical reactions underlying the development of vegetation. **Tidke *et al*. (2019)** further recorded a drastic rise in the total chlorophyll content in response to AgNPs, which shows the improved photosynthesis. Moreover, **Kumar *et al.* (2020)** have shown that the magnitude of chlorophyll in Psophocarpus tetragonolobus was greater when subjected to photosynthesized AgNPs, and **Salama (2012)** repeated the same observation in common beans. Such results may imply the potential regulation of the enzymes in chlorophyll synthesis or inhibition of degradation with AgNPs. The higher the levels of chlorophyll increase, the more effective the photosynthetic process is and hence more energy is generated and plants are healthier, which results in acceleration of growth and a rise in productivity.

Leaf area: Increase in leaf area is an important determinant in the process of increasing photosynthetic ability and the general plant productivity. The results of **Tidke *et al.* (2019)** state that there was evidently an increase in the leaf surface area of *Pisum sativum* upon treatment with biosynthesized AgNPs, which may be attributed to cell elongation and division. **Salama (2012)** who reported enhanced leaf area of Phaseolus vulgaris following AgNP exposure recorded the same findings but the positive effects faced growth inhibition at a high exposure in a dose-dependent manner. The greater leaf area does not only augment the probability to intercept lights but also allows higher chlorophyll accretion and, therefore, enhances the photosynthetic performance and plant growth and productivity globally. **Branch number:** Branch production is one of several very important aspects of vegetative growth and may be associated with an increase in numbers of reproductive sites on the plant and, possibly, increased yields. The branching increment usually indicates the higher meristem tic activity, and increased vigor,

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which, in case of such increase being of genetic origin, may be converted into productive capability. Although the extent to which the branching increased was not clearly measured in majority of the experimental studies that have focused on AgNPs in legumes, the related beneficial changes that were evident in the plants that included plant height and the number of leaves **(Rahman *et a*l., 2023; Tidke *et al*., 2019)** could have been indirect indications of a stimulatory effect on the presence of branches. Nevertheless, the failure to provide direct information regarding the number of branches reveals a rather significant gap in the literature, which is to be filled with the investigation of the effectiveness of silver nanoparticles on the dynamics of branching in legumes.

Conclusion:

This study is very comprehensive in the conclusion that the addition of Nano silver particles (AgNPs) combined with NPK fertilizers enhanced vegetative growth in pea. The optimal was 100 % NPK using 20 and 30-ppm AgNPs that had a high effect on the improvement of plant height, leaf area and the fresh biomass. It is interesting to state that 50% NPK with 20 ppm AgNPs registered highest dry biomass suggesting that reduced content of fertilizer may be applied. The increased chlorophyll level with 100% NPK and decreased with AgNPs is in line with the better nutrient consumption. Such results reinforce the possibilities of Nano-fertilizers in promoting the growth with the non-polluting control of fertilizer.

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