EVALUATION OF EFFECTS OF GOLD NANOPARTICLES IN CHITOSAN ON HYACINTH PLANTS

EVALUAREA EFECTELOR NANOPARTICULELOR DE AUR ÎN CHITOSAN ASUPRA PLANTELOR DE ZAMBILE

CAZACU Ana [1], PEREŞ Cătălina-Iuliana[1], TELIBAN G.C.[1], STOLERU V.[1], BODALE I.[1*]

*Corresponding author e-mail: ilie.bodale@uaiasi.ro

Abstract. *In the research on the influence of nanoparticles on plants, it is important to identify both the optimal concentrations of nanoparticles and the growing stage in which the plant is sensitive to a certain type of treatment. The study on the influence of nanoparticles on hyacinth bulbs was carried out in an experiment in which 4 treatment variants were used simultaneously, each variant having 3 repetitions. Two solutions with different concentrations of gold nanoparticles in chitosan (25 and 50 µg/mL) were used as treatment, and the samples in which either a chitosan solution or water was administered were considered as controls. To each hyacinth bulb, 2 ml of a certain solution was applied. Thus, the aim was to improve the sprouting of hyacinth bulbs and of biometric indicators such as hyacinth height, number of leaves and percentage of flowering. It has been observed that the nanoparticle treatments act differently depending on the growing stage of the plant. From the analysis of the results we can state that the treatment with gold nanoparticles in a concentration of 50 µg/mL is good for stimulating the plant growth, but it is not effective in their development, the most suitable solution as a treatment being that of gold nanoparticles in a concentration of 25 µg/mL.*
Key words: gold nanoparticles, chitosan, hyacinth bulbs, nanoparticles treatment, plant growth stimulation

Rezumat. *În cercetările privind influența nanoparticulelor asupra plantelor este foarte important să identificăm atât concentrațiile optime de nanoparticule, cât şi fenofaza în care planta este sensibilă la un anumit tip de tratament. Studiul privind influența nanoparticulelor asupra bulbilor de zambilă s-a desfăşurat în cadrul unui experiment în care s-au folosit concomitent 4 variante de tratament, fiecare variantă având 3 repetiții. Ca tratament au fost folosite două soluții cu concentrații diferite de nanoparticule de aur în chitosan (25 şi 50 µg/mL), iar ca martori au fost considerate eşantioanele la care s-a administrat fie o soluție de chitosan, fie apă. Fiecărui bulb de zambilă i s-au aplicat câte 2 ml dintr-o anumită soluție. Astfel, s-a urmărit îmbunătățirea încolțirii bulbilor de zambilă şi a unor indicatori biometrici precum înălțimea zambilelor, numărul de frunze şi procentul de înflorire. S-a observat că tratamentele cu nanoparticule acționează diferit în funcție de fenofaza plantei. Din analiza rezultatelor putem afirma că tratamentul cu nanoparticule de aur în concentrație de 50 µg/mL este bun pentru stimularea creşterii plantelor, însă nu este eficient în cazul*

[1]"Ion Ionescu de la Brad" Iasi University of Life Sciences, Romania

dezvoltării acestora, cea mai potrivită soluție ca tratament fiind cea de nanoparticule de aur în concentrație de 25 µg/mL.

Cuvinte cheie: nanoparticule de aur, chitosan, bulbi de zambile, tratament cu nanoparticule, stimularea creşterii plantei

INTRODUCTION

Nanotechnology is a growing research domain, having a wide range of applications in which it can be used. Among the various types of nanoparticles, the ones produced from noble metals are of high interest in material science, biomedicine, drug delivery and even plant science (Vinci and Rapa, 2019). The specific physical–chemical properties of this type of nanoparticles (NPs) offers them a high multifunctionality (Fratoddi *et al*, 2018). Gold nanoparticles (AuNPs) have the advantage of presenting a high stability, their chemical synthesis and surface functionalisation being easily achievable (Neuschmelting *et al*, 2018).

Regarding the exposure of plants to nanoparticles, extensive studies must be made, since the effects of NPs on them can be either beneficial or, on the contrary, harmful. More than this, a certain type of NPs can show good influences on a plant species for a given concentration, but at a higher concentration may inhibit the growth and development of that plant or can even be toxic for it (Siddiqi and Husen, 2021).

The NPs synthesis should be done in a way that the final solution does not contain any toxic component. This is the reason for choosing AuNPs prepared in a biopolymer (namely in chitosan), which is a polymer known for its non-toxic effects, obtained from the chitin of crustacean shells and widely used in research studies (Jiménez-Gómez and Cecilia, 2020).

Hyacinths are ornamental plants grown for their production of cut flowers or as landscaping flowers. In the present study, the impact of nanoparticles treatment on different growing stages of hyacinth was investigated, with the aim of enhancing the flowering percentage.

Bulbs are the underground parts of plants that can grow into flowering plants. Hyacinth bulbs can be divided into two main categories: those that bloom in spring and those that bloom in summer. In our study, we used hyacinth bulbs, the Pink Pearl variety, which bloom in spring.

MATERIAL AND METHOD

The study of the effect of nanoparticles on the growth and development of hyacinth bulbs was conducted under control conditions, to avoid the influence of variations of environmental factors. The controlled conditions were set up in the horticultural greenhouse within the “Vasile Adamachi” farm in Iasi. In order to create a climate favourable to plant growth, automated Priva system was used to optimize the temperature, humidity and air flow throughout the research. The control of the internal climate in the greenhouse influences the growth of crops and is responsible for the prevention of diseases and pests.

In this experiment were used 4 treatment variants simultaneously, each variant consisting of 3 bulbs grown in a single pot in 3 repetitions (9 bulbs per treatment variant), thus using a total number of 36 bulbs.

The synthesis method for obtaining the gold nanoparticles in chitosan was described elsewhere (Lipşa *et al*, 2020).

The treatment variants used were:

- V1 – AuNPs (25 µg/mL) + chitosan 0.1%
- V2 – AuNPs (50 µg/mL) + chitosan 0.1%
- V3 – Chitosan 0.1%
- V4 – H_2O (control).

The treatment was performed by applying 1 mL of solution on each bulb, followed by a waiting time of 15 minutes necessary for an efficient absorption by the bulb, and then applying another 1 mL. Thus, a total amount of 2 mL of solution per bulb was applied, representing an amount of 6 ml solution per pot. This treatment was applied to the root of the bulb to increase the degree of absorption of nanoparticles by avoiding its hydrophobic parts.

The hyacinth bulbs were kept in cold conditions for 12 weeks, from which 5 weeks at 4°C, followed by 5 weeks at 10°C and then 2 weeks at 13°C. The cold treatment had the role of stimulating the growth of hyacinths. After the cold period, the bulbs were planted on the surface of the soil in pots with a diameter of 19 cm and a volume of 3L, a quarter of the volume of the bulb being left uncovered. The planting distance was considered as to keep at least 1-2 cm between the bulbs and, also, from the edge of the pot. The plants were kept at a temperature of about 20°C. The substrate used in the experiment was a mixture of peat (40%), garden soil (42%) and leaf compost (18%). In terms of irrigation, the plants were watered so that the substrate was slightly moist. All plants in the experiment were maintained throughout the experiment in identical environmental conditions.

RESULTS AND DISCUSSIONS

In the research on the influence of nanoparticles on plants, it is important to identify both the optimal concentrations of nanoparticles and the growing stage in which the plant is sensitive to a certain type of treatment. For this purpose, we observed the sprouting process of the bulbs and noted the influence on bulbs for each solution applied.

The sprouting of hyacinth bulbs began on the first day for two of the treatments (V1 and V3) and ended on the ninth day for V2 and the twelfth day for V1 (tab. 1).

Table 1

Results regarding the sprouting of hyacinth bulbs

Sample	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
V1	1	2	4	4	4	5	6	6	7	8	8	9	9
V2	0	0	1	4	4	5	6	8	9	9	9	9	9
V3	1	3	3	4	4	6	7	7	8	8	8	8	8
V4	0	0	2	3	4	4	6	6	7	7	7	7	7

The final percentage of sprouting varied between 77.78% for the control variant (V4) and 100% for the V1 and V2 variants (fig. 1). For the V3 variant, the percentage was 88.89%.

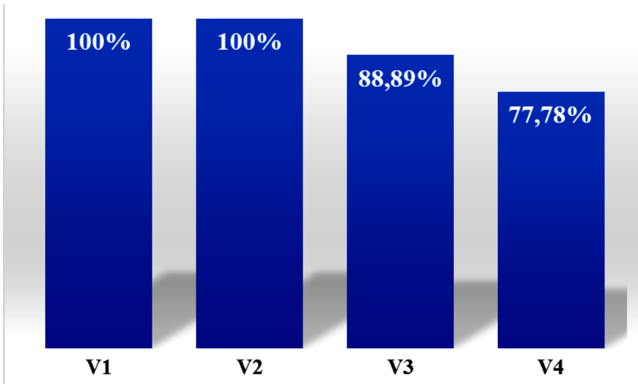

Fig. 1 The influence of nanoparticles on the sprouting of hyacinth bulbs

As can be seen, the treatment with nanoparticles stimulates the sprouting, regardless of the concentration used. The treatment with only chitosan (as the polymer in which the nanoparticles were dispersed) has a lower sprouting rate than the treatment with AuNPs – chitosan solutions. These experimental observations show that gold nanoparticles play an important role in the sprouting growing stage (fig. 1), the development differing as function of the treatment applied.

Regarding the flowering process, measurements were done every two days, to ensure a careful monitoring. The biometric indicators investigated were represented by the hyacinth height, the number of leaves, and the percentage of flowering. Measurements for all these indicators were performed weekly after the first plants reached this growing stage.

The height of the hyacinth was measured using a ruler, starting from the base of the soil to the top of the plant, at 35 days after the start of the experiment. The average data on the height of plants treated with the four variant solutions are presented in table 2, where a significant difference can be observed between the bulbs treated with gold nanoparticles and the ones from the control batch.

Table 2

The average height of hyacinths

Variant	Average height (cm)
V1 (AuNPs (25 µg/mL) + chitosan 0.1%)	10.8
V2 (AuNPs (50 µg/mL) + chitosan 0.1%)	9.6
V3 (Chitosan 0.1%)	7.9
V4 (Control)	7.4

The average height varied between 7.4 for the control batch and 10.8 for the variant treated with a solution of AuNPs (25 µg/mL) and chitosan. Thus, the treatments with gold nanoparticles on hyacinths led to an increase of the height by 45% for V1 and by 30% for V2.

Another bioindicator determined in the experiment was the average number of leaves (tab. 3), which indicate the vigour of the plant.

Table 3

The average number of leaves

Variant	Average number of leaves
V1 (AuNPs (25 µg/mL) + chitosan 0.1%)	7.2
V2 (AuNPs (50 µg/mL) + chitosan 0.1%)	7.5
V3 (Chitosan 0.1%)	6.3
V4 (Control)	6.0

The number of leaves varied between an average of 6.0 for the control variant and 7.5 for the V2 variant. It can be noted that for this bioindicator, the best treatment was the one involving a concentration of the gold nanoparticles of 50 µg/mL, even though good results were also obtained for a concentration of 25 µg/mL of AuNPs.

The flowering rate of hyacinths is very important, because they are used as cut flowers and is necessary for it to be as high as possible. In the case of ornamental flowers, the percentage of flowering (tab. 4.) is the most important indicator that is taken into accounts.

Table 4

The average flowering percentage of hyacinths

Variant	Flowering percentage (%)
V1 (AuNPs (25 µg/mL) + chitosan 0.1%)	83.3
V2 (AuNPs (50 µg/mL) + chitosan 0.1%)	70.8
V3 (Chitosan 0.1%)	66.7
V4 (Control)	51.7

According to table 4, the control variant (untreated) achieved an average flowering percentage of 51.7%. In contrast, variant V1 has an average flowering percentage of 83.3%, the treatment with AuNPs (25 µg/mL) and chitosan being the most effective treatment from the two gold nanoparticles treatments presented in this study.

Although the V2 variant had the highest number of leaves, it showed a lower percentage of flowering. From the analysis of the results, we can state that the treatment with AuNPs (50 µg/mL) – chitosan is good for stimulating plant growth, but is not that effective in their development.

CONCLUSIONS

1. The results show that the nanoparticle treatments act differently depending on the growing stage of the plant, and this is an important aspect to be assessed as function of the final goal (if the plants are grown for their flowers or for other parts).

2. The treatment with gold nanoparticles in a concentration of 50 µg/mL is good for stimulating the plant growth, but it is not effective in their development, the most suitable solution as a treatment being that of gold nanoparticles in a concentration of 25 µg/mL.

3. Using a lower concentration of gold nanoparticles is cost effective and provides improved results for the flowering percentage of hyacinths.

Acknowledgments: This work was supported by the CNCS-UEFISCDI, PN-III-P1-1.1-TE-2016-2336 project.

REFERENCES

1. **Fratoddi I, Cartoni A, Venditti I, Catone D, O'Keeffe P, Paladini A, Toschi F, Turchini S, Sciubba F, Testa G, Battocchio C, Carlini L, Proietti Zaccaria R, Magnano E, Pis I, Avaldi L., 2018** - *Gold nanoparticles functionalized by rhodamine B isothiocyanate: A new tool to control plasmonic effects*, Journal of Colloid and Interface Science, 513, pp. 10-19.
2. **Jiménez-Gómez C.P., Cecilia J.A., 2020 -** *Chitosan: A Natural Biopolymer with a Wide and Varied Range of Applications*, Molecules (Basel), 25(17), pp. 3981.
3. **Lipşa F.D., Ursu E.L., Ursu C., Ulea E., Cazacu A., 2020 -** *Evaluation of the Antifungal Activity of Gold–Chitosan and Carbon Nanoparticles on Fusarium oxysporum*, Agronomy, 10(8), pp. 1143.
4. **Neuschmelting V, Harmsen S, Beziere N, Lockau H, Hsu HT, Huang R, Razansky D, Ntziachristos V, Kircher MF., 2018** - *Dual-Modality Surface-Enhanced Resonance Raman Scattering and Multispectral Optoacoustic Tomography Nanoparticle Approach for Brain Tumor Delineation*, Small, 14(23), pp. 1800740.
5. **Siddiqi K.S., Husen A., 2021 -** *Plant response to silver nanoparticles: a critical review*, Critical Reviews in Biotechnology, pp. 1-18.
6. **Vinci G., Rapa M., 2019** - *Noble Metal Nanoparticles Applications: Recent Trends in Food Control*, Bioengineering (Basel), 6(1), pp. 10.