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Fermentation of alfalfa brown juice and its environmental friendly reusing

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Stockholmi
Ifjúsági Víz Díj
magyar verseny

"Az ifjúság a víz jövője"

GWP Magyarország Alapítvány

SIWI STOCKHOLM JUNIOR WATER PRIZE

Kisvárda

2021

Table of contants

Abstract

The human population is constantly growing on our Earth. Protein sources are in the middle of the interest, deficiency of protein could be a significant problem in the future. Because of this the alternative protein sources will play main role in the nutrition of human population. Nearly the 18% of the alfalfa *(Medicago sativa L.)* is protein, so it is an excellent alternative protein source. The isolation of leaf protein resulted in four products, i.e., green juice, fibre, leaf protein concentrate, and deproteinized juice which is also referred to as brown juice. Fractionation of one-kilogram fresh biomass results in 450–550 mL brown juice which has a harmful effect on the environment. Deproteinized juices are handle like sewage in the whole world. It is produced in a very large amount and difficult to store at room temperature, however there are several advantages if we recycle them. The brown juice entering the sewage cause a lot of environmental problem, because of its nitrogen content. Moreover a preservation procedure was developed and the effect of the brown juice for the growth of sweet basils *(Ocimum basilicum L.)* was examind.

Introduction and Literature review

It is well known, that the human population is constantly growing on our Earth, which could reach nearly 10 billion by 2050. The increase in the world's population leads to several issues that we already have to face, and we must find sustainable solutions for them to save our life on the planet. The alternative protein sources will be a huge help in the future. One of these concerns is the sustainable supply of high-quality protein. To solve the protein issue, several approaches were proposed to identify novel protein sources or alternatives. The leaf protein research started in the Proteomill program in 2001 which was led by Tedej Ltd. We used alfalfa like alternative protein source from which we could create a leaf protein concentrate. The isolation of leaf protein resulted in brown juice which is nearly half of the biomass. The protein coagulation was successful, but beside the protein a so called brown juice by-product was produced, which has a very harmful effect on the environment. The amount of produced BJ during the isolation of leaf protein and the utilization of this product is a huge obstacle for the wider recognition of LPC production. Its colour may be brown, dark brown, yellowish brown and green.

The alfalfa phytoserum has a dry matter content of 4-8% which is influenced by the species, varieties, weather conditions, phenophase, methods of harvest and processing. Furthermore, it is rich in pigments, vitamins, enzymes, minerals and other phytochemicals, thus can be used in various ways. It is very difficult to store the brown juice at room temperature because of its sugar, free amino acid content. Preserving with freezing, drying or lyophilisation are very expensive. The brown juice can be utilized as a biofertilizer at the fields, because they contain macro- (K, N) and microelements.(Zn, Fe, Cu) However, it has some serious environmental risks as well. The excessive amount of nitrogen can drain into soil water and raise its nitrate level or enhance eutrophication. To prevent such negative impact some countries, e.g. Denmark, has limited periods of application (Thomsen et al., 2003). Holmberg (2016) and Lamont et al. (2017) stressed the importance of lactic acid bacteria in preservation of agricultural products and especially in crop cultivation for multiple purposes like enhancing soil fertility, growth regulation or plant protection. According to Santamaria (2018), fermentation using *Lactobacillus salivarius* reduced the pH of green juice making the proteins to precipitate. The results of Thomsen and Kiel (2008) showed that from the examined Lactobacillus strains the *Lactobacillus salivarius ssp. salivarius DSM20555* proved to be the best to sour and conserve phytoserum. Our goal was to fermentated the brown juice with the help of Lactobaccilus strains at room temperature. We wanted to avoid the expensive processes like freezing or lyophilisation to preserve our environment from the by-product. To solve this problem, an environmental-friendly and cheap method was made by us to recycle the harmful by-product. After the appropriate treatment we can utilize the brown juice among others like raw material of biodegradable plastics or feed supplement or as a liquid medium supplement as well.

We can produce L-lizin from the brown juice. The L-lizin is an essential amino acid which we can amend the amino acid and protein recording of poultries and offenses with. Moreover besides the effective food applying, the nitrogen load of environment are also reduced. L-lizin production is happened by *Corynebacteria and Brevibacteria ssp.* in intermittent fermentation (Kiss and Stephanopoulos, 1991).

Polylactic acid (PLA) is a plastic produced by polymerization of lactic acid which can be applied for storage of fruits, vegetables or liquid foods like fruit juices. PLA can be used alone or with other polymers. Surgical sutures, implantable medical devices, drug delivery devices may be produced from PLA (Shogren,1997).

The brown juice can be used for foraging as well. Because of its minerals and amino acids content we can utilized them like cattle feed, as the fibre (Wallace 1975).

Extraction of carbohydrates and amino acid from the brown juice is possible but expensive. The clean amino acid is very valuable. It is approaching the value of leaf protein concentrate, but depends on the amino acid (Paping és mtsai., 2014).

The brown juice can be utilized as a biofertilizer at the fields. It contains nitrogen and potassium, so this application have several disadvantages (Thomsen, 2003). The thoughtless agriculture using can cause a lot of environmental problems. For example, the nitrate surplus can appear in the ground water in a higher concentration. To prevent such negative impact some countries, e.g. Denmark, has limited periods of application (Thomsen, 2003; McDonald, 1974; Foxell, 1977; Heath és mtsai., 1981; McDonald és mtsai., 1981).

There are other ways of utilizations, as well. For example, Manur may be made which produces a well quality soil improver from the low value straw. During the process the dry vegetable substance to be processed is made wet with some brown juice to an extent that the heat arising during the rot will be able to vaporize the moisture. We are going on rewetting until the biomass reaches the state of some dung.

Objective

Our goal was to:

- determine the amount of brown juice
- fermentation the brown juice with the help of Lactobacillus strains at room temperature.
- examine the effect of the irrigation experiments for the growth of different plants

Method and Material

Methodology of brown juice production

Our research was done at Department of Agricultural Botany, Crop Physiology and Biotechnology University of Debrecen The samples originated from the research field of the University. 7 different alfalfa species were used: Tápiószelei-1, Jozsó, Hunor-40, Olympia and Express, Dimitra (Italian), Legend.

Figure 1: Alfalfa biomass

On the second illustration you can see the leaf protein production.

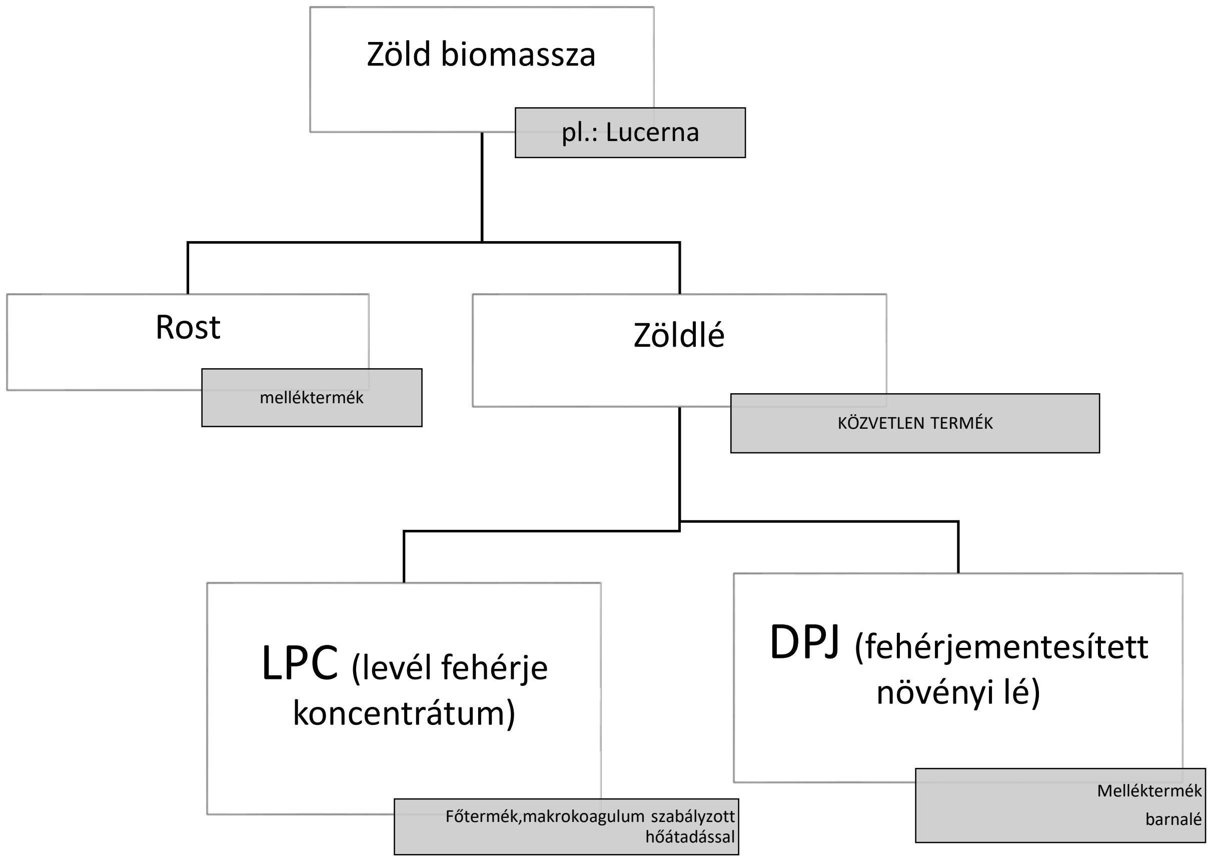

Figure 2 : The Leaf protein production

The fibre and the green juice was produced from biomass with the help of a twin gear juicer machine (Angel Juicer 5500, Eujuicers.com Ltd., Czech Republic). After the coagulation of green juice we got the brown juice, which is nearly the 50% of the biomass. The coagulation happened above 80 degrees of Celsius that is the reason samples were heated by microwave oven for nine minutes. The protein free brown juice was separated from the main product with fractionation.

Methodology of brown juice preservation

The samples had a dry matter content of sucrose expressed in Brix% (measured by manual refractometer (RBR32-ATC, Germany)) of 7.8 and the pH was 4.8 on average. The pH of by-product was measured before the inoculation. The inoculation happened with AdiSil LG-100 Perfect Prepartion at room temperature. This preparation contains 3 homofermentative lactic acid bacteria strains: Pediococcus acidilactic, Lactobacillus paracasei, Lactobacillus plantarum. After inoculation we put the samples into 20 litre cans for fermentation. Then their pH changes were measured with

Voltcraft pH-100 atc device every day (Figure 3.) After that 12g L-1 glucose was added to the samples, it was measured by an analytical balance (Figure 4.).

Figure 3: pH measurement

Figure 4: Glucose was measured by an analytical balance

Methodology of irrigation experiments

The effect of different brown juices were examined for the growth of plants, but the alfalfa brown juice has not been examined yet. The exact elemental composition had to be determined, so we examined the effect of BJ in greenhouse experiment. Every crop were planted into a tile with full of peat. The control crops were indicated with water, there were plants treated with BJ and we also used combined treatment in concentration of 0,5%, 1%, 2,5%, 5%, 10%. Every layout was examined on 15 plants. We indicated them with 2dl BJ and sprayed with 0.25dl solution twice a week. The crops were processed before the flowering. We measured the root and shoot length, the weight of root, leaf and stem with the help of analytical balance. Moreover, we counted the number of leaves. The data were averaged with the help of SigmaPlot program. The averages were compared by Tukey Test.

Results and Conclusions

Quantification of brown juice

This diagram shows the fraction ratio of different species after fractionation and heat coagulation. The results based on the average of three harvest time.

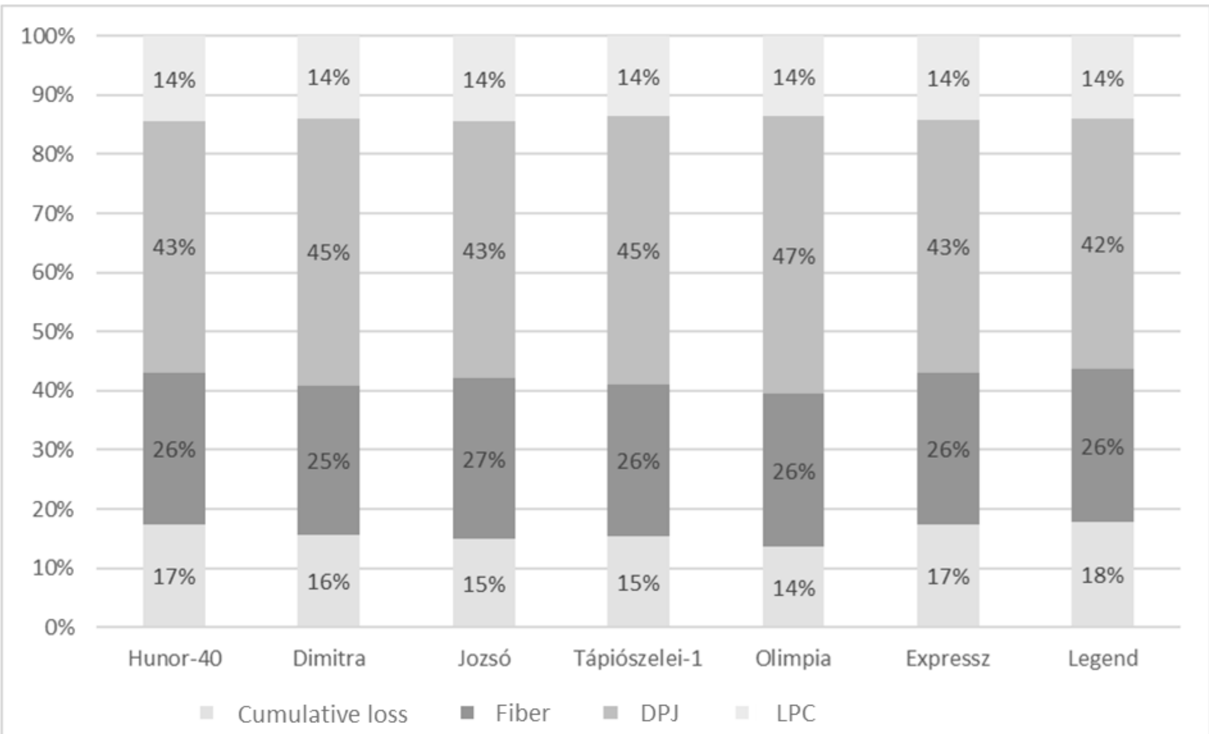

Figure 5: the fraction ratio of different species after fractionation and heat coagulation. n=9

It seems well that the protein concentrate (LPC) is 14%, the fibre is 25-27% and the by-product (DPJ) is 42-47%. So actually you can see that the brown juice is nearly half of the biomass. Our results are very similar to Kohler-Bickoff's and Pirie's (1942) results which confirmed our statement that the brown juice is created in a very large amount. It is proved by this result, that the environmental-friendly recycling of this by-product is very important. Unfortunately, they were handle like effluent in the past, but there are several advantages if we recycle them, because they contain several valuable compounds and nutrients, like enzymes minerals, Phytochemicals, vitamins.

Results of brown juice preservation

On the sixth figure we can observe the pH changes of fermented, inoculated brown juice at room temperature.

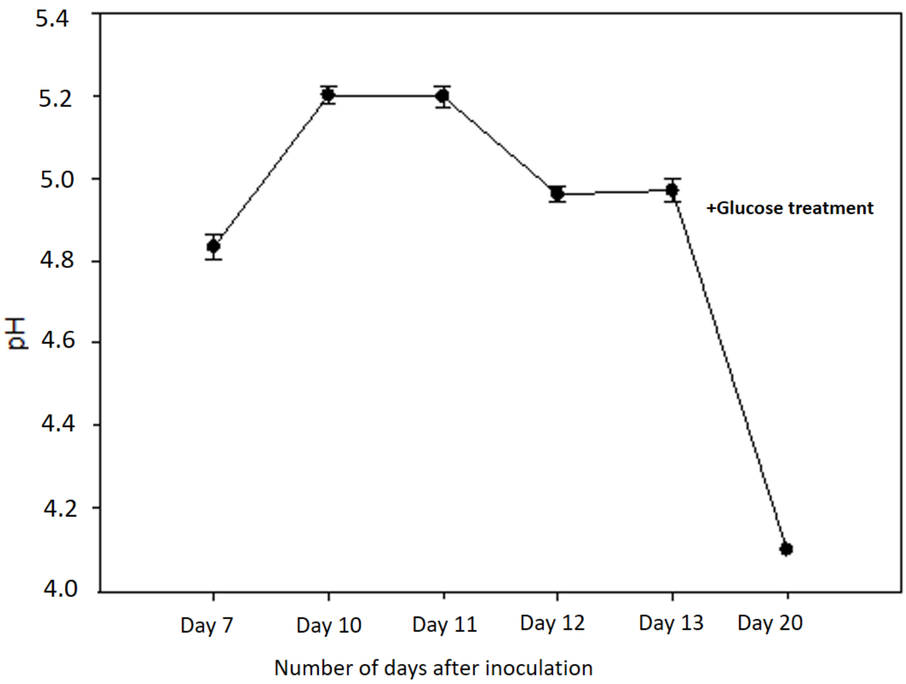

Figure 6: pH changes of fermented, inoculated brown juice at room temperature. n=3

Lactic acid bacteria have a very important role in agriculture as well as in many other aspects of human life. Our results show that as expected, lactic acid bacteria from inoculation material could significantly reduce the pH of phytoserum and make it stable at room temperature for extended storage time. The samples were kept at room temperature and we measured the change of pH every day. It seems well that the pH increased above 5 pH which didn't facilitate bacteria and the shelf-life reduced, so on the 13. day 12 g L^{-1}glucose was added to the inoculated brown juice. This significantly decreased the pH of phytoserum. By the day of 20^{th} the pH of around 5 was reduced to 4.1 pH. So we can say that we stabilized the brown juice at room temperature. We examined the effect of different doses of glucose on the pH of phytoserum and the 12 g L^{-1} treatment proved to be the minimum still efficient for preservation.

Results of irrigation experiments

The brown juice did not results in significant change to the growth of root, but as for the growth of shoot, it grew significantly. The concentration of 1% was the most effective. The stars sign the significant changes compared to control.

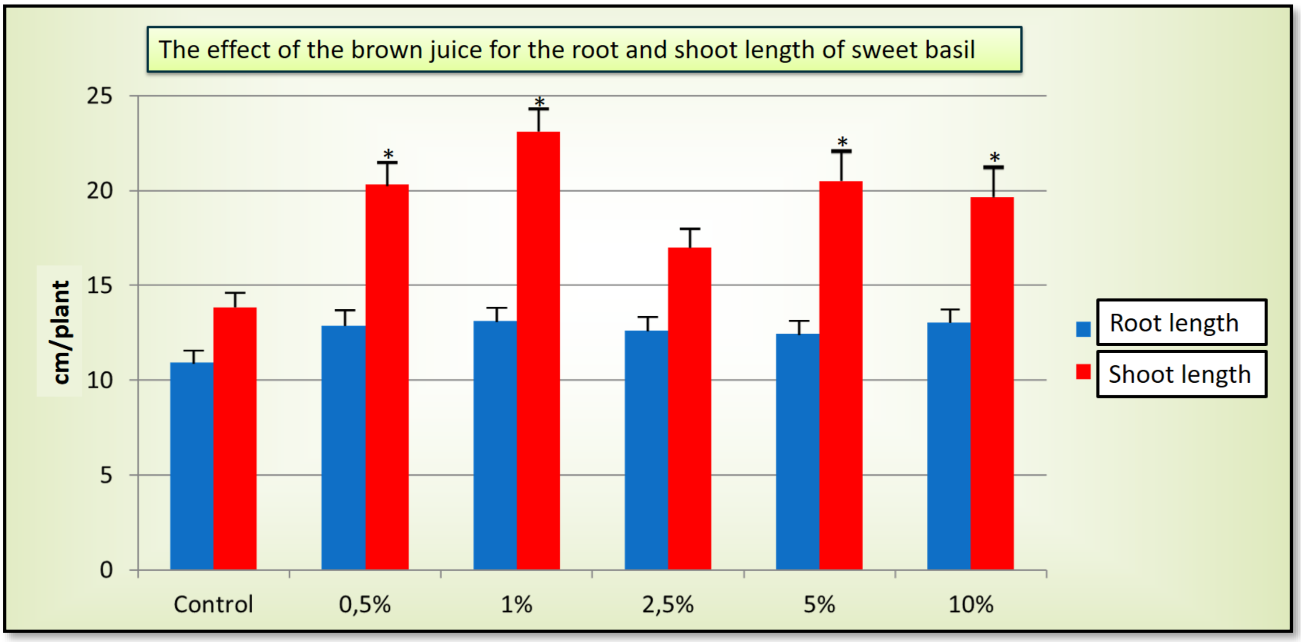

Figure 7:The effect of the brown juice for the root and shoot length of sweet basil

The brown juice affects the weight of organs of plants too. The concentration of 1% was also the best, because the weight of all organs was significantly heavier than the organs of control plant. However, in case of concentration of 10% the growth were disproportionate. The weight of leaves were heavier, but the weight of stems and root did not followed this change.

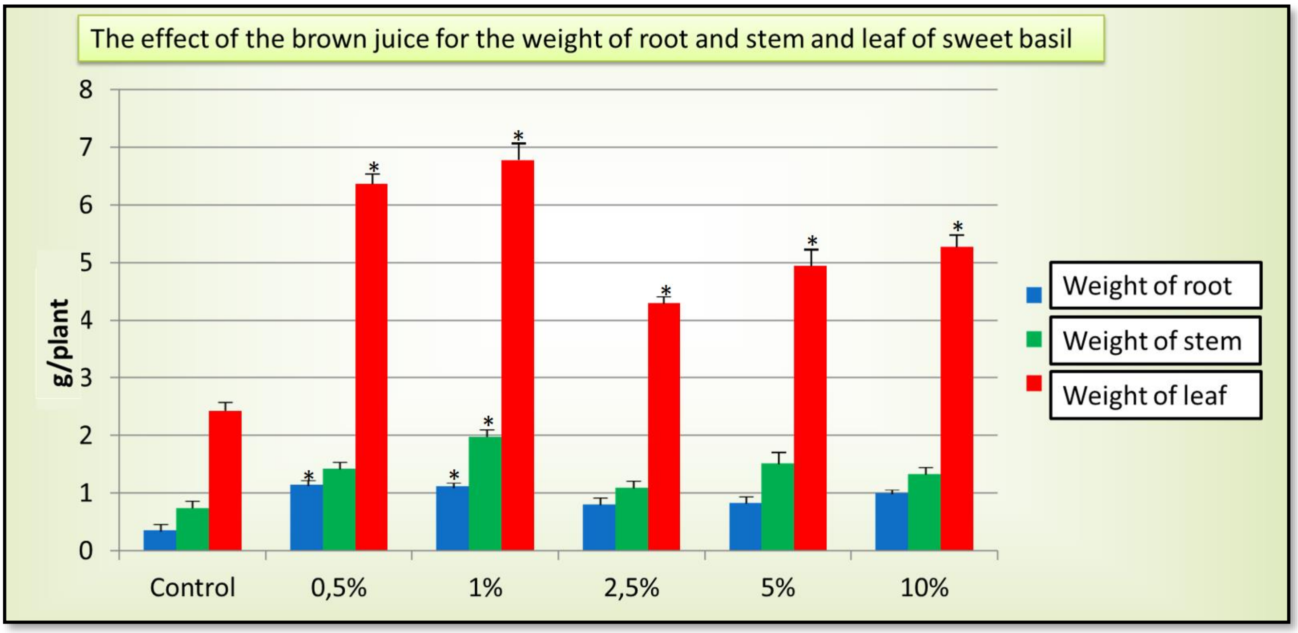

Figure 8: The effect of the brown juice for the weight of root, leaf and stem of sweet basils.

The effect of brown juice for the leaf number were also examined. In this case the concentration of 0,5% and 1% was the most valuable. We can conclude that the effect of the 1% BJ was the most valuable for the growth of sweet basil.

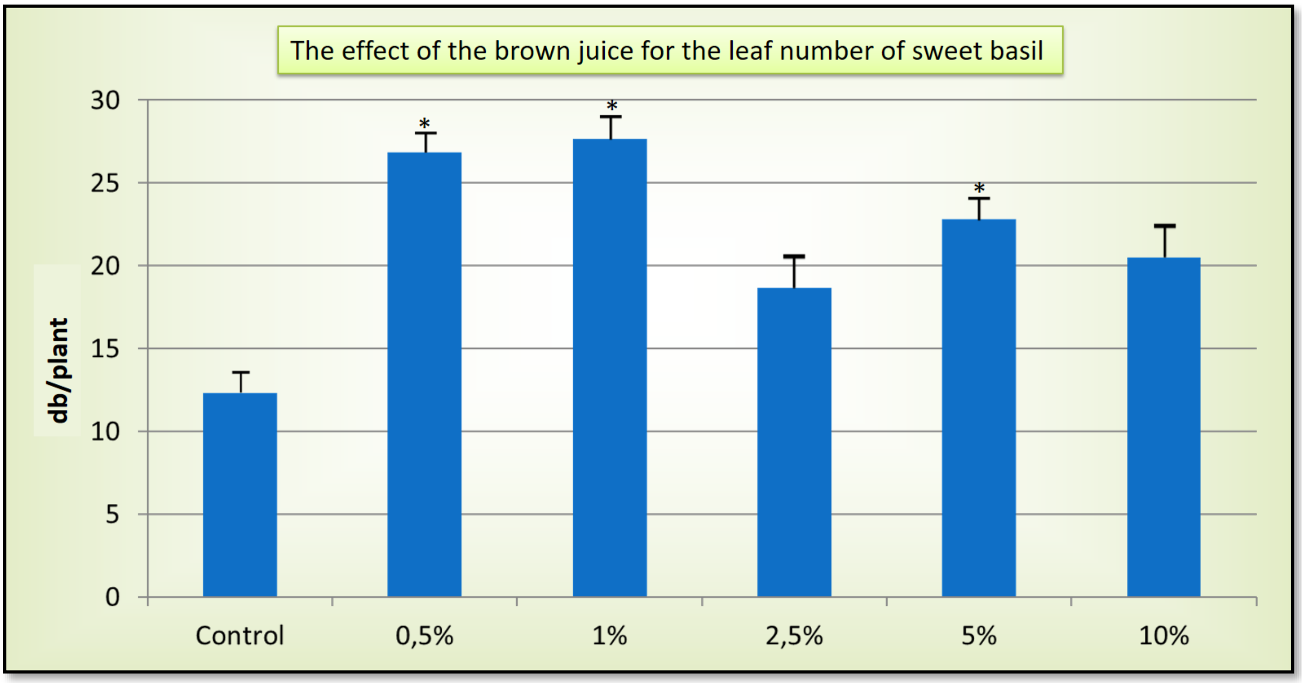

Figure 9: The effect of the brown juice for the leaf number of sweet basils

The effect of combined treatment was also examined. In this case we used Brown juice and spraying too. As for the length of root and shoot the concentration of 1 and 2,5 was the most valuable however the root did not grow significantly either.

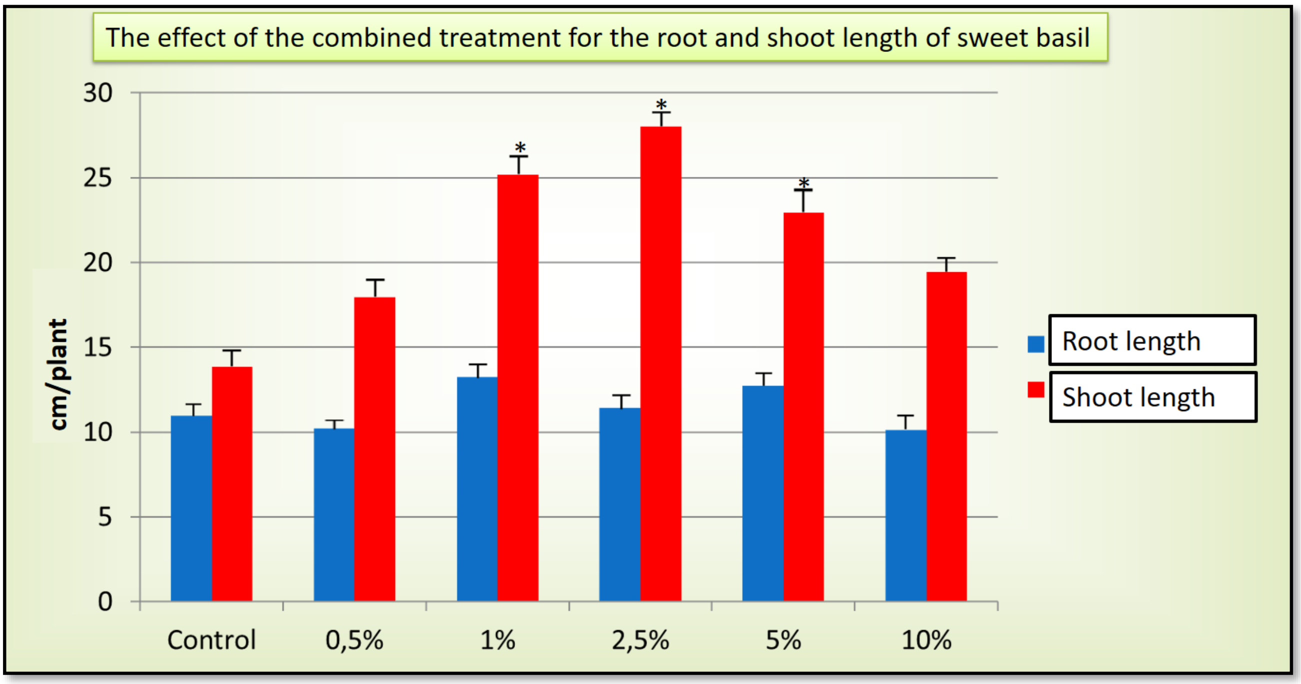

Figure 10: The effect of the combined treatment for the root and shoot length of sweet basils

With respect to organs of crops the concentration of 1, 2.5, 5 caused the most significant changes but taking into account the all organs of plants the treatment with 2.5% solution was the most valuable.

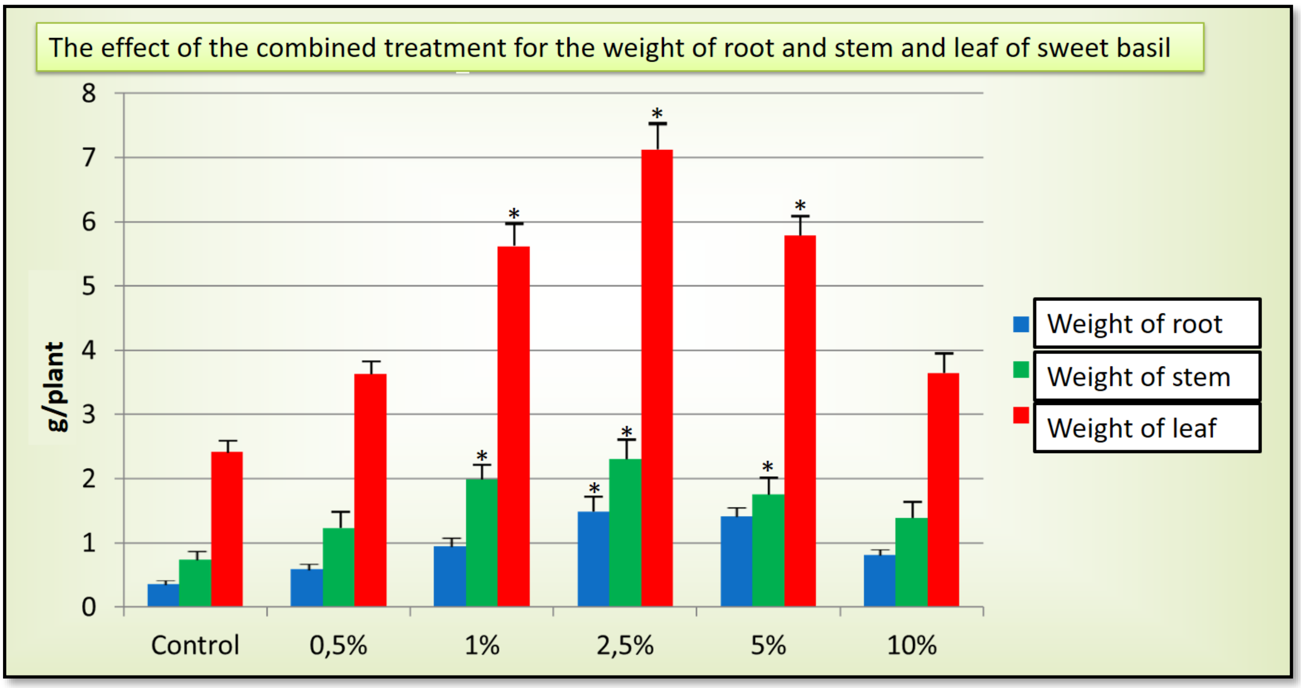

Figure 11: The effect of the combined treatment for the weight of root, stem and leaf of sweet basils

As for the number of leaves the treatment with 1% and 2.5% produced the most significant changes. For the effect of concentration of 5% the weight of leaves grew valuable but the number of leaves did not followed this change so we can conclude that probably decreased the leaf surface which is capable of active photosynthesis.

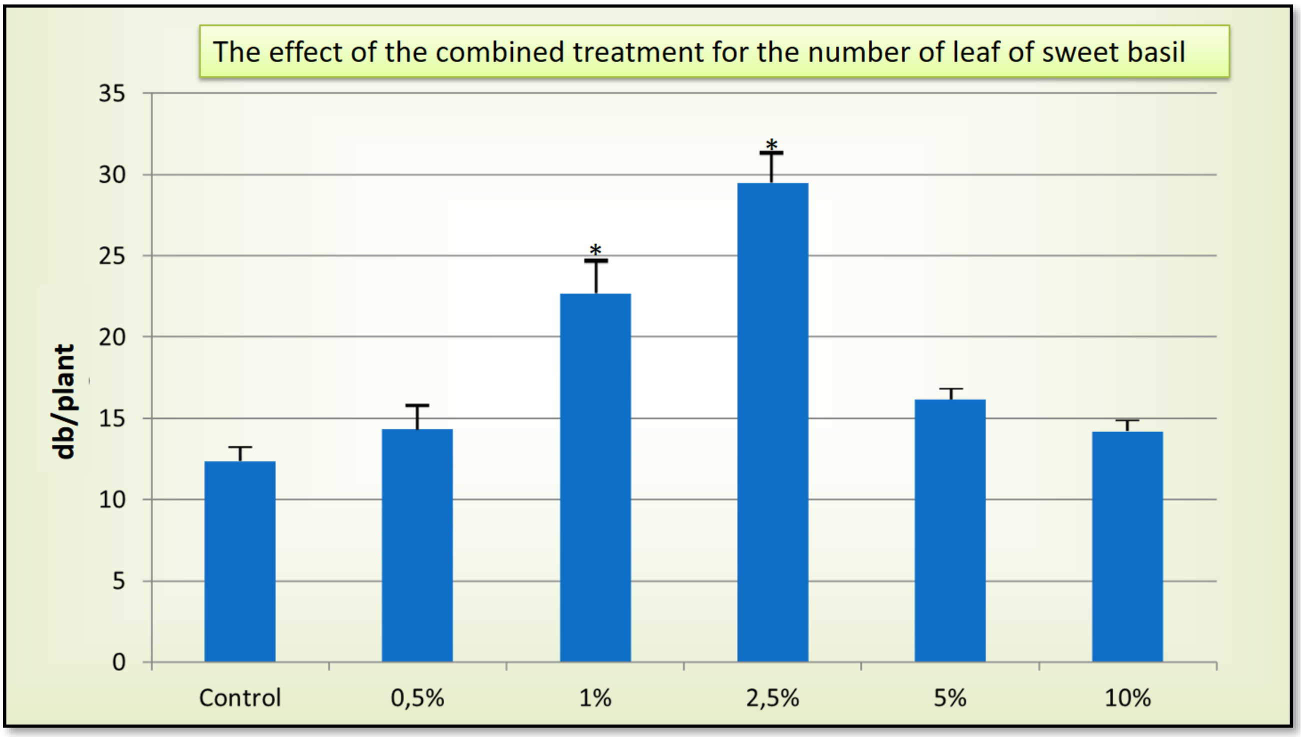

Figure12: The effect of the combined treatment for the number of leaf of sweet basils

Results of the irrigation experiment indicated that the concentrations of 1% and 2.5% were the most valuable. The plants treated with 1% fermented brown juice grew healthy and the organs of plants grew pro rata. The growths were disproportionate in case of higher concentration. The combined treatment did not result in higher growth than the treatment with only brown juice, so the simple process may be sufficient.

Summary

The increase in the world's population leads to several issues that we already have to face, and we must find sustainable solutions for them to save our life on the planet. One of these concerns is the sustainable supply of high-quality protein. To solve the protein issue, several approaches were proposed to identify novel protein sources or alternatives. Alfalfa (Medicago sativa L.) leaf protein concentrate (LPC) is a promising element either in human or animal diet as the human population of the Earth (7.2 billion) is growing rapidly causing a high demand for animal protein. The isolation of leaf protein resulted in four products, i.e., green juice, fiber, leaf protein concentrate, and deproteinized juice or referred to as brown juice (BJ). The amount of produced brown juice during the isolation of leaf protein and the utilization of this product is a huge obstacle for the wider recognition of LPC production. Fractionation of one-kilogram fresh biomass results in 450–550 mL brown juice. Therefore, disposal of brown juice is a big challenge that the leaf protein isolate approach faces. Another reason making disposal of brown juice risky is the high content of several bioactive components such as sugars, free amino acids, minerals and vitamins that brown juice contains. Therefore, finding an alternative use of brown juice is urgent due to environmental concerns, besides maximizing the benefit from this waste that is very rich in several valuable compounds and nutrients. Instead of dumping it we could make a valuable product, thus making a step towards the circular economy concept. The brown juice contains about 40% carbohydrates (mainly monosaccharides, like glucose and fructose) and 3% nitrogen. However, fresh brown juice cannot be stored at room temperature, after a few days it gets spoiled at pH 5–6 due to its high sugar content. Because of the inoculation of brown juice with lactic acid the pH reduce till 4.1.Therefore, brown juice seems to be an ideal component in animal feeding programs as well as plant nutrition and soil stabilization. Fermented brown juice can be applied as a very effective foliar biostimulant, as well. Sweet basil (Ocimum basilicum L.) is a well-known annual herb, member of the Lamiaceae family. It is one of the 150 species of the Ocimum genus, a very important medicinal, spice and fresh vegetable, culinary herb and industrial plant, cultivated for aromatic and medicinal use on large areas in many countries. Sweet basil is popularly used in traditional Chinese medicine, because of its selected purified components and antiviral activity. The 1% fermented brown juice has positive effect for the growth of all organs of sweet basils. However in case of higher concentration the growth were disproportionate.

Declaration

Our research was done at Department of Agricultural Botany, Crop Physiology and Biotechnology University of Debrecen. I has been searching a by-product so that it can be recyclable and storable in order that preserve our environment. We used alfalfa brown juice for this process. We inoculated the samples with lactic acid bacteria and examined the pH changes in the fermentation time. The effect of this brown juice were also examined for the growth of sweet basils. I have been studying it for over a year. After the recognition of literatures, I joined to the research. My task was the preservation of brown juice, processing of sweet basils and the analysis of data. Every research was led by Dr. Bákonyi Nóra. I have been preparing for the competitions with the help of my teacher, Dr. Koncz Gábor. He helped me a lot in order that I can get to know the topic. He explained what I did not understand and corrected my mistakes.

Introduction

My name is Emília Kovács. I am studying in Kisvárdai Bessenyei György Gimnázium és Kollégium. I am in year 10. I am interested in Biology and chemistry. In fact, I adore dealing with these subjects in my free time, as well. Beyond that I enjoy walking and riding in the nature. Environment protection is very important topic for me, especially the water protection, because the contaminated environment can be dangerous for the human health and for the health of plants and animals. I live next to the rivers of Tisza and Kraszna. If I can I am on the beach with my friends or with my dogs, because it has a peaceful feeling. Moreover, I often help in the cleaning of them as well. After the school leaving exam I would like to study medicine or psychology at university, because I would like to help a lot of people one day.

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