SCIENTIFIC REPORT

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Project title: **The use of engineered nanomaterials in plant science (*NanoPlant*)**

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Stage 3	**Investigation of antifungal properties and nanoparticles on soil diseases (Part II)**	**37.950,00 lei**
Activity 3.1	Evaluation of the antifungal activity of nanoparticles on plants infected with fungal spores	

The scientific objectives of this stage have been completely realized: the plant bulbs were inoculated with fungal spores in order to evaluate the effects of different types of nanoparticles on combating them.

The studies carried out at this stage of the project are very important in the current international context of research related to the use of nanoparticles in the field of plant science. Therefore, the value and scientific level of the results obtained so far are high, the statement being confirmed by the scientific paper sent for publication, the patent application sent to OSIM during this stage and by a scientific paper presented at an international conference.

Activity 3.1 Evaluation of the antifungal activity of nanoparticles on plants infected with fungal spores

Fungal material

The study focused on identifying the impact of different nanoparticle (NPs) solutions on hyacinth plants infected with different types of fungi. To identify the optimal dose of NPs needed to control fungal attacks in crops, NPs solutions of different concentrations were used.

In the experiment, three types of fungi that are common in hyacinth bulbs and plants were used. To determine the fungal species found on the surface of infected hyacinth bulbs (*Hyacinthus orientalis*), microscopic preparations were made which were analyzed and measured under an optical microscope, and the determination of micromycete genera was performed using the literature.

The fungal species identified in this experiment were::

1. Fusarium rot, *Gibberella zeae*, fam. *Nectriaceae*, affecting the hyacinth plant in the form of rot of leaves, peduncles and flowers. Light pink to dark red mycelium grows in colonies on plant lesions in humid conditions.

2. Blue mold rots, *Penicillium spp*, fam. *Trichocomaceae*, visually identified by the blue color that appears on the hyacinth bulbs.

3. Gray mold, *Botrytis hyacinthi*, fam. *Sclerotiniaceae*, it often forms yellow to brown areas on hyacinths.

After identifying the pathogens, they were isolated and grown in Petri dishes, being kept at 28ºC. Subsequently, the pathogens were inoculated into lesions created in healthy hyacinth bulbs, subjected to the experiment.

Treatment with nanoparticle solutions

The activities carried out in this stage of the project focused on the study of the antifungal effects of magnetite nanoparticles dispersed in aqueous solution of sodium hydroxide and chitosan, respectively of gold nanoparticles dispersed in chitosan and citrate. The methods of synthesis of the nanoparticles used as well as their characterization have been presented in previous reports.

In this study, we used treatments with different concentrations of nanoparticles to identify the optimal treatment for the most common fungal attacks on hyacinth plants grown in the greenhouse.

The studied hyacinth bulbs were cultivated in the horticultural greenhouse, from the "Vasile Adamachi" farm of USAMV Iași, under controlled conditions, in order to avoid contamination with fungi from other crops. The experiment for each type of treatment was organized on 9 bulbs grown in 3 pots.

The treatment variants used for this activity were:

- v1 – magnetite NPs, GD28 0.18%
- v2 – magnetite NPs, GD28 0.14%
- v3 – magnetite NPs, GD28 0.04% (fig. 1, (c))
- v4 – chitosan-magnetite NPs, GD30 0.21%
- v5 – chitosan-magnetite NPs, GD30 0.157%
- v6 – chitosan-magnetite NPs, GD30 0.052%
- v7 – AuNPs + citrate (5,2 mM)
- v8 – AuNPs + citrate (10,4 mM)
- v9 – Citrate (fig. 1, (a))
- v10 – AuNPs + chitosan (25 µg/ml)
- v11 – AuNPs + chitosan (50 µg/ml)
- v12 – Chitosan (fig. 1, (b))
- v13 – H_2O (control).

(a) (b) (c)

Fig. 1. Nanoparticle solutions of different concentrations: citrate solutions with Au nanoparticles (a), chitosan solutions with Au nanoparticles (b) and magnetite nanoparticles GD28 (c).

The nanoparticle treatment was applied to each bulb in two steps. The amount of solution used for each bulb was 2 ml applied sequentially in two installments in the form of 1 ml doses with a waiting time between the two treatments of 15 min.

Hyacinth bulbs

The experiment was organized in the greenhouse in January, and to simulate the natural conditions of winter, the bulbs were kept in cold conditions for 8 weeks, of which 5 weeks at 4°C, followed by 2 weeks at 8°C and then 1 week at 12°C.

After treatment, hyacinth bulbs were grown in pots with a diameter of 19 cm, distributed so as not to touch the edges of the pot or neighboring bulbs. At the end, they were covered three quarters with substrate (Figure 2 (a)).

(a) (b)

Fig. 2. Hyacinth bulbs planted in pots

Determination of the antifungal properties of nanoparticles on hyacinth cultures

The antifungal properties of the nanoparticle solutions were determined for batches of hyacinth bulbs planted in pots and inoculated with the following fungi: **Fusarium rot**, *Gibberella zeae* of the family *Nectriaceae*; **blue mold**, *Penicillium spp* of the family *Trichocomaceae*, and **gray mold**, *Botrytis hyacinthi* from the family *Sclerotiniaceae*, as can be seen in the images below.

Fig. 3. Hyacinth bulbs inoculated with: Fusarium rot, Blue mold and Gray mold

Determining the negative impact of fungi on hyacinth growth

Fungal colonies can develop in different phenophases of plant growth and development. They depend on environmental conditions, especially humidity and temperature.

In the present study, the impact on different phenophases was determined. In the germination phenophase, it is observed that the development of the colonies differs depending on the treatment.

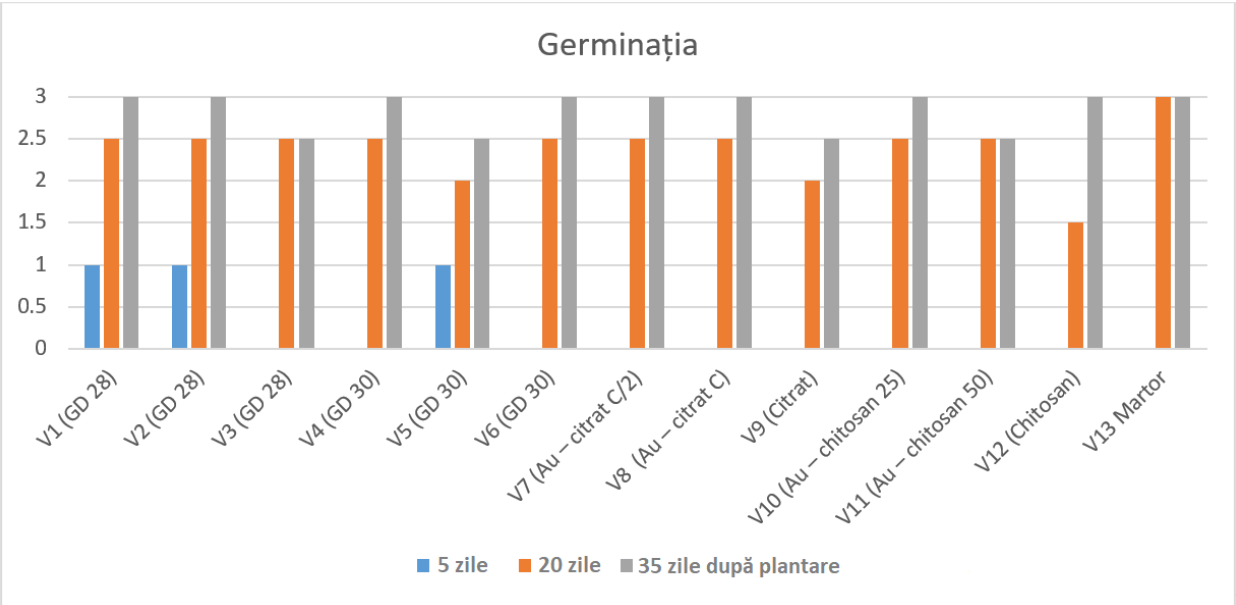

Fig. 4. Germination of hyacinth bulbs infected with different types of fungi

From the image can be seen that the variants with the highest germination capacity were V1 (GD 28 0.18%), V2 (GD 28 0.14%) and V5 (GD 30 0.157%).

In the case of the variants treated with citrate (V7, V8 and V9), it can be seen that the variant with citrate (V9) germinated harder than the V7 and V8 variants in the composition of which there is also gold.

In order to follow the flowering process as closely as possible, determinations were made every two days.

The biometric indicators measured were represented by: the hyacinth height, the number of leaves and percentage of flowering. Measurements for these indicators were performed weekly after the first plants reached this phenophase.

Determination of height

The height was measured, using a ruler, from the base of the soil to the top of the plant (figure 5).

Fig. 5. Determining the height of the hyacinth

Data on the height of hyacinth plants treated with nanoparticle solutions are presented in Table 1.1, where a quite large variation between different treatments can be observed.

Table 1.1. The average height of the hyacinths

Variant	**Average height (cm)**
V1 (GD 28 0.18%)	8,9
V2 (GD 28 0.14%)	13,8
V3 (GD 28 0.04%)	9,6

V4 (GD 30 0.21%)	11,6
V5 (GD 30 0.157%)	10,2
V6 (GD 30 0.052%)	10,6
V7 (Au – citrate 5,2 mM)	11,4
V8 (Au – citrate 10,4 mM)	8,8
V9 (Citrate)	7,4
V10 (Au – chitosan, 25 μg/ml)	10,8
V11 (Au – chitosan, 50 μg/ml)	8,7
V12 (Chitosan)	7,5
V13 Control	8,6

The height ranged from 7.4 for V9 to 13.8 for V2. The variants treated with citrate and chitosan showed a stimulation by 54% and 25%, respectively, of the increase in height for average NP concentrations.

Another bioindicator determined in the experiment was the number of leaves (table 1.2.) which indicates the vigor of the plant.

Table 1.2. The number of leaves

Variant	**Number of leaves (average)**
V1 (GD 28 0.18%)	5,8
V2 (GD 28 0.14%)	8,3
V3 (GD 28 0.04%)	7,0
V4 (GD 30 0.21%)	7,7
V5 (GD 30 0.157%)	6,0
V6 (GD 30 0.052%)	7,5
V7 (Au – citrate 5,2 mM)	7,2
V8 (Au – citrate 10,4 mM)	6,5
V9 (Citrate)	4,7
V10 (Au – chitosan, 25 μg/ml)	6,8
V11 (Au – chitosan, 50 μg/ml)	6,8
V12 (Chitosan)	6,3
V13 Control	6,0

The number of leaves varied between 4.7 in the case of variant V9 and 8.3 in the case of variant V2. It can be seen, from table 1.2., that the control variant achieved an average number of 6.0 leaves, compared to the variants treated with chitosan with an average of 6.8.

The percentage of hyacinths flowering is important, because they are used as cut flowers and it is necessary for it to be as high as possible. Due to the infection of the plants with fungi, some developed until the flowering phase and after that they stagnated (Figure 6).

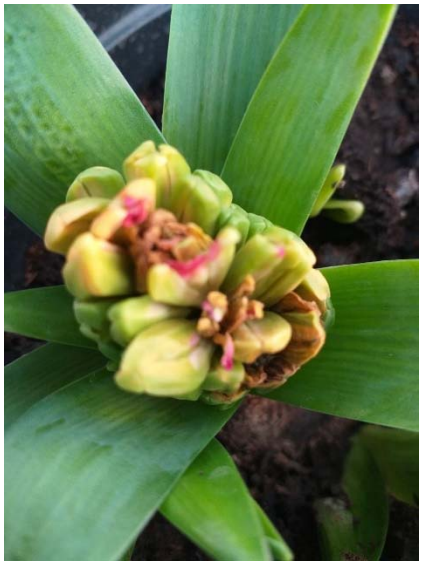

Fig. 6. Effects of the mold on hyacinth flower

In the case of ornamental flowers, the percentage of flowering (Table 1.3.) is the most important indicator. To obtain a high percentage of flowering plants, we studied the treatement of fungi with different nanoparticle solutions.

Table 1.3. Percentage of flowering

Variant	**Percentage of flowering (%)**
V1 (GD 28 0.18%)	63,3
V2 (GD 28 0.14%)	49,2
V3 (GD 28 0.04%)	65,0
V4 (GD 30 0.21%)	100,0
V5 (GD 30 0.157%)	70,8
V6 (GD 30 0.052%)	84,2
V7 (Au – citrate 5,2 mM)	87,5
V8 (Au – citrate 10,4 mM)	68,3
V9 (Citrate)	61,7
V10 (Au – chitosan, 25 µg/ml)	83,3

V11 (Au – chitosan, 50 µg/ml)	66,7
V12 (Chitosan)	66,7
V13 Control	51,7

From table 1.3., can be seen that the control variant (untreated) achieved a flowering percentage of 51.7%, and the lowest percentage of flowering was achieved in the case of variant V2, which was 49.2%.

Instead, the V4 variant has a 100% flowering percentage. The GD 30 magnetite treatment is the most effective nanoparticle treatment of the variants presented in this study.

Although the V2 variant achieved the highest height and the largest number of leaves, it showed the lowest percentage of flowering. From the analysis of the results we can say that the treatment with GD 28 mangentite is good for stimulating plant growth but is not effective in their development.

Conclusions

Nanoparticle treatments work differently depending on the phenophase of plant growth and development. In the case of hyacinth bulbs, treatment with magnetite GD 28, of different concentrations (V1 and V2), and magnetite dispersed in chitosan GD30, of medium concentration (V5), are the most effective in the case of germination capacity. In contrast, the citrate solution (V9) has an inhibitory effect on the germination of hyacinth bulbs.

Citrate has an inhibitory effect on the growth of hyacinth plants, whereas NP of magnetite GD 28 has a stimulating effect. Note that gold NP dispersed in citrate blocks the inhibitory effect of citrate. The same effect, but of smaller magnitude, was reported in the case of chitosan. NP treatments have the same effect in the case of the foliar index.

The treatment with concentrated solution of NP of mangetite (GD 30) dispersed in chitosan has a very good effect in stimulating the flowering of hyacinths. This treatment inhibits the action of all the fungi studied and stimulates the plants to bloom in a maximum percentage.