Effect of new complex bio-fertilizer on seed germination of different varieties of winter vetch

A S Skamarokhova¹, H R Siyukhov¹, ³, N A Yurina¹, ², A I Petenko¹, ², E Yu Anisimova⁴, N I Mosolova⁴ and S A Surkova⁴, ⁵

¹Krasnodar Research Centre for Animal Husbandry and Veterinary Medicine, Krasnodar, Russia
²Kuban State Agrarian University named after I. T. Trubilin, Krasnodar, Russia
³Maikop State Technological University, Maikop, Russia
⁴Volga Region Research Institute of Manufacture and Processing of Meat-And-Milk Production, Volgograd, Russia

⁵E-mail: api.niimmp@mail.ru

Abstract. The article presents the results of studying the effect of new bio-fertilizer on the germination and germination energy of winter vetch seeds (Vicia villosa Roth). Experimental studies were conducted in the laboratory conditions of the Krasnodar Research Centre for Animal Husbandry and Veterinary Medicine (Krasnodar, Znamenskiy settlement). The positive effect of vetch seeds treatment with a new bio-fertilizer on germination energy and seed germination has been established. The germination energy and seed germination when treated with bio-fertilizer compared to the control (water) were higher by an average of 14.6 and 4.0%, respectively. The winter vetch variety Glinkovskaya in terms of seed germination energy turned out to be the best among other varieties. This indicator was higher by 19.35% in comparison with the control. The winter vetch variety Orlan in terms of seed germination turned out to be the best among other varieties. This indicator was higher by 9.0% in comparison with the control. This bio-fertilizer can be used to increase soil fertility and soil recultivation in plant-growing, agriculture.

1. Introduction

Much of the work of the advisory group on international agricultural research focuses on land or water management, agro-ecological approaches that use biological and ecological processes to reduce the use of non-renewable resources, especially chemical fertilizers produced from them. Sparing anti-erosion tillage, soil fertility and land fallow, the use of plant organic fertilizers, the control of the spread of farm pests through the use of biodiversity, biological control, and the reduction in pesticide use could be examples [1].

The intensification of arable farming is associated with air pollution from pesticides, NO₂ and CO₂, while the loss of organic matter in the soil has reduced the system's ability to carbon fixation. International trade contributes to global climate change by transporting arable land over long distances [2].

As the economic share of the primary agricultural sector declined, developed countries (the United States and the European Union, in particular) introduced a number of agricultural support measures to stimulate quality domestic production. These measures make it possible to increase the profitability of...
domestic agriculture by limiting variability and protecting it, for example, from the negative natural impacts. The biologization of agriculture plays an important role in this matter. However, this kind of policy can lead to serious distortions in international agricultural markets (for example, to lower demand for imports) to create long-term economic incentive for agricultural development in developing countries [3].

In addition to increasing agricultural productivity, synthetic nitrogen and phosphate fertilizers used in agricultural land are dramatically change global natural resources consumption, water quality, greenhouse gas balance and their feedback to the climate system. However, due to the lack of geospatial data on fertilizer use, modern research on earth ecosystem modeling is forced to ignore or use oversimplified data, for example, on the use of nitrogen and phosphorus as fertilizers in agriculture over the past ten or one hundred years. The IFA (International Fertilizer Association) and FAO (Food and Agriculture Organization of the United Nations) reviews on fertilizer introduction have become available. Given the expansion of arable land, the use of fertilizers has increased even more [4].

Soil microbial communities play an important role in ecosystem functioning because they influence several important processes, including the use of nutrients such as carbon, phosphorus and nitrogen, and soil formation. Bacteria and archaea make up a significant part of soil microbiome biodiversity and closely relate with biogeochemical cycles, energy flow and biodegradable pollutants. The presence of nutrients and organic substances in environments in which human intervention is small are direct results of microbial activity. This activity is capable of activating the depolymerization and mineralization of nitrogen, phosphorus and sulfur, and, as a rule, is associated with organic molecules, modulating the presence of inorganic forms of these nutrients in the soil, including in the form of ions such as ammonium, nitrates, phosphates and sulfates, which are more preferred plant nutrients [5, 6].

The ecological, social and economic reasons for the use of organic bio-fertilizers indicate that traditional agriculture exert constant pressure on the ecosystem, gradually deteriorating the state of the environment, especially as a result of the active use of resources and high-level technologies. The increasingly significant use of irrigation water is one of the main factors leading to the accumulation of salt in the rhizomes of plants, affecting both the physical, chemical and biological properties of the soil and the productivity of the agroecosystem. In this situation, soil degradation caused by salt exposure the need to develop soil reclamation strategies. The use of humic acids is one of the possible solutions because there is an increasing demand for their use in agriculture [7]. It is the main components of soil organic matter, play an important role in plant growth. And it is the subject of study in various fields of agriculture such as soil chemistry, soil fertility, and plant physiology. To date, the intensification of agriculture has reached a critical point. The negative consequences of this activity have led to irreversible global changes of the climate and ecosystem. New approaches are need. The use of plant biostimulants based on humic substances is one of the possible decisions to this problem [8].

The biostimulating properties of humic substances contribute to structural and physiological changes in roots and stems associated with the absorption and nutrient uptake. In addition, it can cause changes in the primary and secondary plant metabolism associated with abiotic stress resistance. Exogenous humic substances in agronomic systems can use for sustainable intensification of eco-farming [9].

Since most of the humic substances used in agriculture are currently derived from non-renewable resources such as coal and peat, the introduction of this technology requires the development of new sources of humic acids (for example, organic waste). The positive effect of organic or plant biostimulants based on humic substances is an alternative method for developing plant-growing and maintaining optimal soil fertility [10].

The use of humic acid has indirect and direct beneficial effects. Indirect effects are obtained by improving soil aggregation, structure, soil fertility and moisture retention, as well as increasing microbial activity. The direct beneficial effect of humic acids on the growth and development of plants consist of their effect on cell membranes, which lead to increased transport of minerals, improved protein synthesis, which promotes photosynthesis, improved enzymatic activity, high digestibility of micro- and macroelements, and a decrease in the activity of toxic substances. In addition, humic acid is consider a plant hormonal substance. The beneficial effect of humic acids on the growth and yield of
various agricultural plants, for example, beans and broad beans, has been proven. Fulvic acid is the second important humus substance and a good biostimulant for better plant growth and yield Fulvic acid, as an organic fertilizer, is a non-toxic mineral chelating additive and binder that maximizes the uptake of nutrients by plants and stimulates their productivity [11].

Humic products have a certain potential for agriculture, especially in terms of the availability of phosphorus and microelements, as well as soil recultivation. But no recommendations for their use can be made until extensive field trials, comparison of humic products with others, cost-benefit analysis are carried out [12].

Excessive use of chemical fertilizers poses a serious threat to soil and environmental quality. The use of fertilizers containing beneficial microorganisms that promote plant growth is a promising direction for ecosystem restoration [13].

Microbial biofilms are gaining importance in agriculture for their multifaceted agronomic benefits and sustainability to environmental changes. Azotobacter chroococcum and Trichoderma viride and their biofilms have a positive effect on the metabolic activity of soil and plants when growing wheat. The availability of macro- and microelements in the soil increased by 10-40% when using Azotobacter chroococcum and Trichoderma viride. Improved soil biological activity was facilitated by improved biofilm colonization due to the synergism between Azotobacter chroococcum and Trichoderma viride. The promotion of plant growth and soil fertility indicates the usefulness of this biofilm in agriculture [14].

2. Materials and methods
The function of the salt-tolerant strain Azotobacter chroococcum 76A was evaluated as a protective against stress in an important horticultural crop – tomato. The grafting of Micro Tom tomato plants with A. chroococcum 76A increased numerous growth parameters, and also gave protective effects at both moderate (50 mM NaCl) and heavy (100 mM NaCl) salt loads. These advantages were observed mainly with a decrease in nutrient levels and were less noticeable under optimal nitrogen nutrition conditions. It was found that the efficiency of A. chroococcum 76A depends on the nutrient status in the rhizosphere. The grafting with beneficent bacteria such as A. chroococcum 76A can be ideal decision for low-germination systems where environmental restraints and limited use of chemical fertilizers can affect yield potential [15].

Abuse of chemical fertilizers poses a great threat to soil quality and the environmental. The grafting of plants with rhizobacteria, which promote plant growth, has become a great prospect for ecosystem restoration. Of the thirty-nine isolated and tested for their growth potential, thirteen isolates had the ability to fix nitrogen, of which N9 (Azotobacter chroococcum) had the highest acetylene reduction activity at 156.26 nmol / g. The grafting with such a bacterial combination increased the content of alkaline-hydrolysis nitrogen, available phosphorus and available potassium in the soil by 49.46; 99.51 and 19.38%, respectively, and increased the content of nitrogen, phosphorus and potassium in wheat by 97.7; 96.4 and 42.1%, respectively. This bacterial combination is an excellent candidate for bio waste, which can reduce the use of chemical fertilizers without affecting normal wheat growth [13].

The study of complex bio-fertilizer Foshami use efficiency was carried out in the laboratory conditions of the Krasnodar Research Centre for Animal Husbandry and Veterinary Medicine (Krasnodar, Znamenskiy settlement). The object of research is four varieties of vetch (Orlan, Lugovskaya 2, Chernomorka and Glinkovskaya), which are often used in field fodder production in the Central zone of the Krasnodar Territory, for preliminary testing for the responsiveness of varieties of this crop to bio-fertilizer and its subsequent use in the field. A special feature of the vetch is the ability to provide early spring green feed. None of the legumes, except for the Vicia villosa, provides a green crop by the end of May.

Germination was carried out in Petri dishes at an ambient temperature of 20-22 °C by uniform wetting with a certain amount (5 ml) of a solution of the required concentration (0.5 g of extract per 9.5 g of water). The fertilizer includes an extract of poultry litter as a source of humic and fulvic acids, an extract of phosphorite meal as a source of available phosphorus, a shell for enrichment with organic
calcium, a microorganism Azotobacter chroococcum, a fungus Trichoderma viride and a set of mineral components.

The scheme of studies on the germination of vetch seeds in Petri dishes is given in Table 1.

Table 1. The scheme of research on the germination of vetch seeds in Petri dishes.

| Solution name                  | Stage                                      | Stage                                      |
|-------------------------------|--------------------------------------------|--------------------------------------------|
| Water                         | Determination of germination energy (on the 3rd day) | Determination of seed germination (on the 7th day) |
| New complex bio-fertilizer    | according to GOST 12038-84                 | according to GOST 12038-84                 |

We used Petri dishes, the bottom of which was laid with four layers of filter paper and soaked in control with distilled water and a solution of a new complex bio-fertilizer in a volume of 5 ml. 100 vetch seeds were placed in each Petri dishes. Petri dishes were removed to a dark place with t = 20-22 °C and every day 1 ml of solution and water were added in the control variant, respectively. The experiment was carried out in accordance with the requirements of GOST 12038-84 in triplicate. The germination energy of seeds of all varieties of vetch was determined on the third day, the germination of seeds on the seventh day.

3. Results and discussion

According to Table 2, we can judge about a significant increase in seed germination energy when treated with bio-fertilizers (on average by 16%).

Table 2. Germination energy (on the 3rd day), % (n = 3).

| Solution name                  | Winter vetch variety |
|-------------------------------|----------------------|
| (5 ml / l l)                  | Orlan                |
| Control (water)               | 46.00±1.47           |
| New complex bio-fertilizer    | 61.00±0.91***        |

Note: *** p < 0.001.

The results of the germination determination were as follows. All results are reliable (p < 0.001) in the variant with the use of a new complex bio-fertilizer, where the seed germination energy was significantly higher compared to the control (water) by an average of 14.6%: the germination energy was 15% higher in the Orlan variety compared to the control, Lugovskaya 2 – 10%, Chernomorka – 14.75%, Glinkovskaya – 19.35%.

Determination of seed germination (table 3) showed high reliability in almost all variants of research.

Table 3. Seed germination (on the 7th day), % (n = 3).

| Solution name                  | Winter vetch variety |
|-------------------------------|----------------------|
| (5 ml / l l)                  | Orlan                |
| Control (water)               | 80.25±0.63           |
| New complex bio-fertilizer    | 89.25±0.85***        |

Note: *** p < 0.001; ** p < 0.01.

An increase in the number of germinating seeds was also observed in the variant with the use of bio-fertilizer: in the Orlan variety – by 9%, Lugovskaya 2 – by 2.5%, Chernomorka – by 1.75%, Glinkovskaya – by 3%, compared with the control indicators (p < 0.001). On average, seed germination of four varieties of winter vetch increased by 4% in comparison with the control.
4. Conclusion
The results obtained indicate that the seeds of the winter vetch of the Glinkovskaya variety germinate most intensively among the tested varieties on the third day (germination energy) under the influence of complex bio-fertilizer (19.35%). This indicator is high in the Orlan variety also (15%). The seed germination in the Orlan variety (9%) is much higher in comparison with other varieties. Based on the results obtained, it can be assumed that the use of complex biofertilizer allows accelerating the germination energy (on average by 14.6%), thereby making the seedlings more uniform, and has a positive effect on the germination of vetch seeds also (on average by 4%).

Acknowledgments
The work was carried out under grant RSF No 19-76-10010, SSI NIIMMP.

References
[1] World Bank, World Development Report 2008: Agriculture for Development (World Bank, Washington, DC) 155-6
[2] Tilman D, Balzer C, Hill J and Befort B L 2011 Global food demand and sustainable agricultural intensification. Proc. of the National Academy of Sciences of the United States of America 108 20260-4 doi: 10.1073/pnas.1116437108
[3] McCarthy U, Uysal I, Badia-Melis R, Mercier S, O'Donnell C and Ktenioudakid A 2018 Global food security-problems, challenges and technological solutions Trends in food science and technology 77 11-20 doi: 10.1016/j.tifs.2018.05.002
[4] Stoate C et al 2001 Ecological consequences of the intensification of arable land in Europe Journal of Environmental Management 63 337-65 doi: 10.1006/jema.2001.0473
[5] Tian H 2017 Global use of nitrogen and phosphorus fertilizers for agricultural production over the past half century: shifted hotspots and nutrient imbalances The science of the Earth system 9 181-92
[6] Van der Heyden M G A, Bargett R D and van Straalen N M 2008 The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems Ecology Letters 11(3) 296-310 doi:10.1111/j.1461-0248.2007.01139.x
[7] Petenko A I, Zhlobova I S, Aniskina M V, Kucherenko A V, Yurina N A and Skamarokhova A S 2020 Influence of bio-solutions on the growth and germination of seeds of agricultural crops, as well as on changes in their biochemical parameters Agrarian Russia 9 26-9 doi: 10.30906/1999-5636-2020-9-26-29
[8] Borisenko V V, Zhlobova I S, Petenko A I, Gneush A N and Yurina N A 2018 Effect of "ECOSS" BioGumate on the Growth and Development of Winter Wheat of Various Varieties Journal of Pharmaceutical Sciences and Research 10(10) 2626-7
[9] Ouni Y et al. 2014 The role of chemicals in mitigating the harmful effects of soil salinity and increasing plant productivity International Journal of Plant Production 3 353-74
[10] Canellas L P et al. 2015 Humic and fulvic acids as biostimulants in horticulture Scientific gardening 196 15-27 doi: 10.1016/j.scienta.2015.09.013
[11] Fawzy F, El-Bassiony A M, Yunsheng L and Zhu O 2012 Goname Effect of mineral, organic and bio-N fertilizers on the growth, yield and quality of sweet pepper fruits Journal of Applied Sciences Research 8(8) 3921-33
[12] Billingham K L 2012 Humic products-potential or presumption for agriculture? Can chemical products improve my soil? Proc. of the 27th Annual Grasslands Society Conf. in New South Wales 27 43-50
[13] Wang J, Li R, Zhang H, Wei G and Li Zh 2020 The beneficial bacteria that activate the nutrients and promote the growth of wheat in terms of reducing the use of fertilizers Microbiol BMC 20(1) 38 doi: 10.1186/s12866-020-1708-z
[14] Velmumugane K, Prasan R, Chavov G, Naina L and Kumar A 2019 Trichoderma-Azotobacter biofilm inoculation improves soil nutrient availability and plant growth in wheat and cotton
Journal of Basic Microbiology 59(6) 632-44 doi:10.1002/jobm.201900009

[15] Van Oosten J, Di Stasio E, Cirillo V, Siletti S, Vento rino V, Pepe O, Raimonde G and Maggio A 2018 Root grafting with Chromococcus Azotobacter 76A enhances the adaptation of tomato plants to salt load at low N conditions BMC Plant Biol. 18 205 doi:10.1186/s12870-018-1411-5