

# Testing the Concentration of the *Bacillus subtilis* BNt8 Formulation on the Growth of Corn Seeds (*Zea mays* L.) in Vitro

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## ABSTRACT

Corn (*Zea mays* L.) is a food plant that is rich in carbohydrates. *Bacillus subtilis* bacteria are rod-shaped bacteria packaged as formulations. The *B. subtilis* BNt8 formulation is a bacterial culture obtained from Bone Regency, South Sulawesi, which can stimulate the growth of corn plants. The advantage of the *B. subtilis* BNt8 formulation is that the nutrients it contains are more quickly available and easily absorbed by plants. This research aims to determine the effect of various concentrations of *B. subtilis* BNt8 formulations on the growth of corn seeds. This type of research is quantitative research. The research approach used was a laboratory experiment using a one-factor Completely Randomized Design (CRD) with 8 treatments and 3 replications so that there were 24 experimental units, namely 0.5 gr/ml water (P1), 0.75 gr/ml water (P2), 1 gr/ml water (P3), 1.25 gr/ml water (P4), 1.5 gr/ml water (P5), 1.75 gr/ml water (P6), 2 gr/ml water (P7) and control without formulation. The results obtained from this research were that *B. subtilis* BNt8 formulations with various treatment concentrations had no significant effect on root length, sprout length and plant fresh weight.

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## 1. INTRODUCTION

Corn is an important crop for food and feed. More than 120 million hectares of dry land in various areas of the world are the main media for its exploitation. According to Mulyani et al., 2017, in Indonesia, apart from dry land, corn is cultivated in rice fields after rice harvest with productivity reaching around 7.0 t/ha. In Indonesia, corn is the second food crop after rice. In fact, in some places, corn is the main staple food substitute for rice or as a mixture of rice. According to Aprilia, 2016, from a market perspective, the marketing potential for corn continues to increase. This can be seen from the growing development of the livestock industry which will ultimately increase demand for corn as a mixture of animal feed (Septiani & Alexandi, 2014).

Corn is a monoecious plant where the male flowers are separated from the female flowers on one plant, low and efficient in water use (Subekti et al., 2007). On every corn plant there are male flowers and female flowers. The male flowers are found on the flower panicle at the tip of the plant, while the female flowers are found on the corn cob (Rochani, 2007; Subekti et al., 2007; Wandira, 2021). The male flowers at the tip of the plant mature before the female flowers. Corn plants have one or two cobs, depending on the variety. Corn cobs located on each cob consist of 10-16 rows of

seeds, the number of which is always corn kernels is called a caryopsis, the wall of the ovary (Syukur & Azis Rifianto, 2013).

According to (Anwar, 2019) The benefits of planting corn are very large. Apart from seeds as the main product, corn stalks are a very potential animal feed ingredient. Thus, in cultivating corn, apart from getting corn kernels or cobs, you also add the stover which also has high economic value. From a management perspective, the advantage of planting corn is the ease of cultivation. Corn is a plant that is not only a food ingredient, corn is also known as an ingredient in animal and industrial feed.

In relation to decreasing corn production, plant pest organisms (OPT) are an important cause if they infect plants in the vegetative phase. The younger the plant is infected, the greater the chance of yield loss. Next, in the post-harvest phase, the pests that need attention are fungi or fungi that cause disease in corn. Both as feed and as food, it is easily damaged due to external and internal factors, so it is less useful, and can even endanger the health of humans and livestock that consume it (Edy & MP, 2022; Panungul et al., 2023). To overcome the decline in the quality of corn agricultural products, the problem of mycotoxins in food and feed raw materials needs attention. It is hoped that earlier vigilance can be one way to improve the quality of corn products (Pakki & Talanca, 2007).

This can be achieved by combining an understanding of biology, host, distribution, toxicity and other control components (Papavizas, 1985). Plant growth promoting rhizobacteria, which are more popularly called plant growth promoting rhizobacteria (PGPR), are a group of beneficial bacteria that actively colonize the rhizosphere. PGPR plays an important role in increasing plant growth, crop yields and land fertility. Directly, PGPR stimulates plant growth by producing growth hormones, vitamins and various organic acids and increasing nutritional intake for plants (Kenneth et al., 2019). The advantages of *B. subtilis* compared to other types of bacteria are that it is able to suppress various types of plant pathogens, is plant growth promoting rhizobacter (PGPR), and is able to survive extreme environmental conditions. *B. subtilis* is a bacterial isolate originating from the rhizosphere of corn plants. This bacteria has been tested in vitro to be able to suppress the development of pathogens (Zohara et al., 2016).

PGPR is a bacteria that actively colonizes plant roots and has three main roles for plants, namely as a biofertilizer, biostimulant and bioprotectant. PGPR can affect plants directly and indirectly. Directly, namely by fixing nitrogen, dissolving phosphate, producing siderophores and growth hormones, indirectly, namely by improving plant growth conditions (Etesami & Adl, 2020).

The *B. subtilis* BNT8 formulation is a cultured bacterial isolate of *B. subtilis* obtained from Bone Regency, South Sulawesi which is produced using modern equipment which produces ready-to-use bacteria in powder form and makes it easier for us to use. The Bone district area is a temperate climate area where air humidity ranges from 95% - 99% with temperatures ranging from 26oC - 43oC. In the period April to September, easterly winds blow, bringing rain. On the other hand, from October to March the west wind blows during the dry season in Bone district. One of the impacts of chemical pesticides is the presence of pesticide residues in the soil which can affect the balance of the microbial ecosystem in the soil. The presence of the *B. subtilis* BNT8 formulation can help balance the soil ecosystem because it is made from natural ingredients that also come from the soil. Therefore, research was carried out to determine the concentration that effectively stimulates the growth of corn plants.

## 2. METHOD

This research is quantitative research. This research was carried out from March to April 2016 at the Pest and Disease Laboratory of the Cereal Plant Research Institute, Maros. The research approach taken is an experimental approach by applying the principles of laboratory research, especially in controlling things that influence the course of the experiment. This research is a pure experiment (true experimental). This research uses two variables, namely the dependent variable is the growth of corn seeds (*Zea mays* L.) and the independent variable is the formulation of *B. subtilis*.

The data collection methods used in this research were observation, direct measurement and recording of results using electronic devices. The tools used in this research were laminar air flow (LAF), test tube, mortar, spatula, pentri dish, inoculum needle, beaker, Erlenmeyer flask, vortex, glass hockey rod, analytical balance, micro pipette, ruler, isolation tweezers and Bunsen burner.

Testing the concentration of several concentrations of *B. subtilis* is carried out by seed treatment on each corn seed using sterile paper by shaking the seeds evenly. Then the seeds that have been treated are transferred one by one using tweezers to opaque paper that has been marked and coated with heat-resistant plastic, 3 repetitions for each experiment and also 3 opaque paper and inside there are 10 seeds/1 repetition then rolled one by one and no direct contact is given between one seed and another. The treatments include: Treatment 1 (P1.1, P1.2, P1.3), Treatment 2 (P2.1, P2.2, P2.3), Treatment 3 (P3.1, P3.2, P3.3), Treatment 4 (P4.1, P4.2, P4.3), Treatment 5 (P5.1, P5.2, P5.3), Treatment 6 (P6.1, P6.2, P6.3), Treatment 7 (P7.1, P7.2, P7.3) and Control (K.1, K.2, K.3). After everything is wrapped, the bottom of the paper is insulated to prevent direct contamination. After that, stored and grown in paper for 7 days.

### 3. RESULTS AND DISCUSSION

#### 3.1 Research results

Based on the results of research that has been carried out regarding corn plants, the results can be seen in:

a) Leaf length.

Traces of variations in root length of corn plants (*Zea mays* L.) aged 7 days are presented in Appendix Table 1b. The variance test showed that the *Bacillus subtilis* formulation had no significant effect on the root length of corn plants (*Zea mays* L.).

b) Coleoptile Length

The variation in coleoptile length of corn plants (*Zea mays* L.) aged 7 days is presented in Appendix Table 2b. Variant fingerprinting showed that the *B. subtilis* formulation had no significant effect on the coleoptile length of corn plants (*Zea mays* L.).

c) Fresh Weight

The variation of fresh weight of corn plants (*Zea mays* L.) aged 7 days is presented in Appendix Table 3b. The variance test showed that the *B. subtilis* formulation had no significant effect on the root length of corn plants. Average fresh weight of corn plants (*Zea mays* L.)

The growth of corn plants (*Zea mays* L.) is an increase in the size of corn plants, characterized by the formation of an organ in the plant which undergoes changes, including the formation of leaves and stems as measured by root length, coleoptile length and fresh weight. Each of these parameters was measured after planting for 7 days (one week). In this study, corn plants experienced growth because the corn plants in the planting medium experienced changes in the form of increasing root length, coleoptile length and fresh weight which were different for each treatment. According to Agustina (2004), growth occurs due to an increase in the number of cells and cell enlargement. This process occurs due to mitotic division in meristematic tissue.

For example, increasing stem height and number of leaves. Development is the process of specialized cells leading to certain forms and functions that lead to a level of maturity that is qualitative (unquantifiable) and irreversible. For example, the emergence of flowers as a means of reproduction (Purwono, 2005). Growth is the regular increase of all components of an organism. If a liquid seed is planted with microorganism cells, living cells will grow which can be seen in 6 growth phases, namely: adjustment phase, acceleration phase, exponential phase, deceleration phase, zero phase and decline phase. Growth will be greatly influenced by various existing conditions, including the influence of the external environment (Pusdiknakes, 1989). *Bacillus subtilis* is a rod-shaped, gram-positive bacterium that has spores, a facultative anaerobe that can move with peritrichous flagella.

This bacteria is able to live on certain media such as PDPA (Potato Destros Peptone Agar) media which has sufficient nutritional content for the growth of *Bacillus* sp. Bacterial growth was measured after 24 hours using a colony counter by measuring the number of colonies in the media using the CFU count formula. The *B. subtilis* BNt8 formulation is a culture of *B. subtilis* bacteria obtained from Bone Regency, South Sulawesi, which is then processed and produced using modern equipment which produces bacteria that are ready to use in powder form, making it easier for researchers to develop knowledge about the benefits of bacteria in formulation form. This formulation is known to stimulate plant cell growth when given to corn plants at various concentrations.

### 3.1.1 Root Length

The results of observing the root length of corn plants are an indicator that the average root length of corn plants shows that the concentration of B. has no significant effect on the growth of corn seeds, it is possible that the concentration given to each formulation is not much different. It can be seen that the concentration of B. formulation given is 0.5 gr/ml water (P1) then the concentration has the highest root length, namely around 20.83 cm, while the formulation with a concentration of 0.75 roots shows that the administration of formulation B has a significant effect on the growth of root length of corn plants. influenced by environmental factors and growth media factors. This is in accordance with the opinion of Hadi (2000), that the volume factor of the planting media allows it to influence the limited nutrients and space for plant roots to move. has an effect on growth because it directly deals with competition between plants in one clump. A smaller number of seeds per planting point will give the plants room to spread and deepen

This can be seen when measuring the length of plant roots, which indicates competition in corn plants. In control plants, the root length reached 19.33 cm. Compared to plants given the formulation, control plants had lower root length compared to plants with the concentration. This shows that the plants given the formulation had sufficient nutrients for growth. In accordance with the opinion of Soewito (1991) that plants that contain sufficient N, P and K elements, plant nutrient requirements are one of the important factors needed by plants for this. This is in accordance with the ability of *B. subtilis* which can bind the nutrients N, P, K properly so that it can stimulate plant growth and stimulate plant vegetative growth. shows stunted growth. The N nutrient is also useful for the formation of chlorophyll and chloroplasts in leaves which will later be useful for processing. Apart from that, the increase and increase in root length in plants given the formulation is caused by good absorption of nitrogen elements by the roots of corn plants. Plant growth is caused by higher absorption of nitrogen elements

### 3.1.2 Coleoptile Length

The results of the analysis of variations in coleoptile length of corn plants with the *B. subtilis* formulation showed that there was no significant effect on the increase in coleoptile length growth. From table 4.2. It can be seen that the highest increase in coleoptile length was in plants with a concentration of 2 gr/ml water (P7), which was 10 cm and the lowest was 0.75 gr/ml water (P2), which was 6 cm. This shows that the more formulations given, the better it will be for the growth of corn plant coleoptiles. Control plants have a coleoptile length of 9.21 cm (can be seen in Figure 4.2). According to Hadi (2000), normal sprouts are sprouts that show the potential to develop further into normal plants under optimum conditions, while abnormal sprouts are sprouts that do not show the potential to develop into normal plants, if grown in optimum media.

### 3.1.3 Fresh weight

The results of the analysis of variation in fresh weight of corn plants showed that the concentration of *B. subtilis* had no significant effect on the growth of corn seeds. The average results of observations of the fresh weight of corn plants can be seen in Figure 4.3. Based on Figure 4.3, it can be seen that administering the *Bacillus* sp. with concentrations of 0.5 gr/ml water (P1), 1 gr/ml water (P3), 1.25 gr/ml water (P4), 1.75 gr/ml water (P6), 2 gr/ml water (P7) per plant has a higher root length, namely from an average of 15 gr – 17 gr compared to plants given a formulation with a concentration of 0.75 gr/ml water (P2) and 1.5 gr/ml water (P5), which is higher. low, namely an average of 13 gr -14 gr. Meanwhile, the control plants showed the lowest fresh weight, namely 11 gr. This shows that giving the *B. subtilis* formulation has an effect on the growth of corn plants even though it shows no significant changes. This shows that the nutrients in the corn plants given the formulation are sufficient. According to Soewito (1991), nitrogen is the most important vital nutrient for plants. Nitrogen compounds are used by plants to form amino acids which are converted into protein.

## 4. CONCLUSION

The conclusions of this research are as follows: 1. The formulation of *Bacillus subtilis* BNt8 with various treatment concentrations had no significant effect on root length, coleoptile length and fresh weight of corn plants. 2. At a concentration of 0.5 gr/ml water (P1) it has the best effect on the growth of corn plant root length. At a concentration of 1.25 gr/ml (P4) it had the best effect on the growth of corn plant coleoptiles. And at a concentration of 2 gr/ml water (P7) it has the best effect on the fresh weight of corn plants.

## THANK-YOU NOTE

The suggestions I would like to convey are as follows:

1. The *B. subtilis* BNt8 formulation can be used as an alternative fertilizer selection for plants.
2. It is necessary to carry out further research on the concentration of the *B. subtilis* BNt8 formulation so that the optimal concentration can be determined and is suitable for the growth of corn plants.
3. Further research needs to be carried out regarding the application of the *B. subtilis* BNt8 formulation on generative growth up to harvest time so that the data needed to prove the existence of the most valid effect.
4. Further research needs to be carried out on the effect of the *B. subtilis* BNt8 formulation on the growth of corn plants on new media, for example cotton and husks.
5. Further research needs to be carried out on the effect of the *B. subtilis* BNt8 formulation on the growth of corn plants with different types of paper and seed sizes.

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