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COBALT FERTILIZATION IN ORDER TO PROMOTE NITROGEN FIXATION IN ANNUAL FORAGE LEGUMES

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Abstract: The work aimed was to analyze the importance and possibilities of using cobalt for fertilizing annual forage legumes. Cobalt in small concentrations in the soil has a positive effect on the processes of nodulation and symbiotic nitrogen fixation. Therefore, its addition as fertilizer, especially on acidic soils, has a positive effect on the growth, yield, and quality of leguminous plants. Optimum concentrations of cobalt in the soil solution vary depending on the plant species and range from 8 to 50 ppm. Higher concentrations of cobalt in most annual forage legumes result in a toxic effect.

Keywords: cobalt, legumes, nitrogen fixation, nodulation

Introduction

Cobalt is an element necessary for normal leaf development, inhibition of ethylene biosynthesis, stimulation of alkaloid biosynthesis, processes of photosynthesis, plant respiration, etc. (Farooq et al., 2012). Cobalt fertilization is of particular importance for legumes due to its important role in the processes of nodulation and symbiotic nitrogen fixation (Tomić, 2017). Nodulation and nitrogen fixation in leguminous plants, especially on acid soils, depend to a large extent on the availability of cobalt in plants because it is an essential component of several important enzymes (Palit et al., 1994). Various studies indicate that seed vigor, nodule development, and nitrogen content in legumes depend on cobalt content in the soil and rhizosphere (Akbar et al., 2013). Optimum cobalt supply to plants contributes to enhanced nitrogen fixation in all *Rhizobium* species (Collins and Kinsela, 2011). Therefore, with a lack of cobalt in the plant, the organic production of legumes decreases (Vukadinović and

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Lončarić 1997). The aim of the work was to analyze the importance of cobalt fertilization for the process of nitrogen fixation and the productivity of one-year forage legumes, as well as the possibilities of cobalt application.

Effect of cobalt on nodulation and nitrogen fixation

Cobalt is the central atom in the porphyrin structure of cobalamin coenzyme, it is essential for nodulation and development of bacteroids (Akbar et al., 2013). It is a component of vitamin B12, which is included in the composition of enzymes and coenzymes important in the process of nitrogen fixation in leguminous nodules (Palit et al., 1994). Cobalt affects nitrogen fixation through different enzyme systems such as ribonucleotide reductase and methylalonyl coenzyme A mutase (Das, 2000). According to Farooq et al. (2012) several enzymes are activated by cobalt ions: E. coli acetylornithinase, Methanobacterium thermoautotrophicum cyclic 2,3-diphosphoglycerate hydrolase, α-d-mannosidase. However, a high concentration of cobalt in plants is also undesirable because it adversely affects the physiological and biochemical functions of the plant (El-Sheejh et al., 2003). The toxic effect of cobalt on plant morphology is manifested in the form of chlorosis, leaf drop, pale leaf nervure and reduced plant growth (Palit et al., 1994). Cobalt absorption on acidic soils is difficult because it is blocked by numerous other elements (Tomić, 2017).

Application of cobalt in annual forage legumes

The addition of cobalt to the soil during the production of forage pea (*Pisum sativum* L.) in the amount of 10-20 g ha^{-1} in the form of cobalt chloride ($CoCl_2 \cdot 2H_2O$), especially in combination with nitrogen fertilization, effected increasing the number of nodules per plant, the content of cobalt in nodules and shoots, nitrogen content in shoots and stem, flowers number per plant, pods number per plant, thousand grains mass, shoot length, root length, dry matter content in shoots and roots, yield and seed germination (Akbar et al., 2013). Bakken et al. (2004) found that there was no significant positive correlation between cobalt content and nitrogen content in the plant when cobalt was applied at a concentration of less than 0.02%. According to Nadia (2006), the application of cobalt in the amount of 8 ppm affected the increase of nodulation and the number of effective nodules in peas. The authors state that cobalt in the amount of 16 ppm influenced a significant increase in the number and mass of nodules, nitrogen concentration in nodules, leghemoglobin content, total biomass production, and seed yield compared to untreated varieties.

Pattanayak et al. (2000) stated that seed treatment with cobalt in a concentration of 0.008 mg g^{-1} of seed together with *Rhizobium* inoculation in cowpea (*Vigna sinensis* L.) influenced a significant increase in the total and number of effective nodules per plant, the mass of effective nodules per plant, the accumulation of dry matter in plants, number of pods per plant and seed yield per unit area. The authors state that cobalt is an essential element for nodulation and fixation of atmospheric nitrogen in cowpea. Similar results are indicated by Mathur et al. (2006), according to which the treatment of cowpea seeds with cobalt-nitrate in concentrations of 250 and 500 mg kg^{-1}, influenced a significant increase in the number and mass of effective nodules per plant, which was also reflected in an increase in the dry yield matter, of pod number per plant, thousand grains weight and total seed yield. The use of cobalt in cowpea significantly increased the number of nodules, their mass and nitrogenase activity, reducing ethylene production (Jain and Nainawatee, 2000). The application of cobalt in a concentration of 8 ppm over the soil in the form of irrigation influenced a significant increase in the number of nodules per plant, mass of nodules per plant, mass of dry matter of nodules, nitrogenase activity, plant height, number of branches and leaves per plant, leaf area, root length, dry matter of shoots and roots (Nadia, 2012).

Applied in the amount of 0.21 kg ha^{-1} cobalt in the form $CoSO_4 \cdot 7H_2O$ on the soil with 0,03 ppm of cobalt influenced the increase of plant height, number of branches and leaves, leaf area index, dry matter of shoots as well as the yield of peanut pods (*Arachis hypogaea* L.) (Basu, 2006). Banerjee et al. (2005) indicate that cobalt applied in the amount of 0.2 kg ha^{-1} in peanuts influenced the increase of plant height, branches and leaves number, leaf surface index, shoot dry matter, leghemoglobin content, and pod yield. According to Nadia et al. (2012) the application of cobalt in the amount of 8 ppm together with Mo in the form of an irrigation solution affected a significant increase in the dry matter yield of peanuts, especially when it was applied together with nitrogen in the form of urea, ammonium nitrate or ammonium sulfate. Cobalt fertilization also increased the number of nodules per plant, the mass of nodules per plant, the dry matter of nodules, their nitrogenase activity, the number of pods per plant, the mass of pods per plant, the content of oil in seeds and the yield of seeds per plant.

Threshold values for soil cobalt toxicity in beans range from 26 to 72 ppm (Chatterjee et al., 2006). Balachander et al. (2003) indicated that cobalt and molybdenum at a concentration of 50 ppm influenced a significant increase in plant height and biomass production of beans. Fertilization and seed treatment with cobalt increased nodulation, nitrogen fixation, uptake of nutrients, growth and yield of mango bean (*Phaseolus mungo* L.) (Pattanayak et al., 2000).

Cobalt in concentrations higher than 5 to 20 ppm influenced a significant increase in the number of nodules, plant height, number of stems, pod number per plant, dry matter of plants, number of seeds per plant, and seed yield of broad bean (*Vicia faba* L.) (Hala, 2007). An increase in the content of nitrogen, phosphorus and potassium, as well as the content of cobalt, iron, manganese, zinc and copper in the roots, shoots and seeds was also recorded during treatments with cobalt. According to Abdel-Moez and Nadia (2002), a good supply of bean plants with cobalt influenced the better formation of nodules on the roots and the fixation of atmospheric nitrogen by microorganisms that influenced the increase of nitrogen content in the plants.

The application of cobalt in the amount of 0.9 mg $CoSO_4 \cdot 7H_2O$ per pot with 5-8 kg of soil affected the increase in the mass of nodules on the main root of blue lupine (*Lupinus angustifolius* L.) (Dilworth et al., 1979). The number of bacteroids and the content of leghemoglobin in the nodules were directly dependent on the content of cobalamin. Nitrogen fixation activity depended on both cobalt status and cobalamin content in plants. The authors also conclude that lupine crown nodulation is significantly reduced under cobalt deficiency, most likely as a consequence of reduced nodule initiation and that normal nitrogenase activity cannot occur below the critical concentration of cobalt in nodules.

Cobalt in the form of $CoCl_2$ up to a concentration of 50 mg kg^{-1} of soil had a positive effect on the growth of plant organs of soybean (*Glycine hispida* Max.) and the adsorption of nutrients from the soil. Higher concentrations of cobalt than this had a toxic effect on plants and led to less intensive growth (Jayakumar and Jallel, 2009).

Conclusion

Cobalt is a necessary element that, applied in small amounts, especially on acidic soils, can greatly influence the increase in the number of nodules per plant, mass of nodules per plant, dry matter of nodules and their nitrogenase activity in annual forage legumes. Thanks to this, fertilization with cobalt in the form of $CoCl_2 \cdot 7H_2O$ or $CoSO_4 \cdot 7H_2O$ had an indirect effect on many other processes in plants, affecting the increase in root length, plant height, nitrogen content in shoots and stem, number of branches and leaves, shoot dry matter, leaf surface index, leghemoglobin content, number of flowers per plant, number and yield of pods per plant, thousand grains weight, seed yield, seed quality and seed germination. The optimal concentrations of cobalt in the soil solution differed depending on the plant species and ranged from 8-50 ppm. Concentrations higher than those indicated in most annual forage legumes had a toxic effect.

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