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# The effect of using nanosilver-containing microelement fertiliser on the physiological properties of *Avena sativa* L.

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**Abstract:** Since the Green Revolution, higher crop production has caused a significant decrease in available soil elements. Microelement deficiencies have become a factor that limits the productivity of agricultural crops around the world. Recent advances in bionanotechnology have opened the way to the development of biocompatible foliar nanofertilisers with higher nutrient utilization efficiency. It was assumed that the applied foliar fertilisation would have a positive effect on the growth and development of plants. The application of fertiliser positively affected the parameters analysed of plant gas exchange (net photosynthesis rate ($P_N$), transpiration rate ($E$), stomatal conductance ($g_s$), intercellular conductance ($C_i$)) and chlorophyll content and its fluorescence (relative chlorophyll content (*CCI*), maximum quantum efficiency of photosystem ($F_v/F_m$), maximum quantum efficiency of primary photochemistry ($F_v/F_0$), photosynthetic efficiency index (*PI*), total number of active absorption reaction centers (*RC*/*ABS*)). Compared to the control, in most analyses, the most stimulating effect was observed for fertiliser concentrations of 0.25 to 0.30% (except $C_i$ – 0.35%). However, the effectiveness of the applied doses depended on the measurement date. To clearly determine the dose that will have the most stimulating effect on the analysed parameters and at the same time will not be toxic to plants, more research should be conducted, especially under field conditions.

**Keywords:** chlorophyll content, chlorophyll fluorescence, foliar fertilisation, gas exchange, nanoparticles

## INTRODUCTION

Scientific research on the possibilities of using oats (*Avena sativa* L.) focuses mainly on the evaluation of their nutritional value and health-promoting properties of the grain (Yue *et al.*, 2021; Zhang *et al.*, 2021). Oats are a valuable cereal due to the high beta-glucan content in the grain, which has a significant impact on the prevention and treatment of lifestyle diseases (Martnez-Villaluenga and Peas, 2017). From an agrotechnical point of view, it is a cereal with low soil requirements and high resistance to soil acidification. This plant can be successfully grown in marginal areas. It does not require the use of large doses of fertilisers, which allows reducing the costs of grain production (Menon *et al.*, 2016).

Currently, an urgent challenge in agriculture is to improve fertilization efficiency in order to reduce the environmental footprint associated with increased crop production on existing agricultural land. Standard soil fertilization strategies are often not very efficient due to element immobilization and loss of nutrients through leaching or volatilization (Kopittke *et al.*, 2021; Husted *et al.*, 2023). The ineffectiveness of conventional fertilisers is related to the inherent chemical properties of essential plant nutrients. Some nutrients such as P, Mn, Fe and Zn can become unavailable to plants after soil application due to chemical binding and microbial immobilization (Mogollón *et al.*, 2021; Langhans *et al.*, 2022). Despite continued progress in the development of more efficient fertilisers and fertilisation strategies, low nutrient use efficiency (*NUE*) remains. Foliar fertilization offers an attractive complementary strategy because it bypasses unfavourable soil processes, but its implementation is often hampered by poor stomatal penetration, leaf damage, and limited ability of nutrients to translocate within the plant (Peirce *et al.*, 2019; McBeath *et al.*, 2020).

Recent advances in bionanotechnology offer a number of emerging opportunities to overcome these challenges. The development and application of nanotechnology has influenced many disciplines in a wide range of sectors, including communication, medicine, energy, and agriculture. Nanoparticles (NPs) are particles in size from 1 to 100 nm that can be used in many different ways due to their physicochemical properties, such as high surface area, high reactivity, variable pore size, and particle morphology (Sener and Sayğı, 2023). Their effectiveness may depend on application time, concentration, or particle size (Janmohammadi *et al.*, 2016). Metallic nanoparticles have an extremely large surface-to-volume ratio and the ability to regulate electron exchange. Nanoparticles are widely used in environmental remediation, in agriculture as stimulants for plant growth and development, fungicides to prevent fungal diseases, or fruit ripening agents (Geilfus, 2017). In addition, silver nanoparticles (AgNPs) increase chlorophyll content and photosynthetic rate, thereby promoting plant growth and development (Sengottaiyan *et al.*, 2016; Hatami, Naghdi Badi and Ghorbanpour, 2019). There are reports of the use of silver nanoparticles in studies on plant growth and development under field (Hojjat and Hojjat, 2015; Jasim *et al.*, 2017; Mahendran, Geetha and Venkatachalam, 2019; Wasaya *et al.*, 2020; Tung *et al.*, 2021; Sener and Sayğı, 2023) and in vitro (Zarei and Ehsanpour, 2023) and drought stress resistance (Ghavam, 2019; Ahmed *et al.*, 2021; Alabdallah *et al.*, 2021; Akhoundnejad, Karakas and Demirci, 2022; Sarwar *et al.*, 2023). However, it turns out that the introduction of excessive amounts of nanosilver into ecosystems may not be neutral for the environment. Penetrating into the natural environment, they can pose a serious problem for the proper functioning of ecosystems, in particular microorganisms occurring in the soil, and reduce the chlorophyll content in leaves (Iqbal, Waheed and Naseem, 2020; Abdelghany *et al.*, 2022). To ensure sustainable and optimal use of these products and materials, it is important to thoroughly understand the potential negative or positive effects associated with nanomaterials and nanotechnological products (White and Gardea-Torresdey, 2018; Compant *et al.*, 2019; Rodriguez *et al.*, 2019; Sillen *et al.*, 2020).

There are few reports on the effect of silver nanoparticles on chlorophyll fluorescence parameters and plant gas exchange (Sami, Siddiqui and Hayat, 2020; Khina, Lisichkin and Krutyakov, 2024), but they do not focus on a specific multi-component product. Therefore, studies were conducted to assess the effect of different concentrations of multi-component foliar fertiliser containing nanosilver on the course of selected physiological processes of oat plants *Avena sativa* L. Foliar fertilisation is a good and economically justified alternative for plant feeding. It allows for a quick response in the event of deficiencies. Moreover, the use of this type of application does not interfere with the soil environment and allows for a quick supply of nutrients to plants, which may be difficult, for example, due to soil drought. A research hypothesis was put forward in which the exogenous application of multi-component fertiliser containing nanosilver will have a positive effect on gas exchange parameters (net photosynthesis rate ($P_N$), transpiration rate ($E$), stomatal conductance ($g_s$), intercellular conductance ($C_i$)), chlorophyll content (SPAD – soil plant analysis development) and chlorophyll fluorescence ($F_v/F_m$, $F_v/F_0$, $RC/ABS$, $PI$) and will not have a negative effect on plant growth and development.

## MATERIAL AND RESEARCH METHODS

### PLANT GROWTH AND DEVELOPMENT

The pot experiment was conducted in the laboratory of the Department of Plant Production of the University of Rzeszów in January–March 2024. The pots with a diameter of 12 cm were filled with 1.5 kg of soil with a grain size of loamy sand (slightly acidic pH 6.32, content: P – 7.46 mg·(100 g)$^{-1}$, K – 13.9 mg·(100 g)$^{-1}$, Mg – 8.69 mg·(100 g)$^{-1}$ and Ca – 9.52 mg·(100 g)$^{-1}$), and then the seed of the oat cultivar Gepart (Hodowla Roślin Strzelce) was sown. It is a high-yielding cultivar with exceptionally large seeds. The grain is characterised by high uniformity and high grain density and the highest fat content among the registered varieties. It yields well on light soils. The target number of plants after emergence in a single pot was 10. After plant emergence, in the 3-leaf phase, foliar application of SilverPlant (UNI-FARMA Sp. z o.o.) fertiliser containing 0.01% non-ionic colloidal silver was performed at five concentrations within the range recommended by the manufacturer: 0.20, 0.25, 0.30, 0.35 and 0.40%. The spray was carried out with a manual laboratory sprayer with a flow regulation and a dosing volume of 1.2 cm$^3$ ±0.1 during one press (outlet diameter 0.6 mm). An even spraying method was used: the same amount of solution (50 cm$^3$) for each pot until the solution was completely exhausted and the plants were covered. At the same time, deionised water was used in the control sample in the same volume. The fertiliser, in addition to silver, contained: 230 g·dm$^{-3}$ total nitrogen (60 g·dm$^{-3}$ ammonium nitrogen, 110 g·dm$^{-3}$ amide nitrogen, 60 g·dm$^{-3}$ nitrate nitrogen), 4 g·dm$^{-3}$ sulphur, 0.1 g·dm$^{-3}$ boron, 1.0 g·dm$^{-3}$ copper, 0.2 g·dm$^{-3}$ iron, 5.0 g·dm$^{-3}$ manganese, 0.04 g·dm$^{-3}$ molybdenum, 0.1 g·dm$^{-3}$ zinc. The experiment was carried out in four replications in a growth chamber (GC-300/1000, JEIO Tech Co., Ltd.). The chamber temperature was 22 ±2°C, relative air humidity 60 ±3%, photoperiod 16/8 h (light/dark), and maximum light intensity 350 µmol·m$^{-2}$·s$^{-1}$. The moisture of the substrate in the pots was maintained at 60% of the field water capacity. Physiological processes of oat plants were studied on the first and second fully developed leaf. Chlorophyll content and fluorescence were measured three times, on the fourth, eighth and 12$^{th}$ day after foliar spraying of plants. The gas exchange analysis of the plants was performed on the same leaves twice, on the eighth and 12$^{th}$ day after foliar application of fertiliser.

### PHYSIOLOGICAL MEASUREMENTS

#### Relative chlorophyll content measurement

The relative chlorophyll content (*CCI*) was measured using a Minolta SPAD-502 chlorophyll meter and expressed in unmeasured SPAD (soil plant analysis development) units, which are closely related to the chlorophyll content. The instrument measures the difference in light absorption by leaves at 650 and 940 nm. The proportion of these differences indicates the leaf greenness index or chlorophyll content. Measurements were made on three fully expanded leaves per pot (30 measurements per concentration).

#### Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a Pocket PEA continuous excitation fluorometer (Hansatech Instruments) equipped with black shading clips that were applied to the leaf blade away from the leaf vein. Measurements were taken five times in each pot in the mid-leaf area. The fluorescence signal was collected under actinic red light, with a maximum wavelength of the light source of 627 nm and transmitted for 1 s at a maximum available intensity of 3500 µmol (photon) of photosynthetically active radiation (PAR, $m^{-2} \cdot s^{-1}$). Fluorescence measurements were taken three times in each pot on the mid-leaf blade after 30 min of dark adaptation (30 measurements per concentration). The following parameters were measured: maximum quantum efficiency of photosystem II (PSII) ($F_v/F_m$), maximum quantum efficiency of primary photochemistry ($F_v/F_0$), photosynthetic efficiency index (*PI*) and total number of active absorption reaction centers – *RC*/*ABS*)

### Gas exchange measurement

The LCpro-SD photosynthesis measurement system (ADC Bioscientific Ltd.) was used to measure leaf gas exchange. The instrument chamber has a flow accuracy of ±2% of its range. During measurement, the light intensity in the measurement chamber was 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the air temperature was 23 ±2°C. The photosynthetic rate ($P_N$), transpiration rate ($E$), stomatal conductance ($g_s$), and intercellular $CO_2$ concentration ($C_i$) were analysed. Measurements were made on three fully expanded leaves per pot.

### Statistical analysis

The results obtained were statistically analysed using Statistica 13.3.0 (TIBCO Software Inc.). Analysis of variance (ANOVA) with repeated measures (with time assessment as a factor) was used. Tukey's HSD post hoc test was used to determine differences between mean values of the parameters analysed ($p < 0.05$).

## RESULTS

### MEASUREMENT OF RELATIVE CHLOROPHYLL CONTENT

The chlorophyll content (*CCI*) in the oat leaves depended on the applied dose of foliar fertiliser and the measurement date (Fig. 1). A significant increase in chlorophyll content was shown on each date after the application of 0.3% dose of multicomponent foliar fertiliser containing nanosilver, respectively 4.8, 7.06 and 25.6% compared to the control. On the third date, each dose of foliar fertilization was also shown to cause an increase in chlorophyll content compared to the control and the 0.3% dose proved to be the most beneficial. The remaining doses also caused an increase in the chlorophyll content of the leaves, but the differences between them were not significant. The analysis of the measurement date showed that on the third measurement date, a significant decrease in chlorophyll content was observed compared to the first and second measurement dates, regardless of the applied dose of nanosilver-containing multicomponent fertiliser.

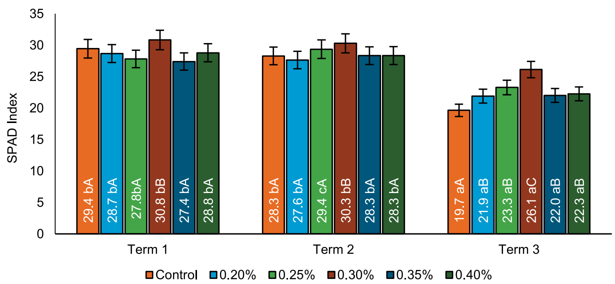


**Fig. 1.** The effect of different concentrations of foliar fertilizer containing nanosilver and measurement date on the relative content of chlorophyll (*CCI*) in oat leaves; capital letters indicate significant differences between means in measurement dates, and lower case letters indicate significant differences between means in subsequent measurement dates ($n = 30$, $p < 0.05$); SPAD = soil plant analysis development; source: own study

### CHLOROPHYLL FLUORESCENCE MEASUREMENTS

In the first measurement date, the application of multicomponent foliar fertiliser containing silver at a dose of 0.25 and 0.40% caused a significant increase in maximum quantum efficiency of photosystem ($F_v/F_m$) compared to the control. On the second date, this relationship occurred at a dose of 0.25 and 0.30%, which was statistically confirmed. Both the applied dose and the measurement date were shown to significantly determine the $F_v/F_m$ values (Fig. 2a).

Statistical analysis showed the effect of applied foliar fertilization doses on the maximum quantum efficiency of primary photochemistry ($F_v/F_0$) index. In the first and third measurement dates, an increase in the index was shown compared to the control at a dose of 0.25 and 0.40%. On the second date, a significant increase was found at a dose of 0.25 and 0.30% (on average of 7.9%). On the third measurement date, this relationship occurred at a dose of 0.25 and 0.40%. In addition to the duration of the experiment, a positive effect of the applied fertilization on $F_v/F_0$ was observed after the application of all doses of the preparation compared to the control (Fig. 2b).

In each of the measurement dates, the application of foliar spraying with multicomponent fertiliser containing nanosilver at a dose of 0.25 and 0.30% caused a significant increase in the *PI* index compared to the control. Both the applied dose and the measurement date were shown to significantly determine the *PI* values (Fig. 2c).

The value of the *RC*/*ABS* index on the first measurement date was higher after the application of multicomponent fertiliser containing nanosilver at doses of 0.20 and 0.30% (by 7.0 and 9.8%, respectively) compared to the control. On the second measurement date, the most stimulating effect of the applied fertiliser was demonstrated for the 0.30% dose (an increase of 12.8% compared to the control). On the third measurement date, all doses of foliar fertilization (except dose 0.35%) caused an increase in *RC*/*ABS* compared to the control, and the highest increase, by 25.5%, was observed for dose 0.30% (Fig. 2d).

### MEASUREMENT OF GAS EXCHANGE PARAMETERS

On the first measurement date, a decrease in plant transpiration ($E$) was observed after the application of all doses of multicomponent fertiliser containing nanosilver compared to the control, except for the dose of 0.25% (an increase of 14%). The 0.25% dose of the preparation also caused an increase in the value of the analysed parameter on the second measurement date, but this increase was smaller and amounted to 3.1%. An increase in transpiration was observed with measurement time (term 2), regardless of the applied fertiliser dose (Fig. 3a).

The stomatal conductance ($g_s$) depended on the applied fertilisation dose and measurement time. On the first date of

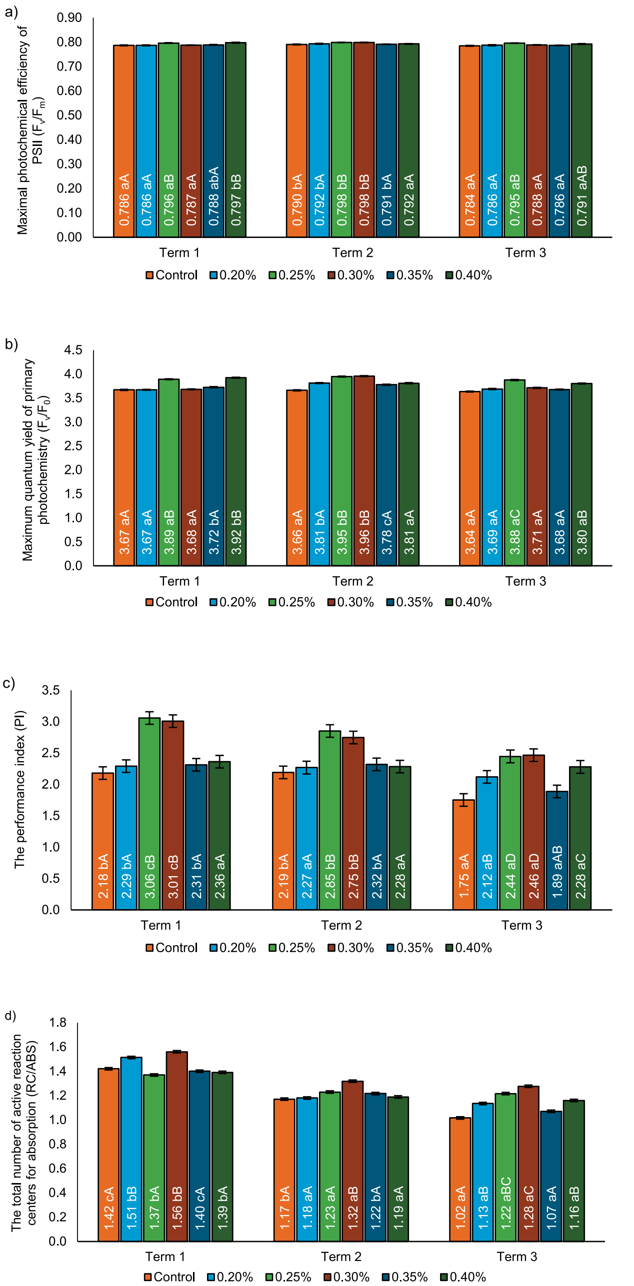


**Fig. 2.** The effect of different concentrations of foliar fertilizer containing nanosilver and measurement date on chlorophyll fluorescence parameters: a) maximal photochemical efficiency of photosystem II (PSII) ($F_v/F_m$), b) maximum quantum yield of primary photochemistry ($F_v/F_0$), c) performance index (*PI*), d) total number of active absorption reaction centers (*RC*/*ABS*) in oat leaves; capital letters indicate significant differences between means in measurement dates, and lower case letters indicate significant differences between means in subsequent measurement dates ($n = 30$, $p < 0.05$); source: own study

measurement, plants obtained significantly the highest $g_s$ index values after applying the 0.25% dose. This relationship also occurred on the second measurement date. Both the dose of fertiliser used and the duration of the experiment significantly conditioned the value of the analysed parameter (Fig. 3b).

At the first measurement date, statistical analysis showed an increase in $P_N$ after foliar application of multicomponent fertiliser containing nanosilver at a dose of 0.25% (an increase of 29% compared to the control). An increase also occurred on the

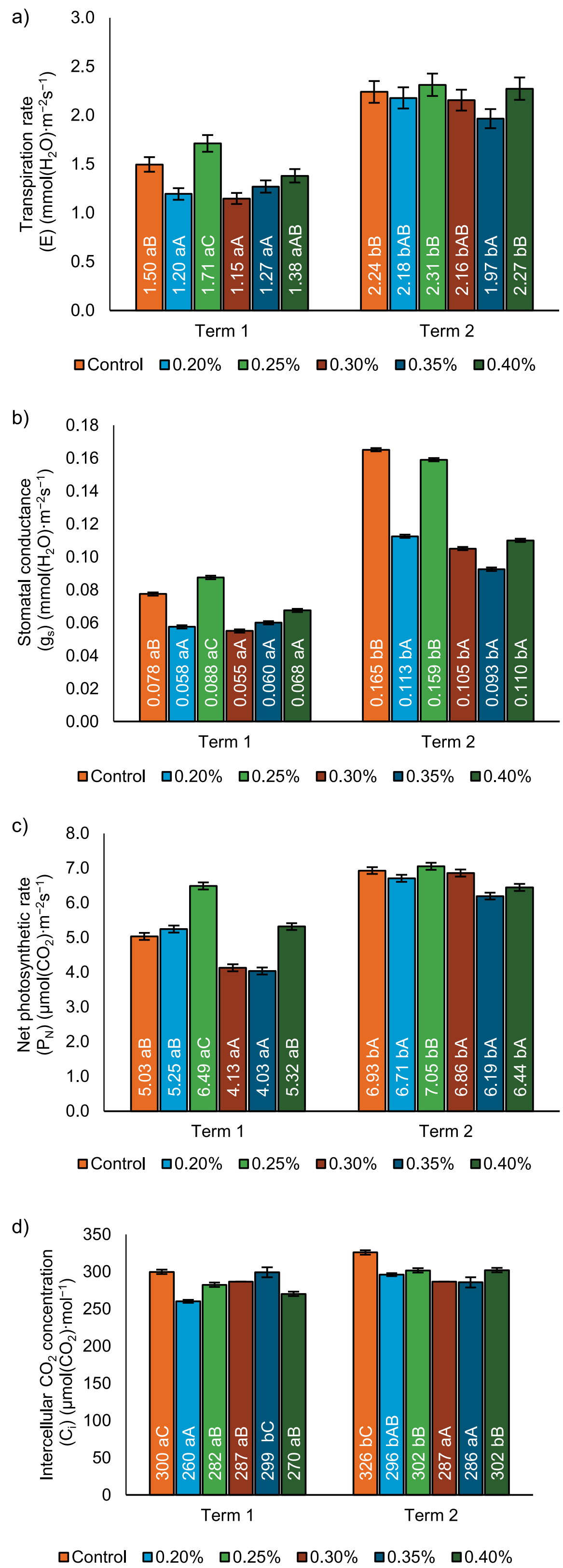


**Fig. 3.** The effect of different concentrations of foliar fertilizer containing nanosilver and the measurement date on gas exchange parameters: a) transpiration rate (*E*), b) stomatal conductance ($g_s$), c) photosynthesis rate (*PN*), d) intercellular $CO_2$ concentration ($C_i$) in oat plants; capital letters indicate significant differences between means in measurement dates, and lower case letters indicate significant differences between means in subsequent measurement dates ($n = 30$, $p < 0.05$); source: own study

second measurement date. With the passage of measurement time, the value of the $P_N$ index increased after the application of all the doses of foliar fertilization analysed. The greatest increase, compared to the first date, was observed after applying a dose of 0.30% (Fig. 3c).

On the first measurement date, the highest $C_i$ values were observed after the application of a 0.35% spray of multi-component fertiliser containing nanosilver. On the second date, a significant decrease in the $C_i$ index was observed compared to the control after the application of each dose of the preparation. It was the highest on the object where 0.35 and 0.40% of the Silverplant spray was applied (average decrease of 12%). By analysing the measurement time, the $C_i$ index on the second date was significantly lower compared to the control (Fig. 3d).

## DISCUSSION

To support sustainable agricultural intensification, innovative solutions for crop production must be introduced. The introduction of nanotechnology to plant science opens the way to the creation of new strategies for nutrient application (Wang *et al.*, 2021; Akhoundnejad, Karakas and Demirci, 2022). Many researchers believe that nanoparticles (NPs), due to their originally small size and adaptable surface properties, enable the delivery of essential plant nutrients in a form fundamentally different from their counterparts in the form of classical mineral ions. Their effectiveness may depend on the time of application, concentration, or particle size (Janmohammadi *et al.*, 2016; Januszkiewicz, Kulczycki and Samoraj, 2023). NPs enable better adhesion to the leaf surface and its penetration, as well as transport through various cellular barriers (Molnár *et al.*, 2020; Yue *et al.*, 2021).

Measurements of gas exchange, chlorophyll content, and fluorescence are valuable tools to understand photosynthesis and plant responses to abiotic stresses (Bucher, Bernhardt-Römermann and Römermann, 2018; Dąbrowski *et al.*, 2019). The study was conducted to investigate the effect of foliar application of a multi-component fertiliser containing nanosilver. The doses used (0.20, 0.25, 0.30, 0.35 and 0.40%) did not negatively affect the growth and development of plants. In comparison with the control, the applied preparation stimulated the development of plants expressed by their physiological properties. Plant growth and development depend on environmental conditions, and photosynthesis is one of the most important biochemical pathways by which plants convert solar energy into chemical energy (Janssen *et al.*, 2014). In the analysed studies, a positive effect of exogenous application of multi-component fertiliser containing nanosilver on gas exchange parameters expressed as transpiration rate ($E$), net photosynthesis rate ($P_N$), stomatal conductance ($g_s$), and intercellular conductance ($C_i$) was found. An increase in the values of the analysed parameters was observed both in relation to the applied fertilization doses and the duration of the experiment. Jaskulska and Jaskulski (2020) demonstrated a similar relationship. The analysis of chlorophyll content and fluorescence, as well as gas exchange, plays an important role in understanding the main mechanisms of photosynthesis and plant responses to environmental changes. These parameters are important markers of the photosynthetic state of the plant, especially in response to environmental stresses (Goltsev *et al.*, 2016; Kalaji *et al.*, 2016; Kalaji *et al.*, 2018). In the conducted studies, foliar application of fertiliser with nanosilver caused an increase in chlorophyll content in oat leaves at each measurement date, and the most effective dose was 0.3%. A positive effect of the fertiliser on chlorophyll fluorescence parameters $F_v/F_m$, $F_v/F_0$, $RC/ABS$ and $PI$ was also demonstrated, depending on both the doses of the fertiliser used and the measurement dates. Studies indicate that silver nanoparticles (AgNPs) increase the chlorophyll content and photosynthetic rate, thus promoting plant growth and development. They contribute to the increase in indole acetic acid (IAA) (Razzaq *et al.*, 2016; Sengottaiyan *et al.*, 2016; Hatami, Naghdi Badi and Ghorbanpour, 2019). In studies on *Trigonella foenum-graecum* L., AgNPs improved plant growth, increased photosynthetic pigment content (that is, chlorophyll and carotenoid content), and indole acetic acid content, which in turn resulted in increased yield and quality (Sadak, 2019).

The effect of AgNPs depends on the plant species, its growth stage and fertiliser concentration and particle size and age, as well as on the concentrations and size of nanoparticles. AgNPs are considered a promising active material because they have antibacterial, antifungal effects and show a positive effect on plant growth and development (Hojjat and Hojjat, 2015; Jasim *et al.*, 2017; Dahlous *et al.*, 2019; Mahendran, Geetha and Venkatachalam, 2019; Acharya *et al.*, 2020; Alabdallah *et al.*, 2021; Amna *et al.*, 2021; Abdelsalam *et al.*, 2022; Khafaga *et al.*, 2022; Sabra *et al.* 2022). Some researchers have found that ions of some metals, e.g. silver, accumulate in high concentrations in plant tissue and cause polymerization of phenol with the peroxidase enzyme, which chelates heavy metals, which leads to toxicity (Nejatzadeh-Barandozi, Darvishzadeh and Aminkhani, 2014). In our studies, the most beneficial effect, similar to chlorophyll fluorescence, was obtained after foliar application of fertiliser in the amount of 0.25–0.30%. Higher doses, especially 0.4%, caused a decrease in parameters, which may indicate toxic effects of the preparation, which was also shown in studies by other authors (Gruyer *et al.*, 2014). High concentrations of AgNPs limit the content of chlorophyll and carbohydrates and reduce the assimilation area of leaves and contribute to the development of oxidative stress induced by reactive oxygen species. In addition, plants exposed to high concentrations of nanosilver may experience water balance disorders, cell damage and reduced photosynthesis. Chlorophyll content decreased at high concentrations of nanosilver in *Oryza sativa* (AgNPs concentration 0.2–1.0 mg·dm$^{-3}$) (Nair and Chung, 2014) and *Vigna radiata* (AgNPs concentration 20 mg·dm$^{-3}$) (Nair and Chung, 2015), especially when these plants were grown under drought stress (AgNPs concentration 100 mg·dm$^{-3}$) (Akhoundnejad, Karakas and Demirci, 2022). Nanotechnology has revolutionised conventional fertilisation science. The use of fertilisers containing elements in the nano form is an innovative solution in plant production. The use of nanotechnological solutions can affect the bioavailability of elements in the environment. However, it is necessary to take care of all aspects related to their proper use.

## CONCLUSIONS

Modern technologies, including the use of fertilisers containing nanoparticles, are considered an important tool for improving the quality of agricultural crops. The conducted studies have shown a positive effect of foliar feeding of oat plants with a multicomponent foliar fertiliser containing nanosilver on the physiological parameters of plants. In most analyses, the use of fertilisers in doses from 0.25 to 0.30% resulted in an increase in the values of the analysed gas exchange parameters (net photosynthesis rate ($P_N$), transpiration rate ($E$), stomatal conductance ($g_s$), intercellular conductance ($C_i$)), chlorophyll content ($SPAD$) and chlorophyll fluorescence (relative chlorophyll content ($CCI$), maximum quantum efficiency of photosystem ($F_v/F_m$), maximum quantum efficiency of primary photochemistry ($F_v/F_0$), photosynthetic efficiency index ($PI$), total number of active absorption reaction centers ($RC/ABS$)) compared to the control. The effectiveness of the fertiliser used was also influenced by the date of the experiment. Compared to the control, no negative effect of the fertiliser used on the growth and development of plants was observed regardless of the concentration used. The use of nanoparticles in agriculture can improve plant resistance to diseases and increase their efficiency in terms of absorption or uptake of more nutrients. To determine the dose that will have the most stimulating effect on the analysed parameters, more research is needed, especially under field conditions.

## CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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