

The influence of the microbiological fertilizer – Slavol on cauliflower growth

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Abstract

The microbiological fertilizers play an important role in the yield and quality of crops, having in the same time a reduced impact on environment. The aim of the present study was to evaluate the influence of two methods of application of microbiological fertilizer Slavol on leaf length, leaf width and stomatal density in cauliflower plants. Two different methods of fertilizer application were employed: application through irrigation water (drip irrigation system) and foliar application. The effects of the application methods were evaluated using the following parameters: leaf length and width and stomatal density. A statistical tool (ANOVA test) was employed for data analysis. Foliar and drip irrigation system application of the microbiological fertilizer Slavol determinates an increase in stomatal density and leaf length of cauliflower plants. Also, drip irrigation system application Slavol produced a positive effect on cauliflower leaf length and width. No significant differences were observed between the stomatal density in plants treated by foliar application and plants treated with Slavol through a drip irrigation system.

Keywords: cauliflower, leaf, microbiological fertilizer, stomatal density

1. Introduction

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is a vegetable in the *Brassicaceae* family. For many years scientists have investigated the beneficial effects of microorganisms' activities such as biological N fixation, organic matter decomposition, mineralization, nitrification and fermentation. Application of microbiological fertilizers in plant production can contribute to reduction of mineral fertilizers applications, which has not only economic benefit, but also a very important safety production. Slavol is a natural bio-organic fertilizer composed of nitrogen fixing and phosphorus mineralizing bacteria (*Azotobacter chroococcum*, *A. vinelandi*, *Derxia* sp., *Bacillus megaterium*, *B. lichenformis* and *B. subtilis*). Application of the microbiological fertilizer Slavol showed positive effect on plant production [1-7]. Although the microbiological fertilizers are widely used, their implementation still requires research for understanding their impact on the soil, flora and fauna as well as on human health [8].

The final size of plant organs, such as leaves, is tightly controlled by environmental and genetic factors that must spatially and temporally coordinate cell expansion and cell cycle activity [9]. Namely, the two effector systems, cell division and cell expansion contribute to the final organ size. Also, it is important to note that cell division normally cooccurs with cell expansion to maintain cell size homeostasis [10]. Smaller leaves are produced when the number and / or size of cells are decreased [11]. Velmurugan et al. conducted an experiment on the effect of different organic manures and bio-fertilizers on the cauliflower recorded length of

leaves (30.55 cm), width of leaves (15.46 cm), leaf area (472.303 cm²) and leaf area index (0.175) [12]. Verma and Yadav [13] conducted an experiment on cauliflower cv. Pusa Snowball K-1 and reported significant improvement in plant height, leaf length, leaf width, leaf weight per plant and curd diameter with combination of PSB + 75% P + recommended dose of NK (120 : 60 kg/ha) through chemical fertilizers.

Leaf stomata are considered to play an important role in plant adaptation to different environmental conditions. [14]. Also, the number of stomata varies according to the plant species. According to Ryugo, stomatal densities can range from 125 to 1000 stomata per square millimeter [15]. The stomatal density is influenced by different factors such as drought, photosynthesis, precipitation, vegetative developmental phases, grafts on different rootstocks, altitude, fertilizers [16, 17, 18, 19, 20, 4].

There is no information regarding the effect of the different methods of application of microbiological fertilizer Slavol on the cauliflower growth. Therefore, the focus of this study was to evaluate the effect of two different methods of fertilizer application, foliar application and drip irrigation system microbial fertilizer Slavol on the cauliflower leaf length, leaf width and stomatal number.

2. Materials and Methods

The research was conducted on alluvial soil at a private farm in Jurumleri near to Skopje, Macedonia. Cauliflower “Barcelona F₁” (*Brassica oleraceae* var. *botrytis*) plants were employed to be analyzed the influence of different methods of application of microbiological fertilizer Slavol on leaf length, leaf width and stomatal number.

After a period of 45 days the seedlings produced in a greenhouse, were treated with Slavol (10 mL/L) for a few minutes, afterwards they were planted in rows. During the vegetation period (from 21th of August until 1th of October 2012) the cauliflower plants were treated with Slavol by employing two different application methods: foliar application (10 mL/L, applied at 7 day intervals) and drip irrigation system (1mL/L applied at 2 day intervals, flow: 2L /min/m²) Unfertilized plants were used as a control. The leaf length and leaf width were determined by using 30 randomly selected leaf samples from cauliflower plants one month after the last application of Slavol. Also, leaf samples from control plants were used for comparison. The leaf length (cm) was measured from lamina tip to the point of the intersection of the lamina and the petiole, along the midrib of the lamina. The leaf width was measured from end to end between the widest lobes of the lamina perpendicular to the lamina mid-rib. The density of stomata (number per 1 mm² of leaf area) on lower and upper leaf surface, on the middle portion of the leaf, was assessed by preparation and microscope examination of nail polish replicas [21]. Average stomatal number was determined, based on 10 leaves per treatment using counts from three areas per leaf. The obtained data were subject to analyses of variance (ANOVA). The differences among treatments were analyzed by least significant differences (LSD) at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results and discussion

In the present study, Slavol a microbiological liquid fertilizer was applied to cauliflower plants using two different methods: foliar application and drip irrigation system in order to evaluate the effect of two different methods on leaf length and width and stomatal number.

Previous study reports indicate positive effect of microbiological fertilizer Slavol on plant growth of wheat, rosemary, apple, strawberry, lettuce, Swiss chard and broccoli [1-7]. According to Molnar [22], the application of fertilizers in vegetable crops is important for obtaining higher yields. Foliar fertilizers are dilute fertilizer solutions applied directly to plant leaves.

One of the benefits of foliar fertilization is the increased uptake of nutrients from the soil [23]. Kolota and Osinska recognized that supplementary foliar fertilization during crop growth can improve the mineral status of plants and increase the crop yield [24]. On the other hand, application through drip irrigation system refers to the application of water soluble fertilizers through irrigation water. This application method of fertilizers is expensive, but it saves water and fertilizer by allowing water to drip slowly to the roots of plants. Also, the advantages of drip fertigation are: the supply of nutrients can be more carefully regulated and monitored, minimal losses of water and plant nutrients, decrease leaching and volatilization losses and minimize the chances for ground water pollution, improved fertilizer use efficiency- FUE, improved yield and water use efficiency, improved yield quality parameters [25].

Table 1. Average cauliflower leaf length (cm) in plants treated with Slavol employing foliar application and through drip irrigation system versus control plants

	Plants by foliar application of Slavol	Plants treated with Slavol through drip irrigation system	Control plants
Average leaf length (cm)	52	56.26	45.8
Minimum	67	67	62
Maximum	43	44	34

Our study showed that both application methods tested had positive effects on leaf length and width of cauliflower plants treated with Slavol (Table 1 and Table 2). According to the obtained data, it could be concluded that an increased average leaf length and width in plants treated with Slavol was obtained compared to the control plants.

Previous reports indicate that repeated foliar application of small units of fertilizers stimulates plant metabolism and increase nutrient uptake via the roots [23].

The maximum average leaf length (56.26 cm) and maximum average leaf width (31.53 cm) were observed in plants treated with Slavol by drip irrigation system (Table 1 and Table 2).

The difference between average leaf lengths in plants by foliar application of Slavol and control plants was statistically significant ($p < 0.001$). Also the difference between the average leaf length in plants treated through drip irrigation system and control plants was statistically significant ($p < 0.001$). The difference between the average leaf lengths of plants treated by foliar application of Slavol and plants treated through drip irrigation system was statistically significant ($p < 0.05$).

The difference between average leaf widths in plants by foliar application of Slavol and control plants was not statistically significant. On the other hand, the difference between the average leaf widths of plants treated with Slavol through drip irrigation system and control plants was statistically significant ($p < 0.01$). The difference between average leaf widths of plants treated by foliar application of Slavol and plants treated through drip irrigation system was statistically significant ($p < 0.05$).

Table 2. Average cauliflower leaf width (cm) in plants treated with Slavol employing foliar application and through drip irrigation system versus control plants

	Plants by foliar application of Slavol	Plants treated with Slavol through drip irrigation system	Control plants
Average leaf width (cm)	29.26	31.53	28.06
Minimum	47	41	35
Maximum	20	23	22

The size of plant organs, such as leaves, is tightly controlled by environmental and genetic factors [9]. The increased leaf width and length resulted in the present study could be the result of nitrogen fixing bacteria and phosphorus mineralizing bacteria present contained in the microbiological fertilizer. These beneficial organisms probably increase the supply of plant nutrients that improve the nutritional status of plants and increase leaf length and leaf width in treated plants. Also, plant hormones play key role in growth regulation. Namely, according to Najdenovska et al. [26] nitrogen fixing bacteria (*Azotobacter chroococcum*) stimulate the synthesis of phytohormones so that they improve the growing and development of potato tubers (*Solanum tuberosum*). Also, Govedarica et al. confirmed the positive effect of *Azotobacter chroococcum* in increasing yield of corn [27]. Govedarica et al. found that *Azotobacter chroococcum* increased the yields of roots and crystal sugar in sugar beet varieties (“Dana” and “Alina”) [28]. From our data it can be concluded that highest average values of leaf length and leaf width were obtained in plants treated with Slavol through drip irrigation system.

Table 3. Average stomatal density (stomata/mm²) of cauliflower leaves in plants treated with Slavol employing foliar application and through drip irrigation system versus control plants

	Control plants	Plants with foliar application of Slavol	Plants treated with Slavol through drip irrigation system
Average stomatal density on upper epidermis (stomata/mm ²)	123	150	166
Average stomatal density on lower epidermis (stomata/mm ²)	191	297	293
total stomatal density (stomata/mm ²)	314	447	459

Also, Verma and Yadav [13] conducted an experiment on cauliflower cv. Pusa Snowball K-1 and reported significant improvement in plant height, leaf length, leaf width, leaf weight per plant and curd diameter with combination of PSB + 75% P + recommended dose of NK (120 : 60 kg/ha) through chemical fertilizers.

Najdenovska et al. concluded that foliar application of Slavol in broccoli plants had a positive effect on plant growth [7]. Slavol application through drip irrigation system increased the average of leaf area. Similar results were obtained in strawberry [4].

The stomatal density on upper and lower epidermis in cauliflower plants treated with Slavol using both application methods versus control plants are presented in Table 3.

Statistical analysis data are indicating significant differences between control plants and treated plans regarding stomatal density ($p < 0.001$). No significant difference was detected between total stomatal density in plants treated with Slavol by foliar application and plants treated through a drip irrigation system. Increased stomatal density enhances leaf photosynthetic capacity by modulating gas diffusion. The genetic basis for stomatal frequency is still poorly understood [29]. Stomatal density is influenced by radiation, water status or developmental stage [30].

Cauliflower has amphistomatous leaves i.e. stomata are on the upper and lower epidermis. The stomata on upper epidermis are more sensitive to environment factors [31]. As it was expected, the stomatal density on lower epidermis was higher than on upper epidermis. The average stomatal density on upper and lower epidermis of control plants (123 / 191 stomata/mm²) was lower than in plants treated with Slavol through drip irrigation system (166 / 293 stomata/mm²) and than in plants treated with Slavol by foliar application

respectively (150 / 297 stomata/mm²) (Table 3). There are studies reporting that microbiological fertilizer Slavol increases the stomatal density in strawberry [4] as well as in broccoli [7]. Najdenovska et al. found the highest stomatal density in broccoli plants treated with Slavol through a drip irrigation system [7]. The study also indicated significant differences between the stomatal density in broccoli plants treated with Slavol through a drip irrigation system and broccoli plants treated by foliar application of Slavol ($p < 0.05$).

4. Conclusion

Foliar and drip irrigation system application of the microbiological fertilizer Slavol determinates an increase in stomatal density and leaf length of cauliflower plants. Also, drip irrigation system application of Slavol produced a positive effect on cauliflower leaf length and width. No significant differences were observed between the stomatal density in plants treated by foliar application and plants treated with Slavol through a drip irrigation system.

The research results could contribute to a better understanding of the effects of the microbiological fertilizer Slavol on plant growth. Our results suggest that the microbiological fertilizer Slavol improved plant growth but more studies are needed to better understand the influence of microbiological fertilizers on living organisms and soil.

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