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The effect of the application of cyanobacterial biofertilizers from the genus *Nostoc* on the quantitative parameters of the species *Monarda fistulosa* L.

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Abstract. The present study evaluates the effects of cyanobacterial biofertilizers from the genus *Nostoc* (N. gelatinosum, N. punctiforme, N. linckia) on the growth, biomass accumulation, and essential oil content of *Monarda fistulosa* L. cultivated under open-field conditions. Experiments were conducted in 2024 in Dobrogea, Sîngera, Moldova, using three application rates (20, 30, and 40 kg/ha) with untreated plants as control. Results indicated that cyanobacterial biofertilizers significantly increased plant height and green biomass, with the highest effects observed at 40 kg/ha. Essential oil content showed a slight, non-significant increase (0.02–0.05 %) compared to control, likely influenced by drought conditions. The study confirms that *Nostoc*-based biofertilizers enhance plant productivity and represent an ecologically sustainable alternative to chemical fertilizers, improving soil quality and promoting growth of medicinal plants.
Keywords: cyanobacteria, biofertilizers, *Monarda fistulosa*, biomass, essential oil.

Efectul aplicării biofertilizanţilor cianobacterieni din genul *Nostoc* asupra parametrilor cantitativi ai speciei *Monarda fistulosa* L.

Rezumat. Prezentul studiu evaluează efectele biofertilizatorilor cianobacterieni din genul *Nostoc* (N. gelatinosum, N. punctiforme, N. linckia) asupra creşterii, acumulării de biomasă şi conţinutului de ulei esenţial la *Monarda fistulosa* L. cultivată în câmp deschis. Experimentele au fost realizate în anul 2024 în satul Dobrogea, or. Sîngera, Moldova, utilizând trei doze de aplicare (20, 30 şi 40 kg/ha), cu lot netratat drept martor. Rezultatele au arătat că biofertilizatorii cianobacterieni au determinat creşteri semnificative ale înălţimii plantelor şi biomasei verzi, cele mai mari efecte fiind obţinute la doza de 40 kg/ha. Conţinutul de ulei esenţial a înregistrat o creştere nesemnificativă (0,02–0,05 %) faţă de martor, probabil influenţată de condiţiile secetoase din anul de experimentare. Studiul confirmă că biofertilizatorii pe bază de *Nostoc* reprezintă o alternativă ecologică şi durabilă la fertilizarea chimică, contribuind la creşterea productivitĭii plantelor medicinale.
Cuvinte-cheie: cianobacterii, biofertilizanţi, *Monarda fistulosa*, biomasă, ulei esenţial.

1. Introduction

Medicinal plants have been of interest in herbal medicine for over 5000 years, being used by the early Indian, Chinese and Egyptian civilizations, and later by the Greek, Roman and Persian civilizations, as well as in American, Arab and Japanese cultures [11]. Due to their demonstrated beneficial effects and advantages resulting from their use, medicinal plants are still widely used in herbal medicine. According to the World Health Organization (WHO) reports on traditional and complementary medicine, over 80% of the population of approximately 170 countries use traditional practices, such as herbal medicine and other specific procedures. At the same time, a wide range of modern pharmaceutical products, including aspirin, artemisinin and some treatments for childhood cancer, are derived from natural sources and traditional knowledge [14]. Research indicates that, during the period 2010–2023, the global value of medicinal plant exports increased by 98.70%, reaching USD 4,178,138,000 [15]. The increasing applicability of medicinal plants has led to the expansion of the areas cultivated with these crops, which, in turn, stimulates the development of new cultivation technologies.

Currently, the process of cultivating medicinal plants largely uses chemical fertilization, which leads to an increase in biomass, but causes soil degradation, the accumulation of toxic substances in the biomass of medicinal plants and limits the sustainable development of this sector [9].

In this regard, there are emerging solutions that can contribute to improving crop yields by using cyanobacteria, which have the potential to revolutionize agricultural systems and solve problems both today and in the long term [1], [5]. Cyanobacteria are preferred as biofertilizers because they represent some of the most archaic organisms (appeared over 3.5 billion years ago), have a high tolerance to environmental factors, easily adapt and can grow abundantly on practically all types of soil. As a result of the activity of most cyanobacterial species, there is an improvement in the physical, chemical and biological state of the soil, strengthening plant health, as well as increasing the quality and quantity of crops [2].

Our recent research has demonstrated that, for the selection of cyanobacterial species used as biofertilizers in plant cultivation, it is necessary to take into account the physiological characteristics of the species (tolerance to drought, temperature fluctuations and strong solar radiation, ability to quickly switch between vegetative and dormant stages, minimum cell volume, regeneration capacity in 8–12 hours, accelerated reproduction, proliferation on the surface and in the depth of the soil, possibility of selection in culture and cultivation on an industrial scale, nitrogen fixation capacity and nutrient mobilization, as well as the possibility of inducing "soil flowering"), ecological characteristics (dominance, distribution of species or groups, geographical distribution, specific resistance to environmental factors, biological specificity and high tolerance to environmental conditions) and the recommended application rate (between 5 and 258 kg/ha). Taking into account these criteria, the cyanobacterial species that can be preferentially used as biofertilizers were recommended. Among these are representatives of the genus *Nostoc*, with the recommended species: *N. linckia*, *N. punctiforme, N. gelatinosum, N. microscopicum, N. commune, N. paludosum, N. minutum, N. foliaceum* and *N. flagelliforme*, all of which are of particular interest in agricultural applicability, including the cultivation of medicinal plants [5].

Monarda fistulosa is a medicinal plant of interest, used since ancient times to treat colds, bronchial diseases, headaches and abdominal pain, as well as against acne. The first European settlers used it as an antiemetic and carminative. The plant contains significant amounts of essential oil, which has antimicrobial, antioxidant, hepatoprotective, anti-inflammatory and anticancer potential [3], [8]. The biomass of the plant contains phenolic acids (rosmarinic, caffeic, neochlorogenic and chlorogenic acid) and various flavonoids (luteolin, apigenin, acacetin, linarin, didymine, etc.). The aerial part can be used to obtain teas, spices and functional food products [6], [12].

To obtain phytotherapeutic products or food supplements from *Monarda fistulosa* biomass, it is very important that the plant is cultivated in an ecological system, using cyanobacterial biofertilizers.

Based on these considerations, the present study aimed to investigate the effect of the application of cyanobacterial biofertilizers from the genus *Nostoc* on the quantitative growth parameters of *Monarda fistulosa* L. cultivated under ecological conditions.

2. Materials and Methods

The experiments were conducted in 2024 under open-field conditions on a *Monarda fistulosa* plantation located in the village of Dobrogea, Sîngera town, Chișinău Municipality (Republic of Moldova), within the "Melnic Cristin Victor" Agricultural Farm. A combined live cyanobacterial biomass consisting of equal proportions (1:1:1) of *Nostoc gelatinosum, N. punctiforme* and *N. linckia* was used as the biological treatment. The cyanobacterial biomass was harvested separately from laboratory cultures maintained on standard Detmer and Kratz and Myers nutrient media, collected during the exponential growth phase, separated from the culture medium, mixed in equal proportions and applied to the soil surface in April. Three application rates of the combined cyanobacterial biomass were tested: Variant 1 – 20 kg ha^{-1}, Variant 2 – 30 kg ha^{-1} and Variant 3 – 40 kg ha^{-1}. An untreated plot served as the control.

The experiment was arranged in three replicates. Each experimental plot had an area of 30 m^2 and contained 255 *M. fistulosa* plants. During the growing season, observations were performed on plant morphological characteristics at different phenological stages, biomass yield and total essential oil content according to the methods described previously [16], [17]. The initial soil characterization was performed using standard soil analysis methods [7]. The mathematical processing of the results was carried out by applying the computer program "Microsoft Office 2010" with the determination of the arithmetic mean (M) and the standard error (m).

3. Results and discussion

The soil in the experimental plots is of typical moderately humiferous chernozem type, with the physicochemical composition presented in Table 1.

Table 1. General physicochemical composition of the soils used in the experiments

Depth	**pH**	**Hygroscopicity, %**	**Humus, %**	**$CaCO_3$, %**	**Ca, me/100g**	**Mg, me/100g**
0-10	7.94	5.62	4.50	–	32.78	5.04
30-40	7.48	5.82	3.30	–	30.19	5.07
50-60	7.21	7.41	2.80	–	29.41	4.32

The soils in the experimental plantations fall into typical moderately humus-rich chernozems, characterized by neutral pH and a high content of humus, calcium and magnesium – essential substances for the cultivation of the *Monarda fistulosa* L. These soils are suitable for this crop (Table 1). An important aspect that determines the quantitative effect of the application of cyanobacterial biofertilizers on plants is the monitoring of their height, because the increase in the amount of biogenic elements in the soil stimulates the height development of plants.

The application of cyanobacterial biofertilizers generated a positive effect, manifested by an increase in the height of *Monarda fistulosa* stems in all vegetation phases. The highest plant height values were recorded in Variant 3, followed in descending order by Variants 2 and 1. In the stemming phase, the height of plants in the variants treated with cyanobacterial biofertilizers was 0.03–0.16 cm higher compared to the control group. In the budding phases and until mass flowering, the height of treated plants was higher, reaching values higher by 0.19 cm (Variant 1) and 6.93 cm (Variant 2) compared to the control. At the end of the flowering phase, the height of the plants in Variant 3 was 7.22 cm higher than in the control group, and in Variants 2 and 1, 4.13 cm and 1.04 cm higher, respectively (Figure 1).

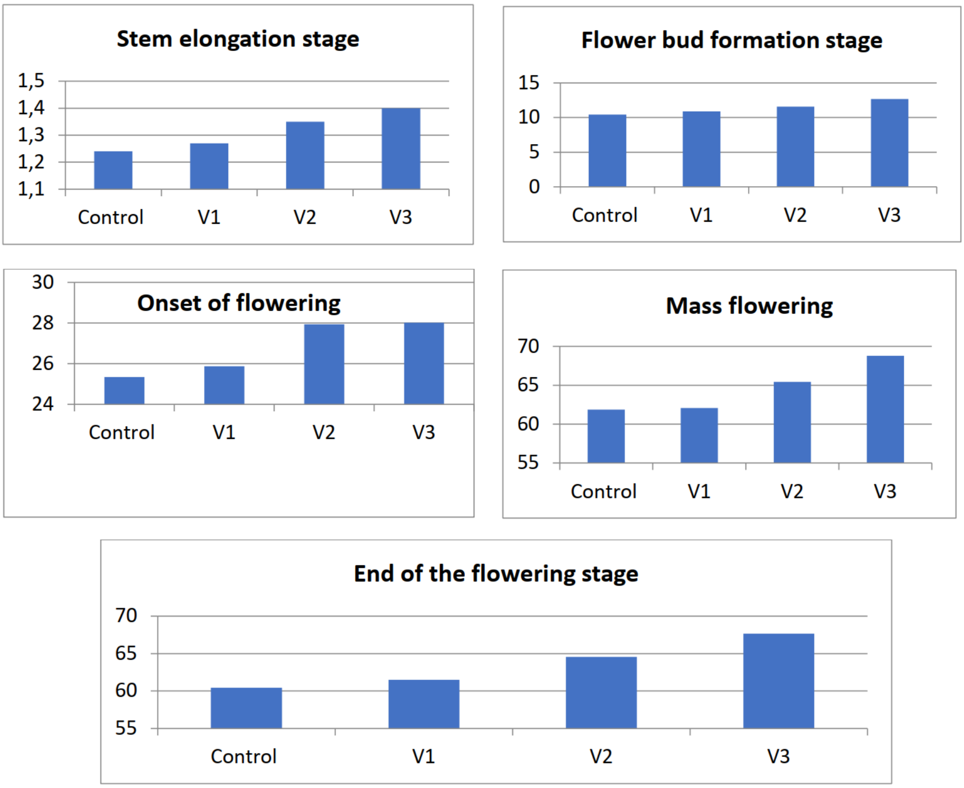

Figure 1. Influence of cyanobacterial biofertilizers from the genus *Nostoc* on changes in the height of the *Monarda fistulosa* stem during phenological phases, cm

The results obtained indicate that the application of cyanobacterial biofertilizers had the greatest effect on the increase in plant height with increasing doses of biofertilizers from the genus *Nostoc*.

Research conducted by Salama A., Sabry R. and Sharaf Eldin M. revealed that the application of nitrogen fertilizers causes an increase in plant height by over 12.53 cm, as well as an increase in biomass and essential oil content in *Monarda citriodora* [10]. It is well known that cyanobacteria of the genus *Nostoc*, used by us as biofertilizers, have the ability to fix atmospheric nitrogen in the soil and to mobilize soil biota, including partially other biogenic elements (P and K). The increase in plant height observed in our experiment is due to the effect generated by cyanobacteria of the genus *Nostoc*, in particular by facilitating access to biologically fixed nitrogen from the atmosphere and to other nutrients mobilized from the soil by them and other soil microorganisms that develop abundantly together with cyanobacteria.

After the start of the mass flowering phase of the plants, the biomass was harvested and the necessary quantitative calculations were performed. The results obtained indicate that the application of cyanobacterial biofertilizers determined an increase in the green biomass of the plant by 2–200 kg/ha. The highest values were recorded when applying biofertilizers from the genus *Nostoc* at a dose of 40 kg/ha, followed by doses of 30 and 20 kg/ha (Figure 2).

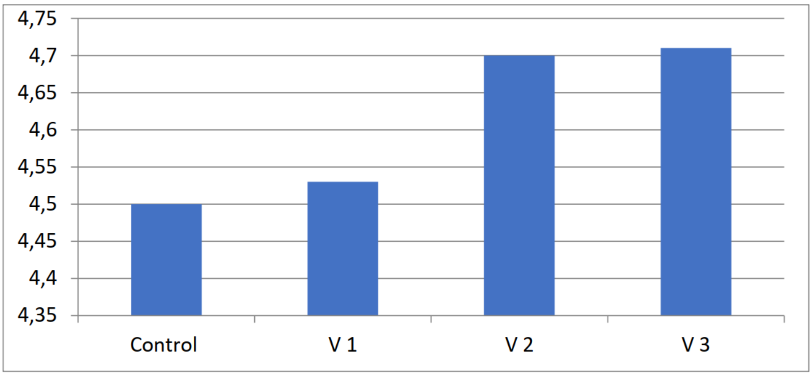

Figure 2. Effect of applying biofertilizers from the genus *Nostoc* on the biomass of *Monarda fistulosa* L., t/ha

The increase in biomass is correlated with the fact that the plants in the variants treated with cyanobacterial biofertilizers had higher heights, which stimulated the accumulation of biomass.

In our previous research, we demonstrated that the combined application of biofertilizers from the genus *Nostoc* (species *N. gelatinosum, N. punctiforme* and *N. linckia*) generated a quantitative increase in the biomass of *Echinacea purpurea* (L.) Moench., by 0.18–0.99 t/ha [4]. Other research that followed the effect of applying cyanobacterial biofertilizers found that their use leads to improved soil quality, stimulation of crop growth and increased yield [13]. In the research presented in the paper, it was also found that the productivity of *Monarda citriodora* plants increased as a result of the application of cyanobacterial biofertilizers from the genus *Nostoc*, which allows us to state that these cyanobacteria had a quantitative beneficial effect on the plants studied.

Quantitative analyses of essential oil from *Monarda fistulosa* plant biomass indicate that the application of cyanobacterial biofertilizers leads to a non-significant increase in total oil content (Table 2).

Table 2. Influence of cyanobacterial biofertilizers from the genus *Nostoc* on changes in essential oil content in *Monarda fistulosa* biomass, %

Control	V1	V2	V3
0.60 ± 0.01	0.62 ± 0.01	0.63 ± 0.01	0.65 ± 0.01

The highest values were recorded when applying biofertilizers from the genus *Nostoc* at a dose of 40 kg/ha (0.65 ± 0.01 %), followed by doses of 30 kg/ha (0.63 ± 0.01 %) and 20 kg/ha (0.62 ± 0.01 %). These values are 0.02–0.05 % higher compared to the control group (0.60 ± 0.01 %), which indicates an increasing trend, but not significant.

The insignificant increase in essential oil content can be attributed in particular to the fact that 2024 was a dry year, which directly influenced the development of cyanobacteria and, implicitly, their effect on the soil and plants studied, since in this case the soil-plant relationship is strictly respected.

4. Conclusions

The application of cyanobacterial biofertilizers based on *Nostoc gelatinosum, Nostoc punctiforme* and *Nostoc linckia* promoted the growth and development of *Monarda fistulosa* plants, resulting in increased stem height throughout all phenological stages compared with the untreated control. The effect was dose-dependent, with the application rate of 40 kg ha^{-1} producing the highest values of the investigated growth parameters. Cyanobacterial biofertilization also stimulated green biomass accumulation, increasing biomass yield by 2–200 kg ha^{-1} relative to the control. The essential oil content showed a slight increase (0.02–0.05%) following biofertilizer application; however, these differences were not statistically significant, possibly due to the dry climatic conditions during the 2024 growing season. Overall, the results indicate that cyanobacterial biofertilizers have the potential to enhance the growth and biomass productivity of *M. fistulosa* under the experimental conditions of this study.

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