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Research on the production of fermented kombucha tea and its influence on the germination capacity for different vegetable species

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Abstract Kombucha is a fermented beverage that is currently sold and home-brewed on all continents, with a history of 2000 years. Its exact origin remains unknown, the current hypothesis being that it was formed by the spontaneous symbiosis of some bacteria and yeasts. Because Kombucha fermented tea is a very complex substance containing vitamins, enzymes, beneficial bacteria, amino acids, phenolic compounds and organic acids, a possible bio-stimulating effect of the fermented tea on plants was considered. In the first stage of the research, the aim was to monitor two quality parameters during the fermentation process of kombucha tea on a sweetened green and black tea substrate. This step confirmed the proper deployment of the fermentation process, herein obtaining a high-quality tea necessary for the next stage. In the second stage of the research, we tested the influence of kombucha fermented tea on the germination rate of peas, cucumbers, lettuce and radishes. Each species was treated with five aqueous solutions with a very wide range of concentrations, along with a control treated with filtered water. In the case of seeds treated with kombucha tea solutions of low concentrations (1% and 3%) a positive influence on germination was observed compared to the control, both by an increased germination speed and an increased total number of germinated seeds in some cases. The solutions of high concentrations (10% and 25%) had negative effects on the germination capacity. The processing of the results allows us to make recommendations regarding the recipe for the preparation of kombucha fermented tea and the range of concentrations with a possible bio-stimulatory effect, especially considering the fact that there is currently no research in the Romanian literature considering this possibility.

Key words

Kombucha, biostimulant, seeds, germination, fermented tea

Kombucha, also known as "mushroom tea" or "Manchurian mushroom", is a fermented beverage, slightly effervescent and with a low alcohol content. It is obtained by the action of the culture on certain types of tea (green and black) and is usually consumed for the known and alleged health benefits. The drink is usually called "kombucha tea" to differentiate it from the membrane that is formed by the action of yeasts and bacteria during fermentation [3, 10]. Although the membrane is often referred to as the "tea mushroom" or "kombucha mushroom", it is actually a symbiotic growth of acetic bacteria and osmophilic yeasts organized in a biological cellulosic membrane [7, 8]. The exact origin of kombucha is unknown, but Manchuria is most likely the place of origin [4, 14]. It may have appeared as recently as 200 years ago, or as late as 2000 years ago [5]. The population of microorganisms in a biological membrane varies. It is known that kombucha contains a wide range of yeast and bacterial species, comprising several genera, including species of *Zygosaccharomyces, Candida, Torulaspora, Kloeckera / Hanseniaspora*, *Pichia*, *Brettanomyces / Dekkera*, *Lachancea*, *Saccharomycoides*, *Saccharomyces*, *Schizosaccharomyces* and *Kluyveromyces* [2, 15, 16, 6]. The vast majority of prokaryotes in this culture belong to the genus *Acetobacter* and *Gluconobacter*. The basic bacterium is *Acetobacter xylinum* (formerly called *Gluconacetobacter xylinus*) which ferments alcohols produced by yeast in acetic acid and other acids, thus limiting the ethanol content of the drink [12]. The cellulosic mass is a secondary metabolite of kombucha fermentation, and also one of the unique characteristics of the culture, hosting the symbiotic microorganisms [11, 13]. Bauer-Petrovska and Petrushevska-Tozi (2000) [1] quantified the water-soluble vitamins in kombucha prepared with 7% sucrose and 5 g / L of black tea. The values were as follows:vitamin B1 74 mg / 100 mL, vitamin B6 52 mg / 100 mL, vitamin B12 84 mg / 100 mL, and vitamin C

151 mg / 100 mL. Malbasa et al. (2011) [9] measured the maximum value of vitamin B2 in samples obtained by classical fermentation with black tea (8.3 mg / 100 mL) and green tea (9.6 mg / 100 ml). In that investigation, the amount of vitamin C steadily increased in all samples and reached a maximum of 28.98 mg / L on day 10 in a beverage prepared with a combination of acetic bacteria and S. cerevisiae isolated from kombucha. This value was slightly lower (27.86 mg / L) in the drink traditionally prepared, at the same stage of fermentation [9].

The present research pursues two main objectives. The first is to test, observe and monitor the culture of kombucha in containers on sweetened green and black tea substrate to clarify the possibility of starting the culture in household conditions with material obtained from local growers, and to obtain a high-quality fermented tea necessary in the next step. During the 14 days of fermentation of the four variants, the daily documentation of two quality parameters was followed, respectively the pH and the Brix sugar content (%).The second objective of the research is to test the influence of kombucha fermented tea on the germination capacity of peas, cucumbers, lettuce and radish. Each species was tested with five variants of aqueous kombucha tea solutions, respectively 1%, 3%, 5%, 10% and 25%, next to a control treated with filtered water. The possibility of a bio-stimulating effect was considered due to the very complex composition of the tea. The fermented tea used in this part of the experiment was produced under controlled conditions in the first stage of the research. There is currently no research on this aspect in Romanian literature, so filling this gap and introducing the organism called kombucha in the world of horticulture is one of the motivations of this research.

Material and Method

The experiment took place inside an apartment, in Bucharest. For the experience, a dedicated table was arranged, protected from temperature and humidity variations, and from direct sunlight. The temperature and relative humidity of the air had constant values between 22-24 ℃ and 55-70% RH, respectively. Taking into account the biological nature of the experiment and the possibility of contamination of the samples, throughout the research strict hygiene conditions were imposed on the dedicated table and in the entire apartment. Surfaces, dishes, tools and the room were cleaned and disinfected. Sampling for observation was done as minimally invasively as possible, and during the experiment there were no events that adversely affected the environmental conditions or accidents that could compromise the results of the analyzes.

1. The study of the evolution of pH and sugar content (Brix%) during fermentation

In this part of the experiment, four different kombucha tea recipes were fermented for 14 days. The „mother" culture needed to start fermentation was obtained from two sources from local growers. Variations in recipes consisted of different amounts of added sugar, the use of two types of tea (green and black) and different concentrations of infusions. The culture was obtained in glass jars with a volume of three liters each.

In the case of kombucha tea, under the conditions of a normal fermentation process it is expected that the pH values and the sugar content decrease, these being two indicators of a well deployed fermentation process. With regards to this, the sugar content (Brix%) and the pH of the four recipes were measured daily to observe and confirm the fermentation process.

Studied formulations:

Formula 1: 70 g sugar / L + 8g green tea / L
Formula 2: 90 g sugar / L + 8g green tea / L
Formula 3: 90 g sugar / L + 8g black tea / L
Formula 4: 70 g sugar / L + 5.5g black tea / L

Working method

In order to achieve the research's objectives, a total volume of 12 liters of fermented tea was prepared, distributed in four vessels of three liters each. For variant one, a total of 210 grams of sugar was added, equivalent to 70 grams of sugar per liter of infusion. This is one of the most popular and used recipes for making kombucha drink. For variant two, the sugar concentration was higher, totaling 270 grams of sugar per three liters of infusion, equivalent to 90 grams per liter. After the sweetened tea has been brought to normal concentrations and cooled, for a three-liter vessel, it is continued by adding 150-250 milliliters of starter liquid (5-8%) from a previous culture. This step has a very important role because it lowers the pH of sweetened tea and populates it with specific bacteria and yeasts, thus protecting of contamination. Finally, the microbial membrane ('mother') can be added, keeping the maximum level of tea close to the large diameter of the container to ensure proper oxygenation.

In the case of variants three and four, prepared with black tea, the infusions for the two culture jars were prepared separately, with different concentrations of tea. For variant three we used 24 grams of black tea (corresponding to 8 grams for a liter of water), and 270 grams of white sugar (corresponding to 90 grams for a liter of infusion). Compared to variant two, which has the same sugar content, this variant will indicate the influence of different types of tea, being prepared exclusively with black tea. For the preparation of variant four, 16.5 grams of black tea (equivalent to 5.5 grams for a liter of water) and 210 grams of sugar (equivalent to 70 grams for a liter of infusion) were used. The working method for variants three and four was identical to that for variants one and two, these being prepared on the same day and the samples placed in the same conditions.

The experiment continued for 14 days with the daily observation of cultures and collection of samples for

analysis. Daily measurements included determination of sugar content (Brix%) using an optical refractometer and determination of pH using a high-precision digital pH meter. Very non-invasive tools (pipette and syringe) were used to take the liquid samples, so as not to disturb the process of the formation of the new cellulosic membrane on the surface of the tea.

2. The analysis of the influence of the kombucha fermented tea on the germination capacity of peas, cucumbers, lettuce and radishes.

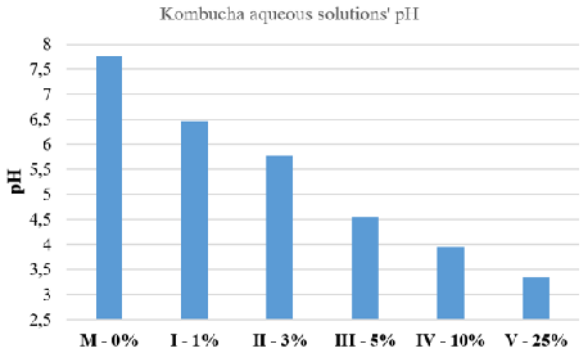

Fig. 1: pH of kombucha solutions used for germination

In this final part of the experiment, a possible bio-stimulating effect of the fermented tea on plants was considered, investigating the influence of the tea on the germination capacity for four species of vegetable plants, namely peas (*Pisum sativum)*, cucumbers (*Cucumis sativus)*, lettuce (*Latuca sativa)* and radishes (*Raphanus raphanistrum* subsp. *sativus*). Following this goal, with the help of the product obtained in the first stage of the research, 5 aqueous solutions of different concentrations were prepared and tested, alongside a control with filtered water.

Working method

The experiment began by sanitizing the space and disinfecting the necessary vessels and tools. The experiment aimed to test the influence of kombucha tea on the germination capacity of peas, cucumbers, lettuce and radishes. This was achieved by the comparative study of five solutions of different concentrations, alongside a control with filtered water, for each of the target species during seven days. The concentrations studied were: variant one - 1%, variant two - 3%, variant three - 5%, variant four - 10%, variant five - 25%, and control - 0%. Therefore, for each of the four studied species, six Petri dishes with solutions of different concentrations were prepared, resulting in a total of 24 dishes. The number of seeds used in each vessel was 30, resulting in a total of 720 seeds. The vessels were labeled with a unique identification number and placed in rows on the table dedicated to the experiment. During the research, several measurements were made such as the pH for each of the five fermented tea solutions, the pH of the water for the control sample, the temperature and relative humidity of the air and the daily monitoring of germination.

The germination technique used in the experiment involved soaking the seeds at the beginning of the process for 12 hours in the solution corresponding to the variant studied. For this purpose, the seeds were kept for 12 hours in Petri dishes, soaked in 50 milliliters of the solution corresponding to the studied variant. This first step aims at complete hydration of the seeds, which quickly triggers the germination process. The first count of germinated seeds was done after 12 hours of hydration, this time representing "day 1". After counting, the seeds were placed between two layers of absorbent paper in the same Petri dishes. The paper was kept permanently moist by adding the solution corresponding to each variant using a 10 ml syringe. The experiment continued with daily observation and monitoring of germinated seeds, and all measurement data were noted in an Excel table to be processed into graphs that will show the influence of kombucha tea on germination in the case of the studied species. During the experiment the temperature and humidity in the room remained constant, with small variations. Wetting the seeds was necessary twice a day.

Results and Discussions

During the experiment, the correct fermentation of the culture and the obtaining of a quality drink were achieved. In the case of all four variants, a decrease in pH and a decrease in sugar content could be observed.

The cultures behaved normally and multiplied, forming a new biological cellulose membrane on the surface of the liquid. Sugar content and pH were measured daily for all four variants, indicating correct fermentation in all cases. Below are the graphs that illustrate the evolution of the two quality parameters studied and the general results obtained from the experiment.

1. The evolution of pH and sugar content (Brix%) during kombucha fermentation

The pH and sugar content (Brix%) were measured daily for 14 days for each of the four variants studied.

A significant reduction of the values of both parameters was observed during fermentation in the case of all studied variants.

1.1 Evolution of sugar content (Brix%)

In the case of the sugar content, the largest differences appeared in the case of variant four, which decreased from 9.1 to 7.7 Brix (%), with a difference of 1.4 Brix (%). This indicates that the fermentation process makes better use of dissolved sugar when using a black tea substrate, sweetened with 70 grams of sugar per liter. The smallest changes in sugar content were observed for variant two, the value decreasing from 11.2 Brix (%) to 10.9 Brix (%). This indicates that sugar is used more heavily in the fermentation process when using green tea sweetened with 90 grams of sugar per liter.

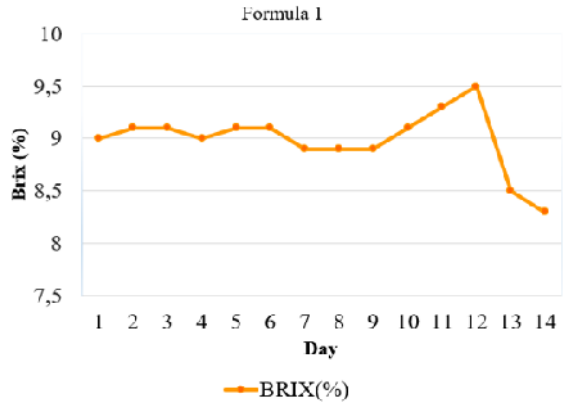

Fig. 2: Brix(%) during fermentation - Formula 1

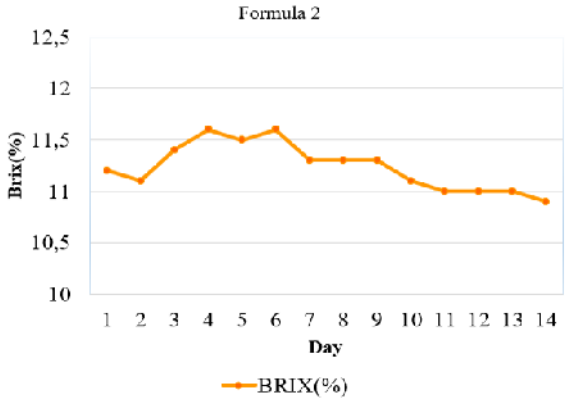

Fig. 3: Brix(%) during fermentation - Formula 2

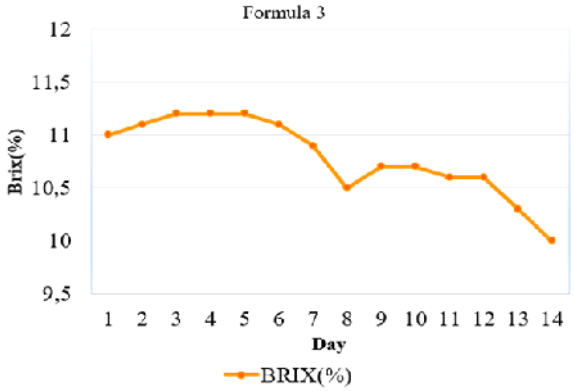

Fig. 4: Brix(%) during fermentation - Formula 3

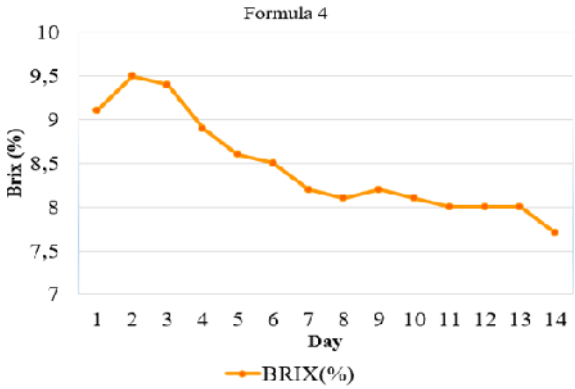

Fig. 5: Brix(%) during fermentation - Formula 4

1.2 The evolution of pH

The largest changes in pH occurred in the case of variant one studied, which decreased from 3.55 pH to 2.77 pH, a difference of 0.78 pH. A very interesting thing was observed in the case of the other three variants, their pH values decreasing by 0.64 pH in all cases. In the graphs below you can see how in all cases there was a more or less pronounced decrease in pH and sugar content.

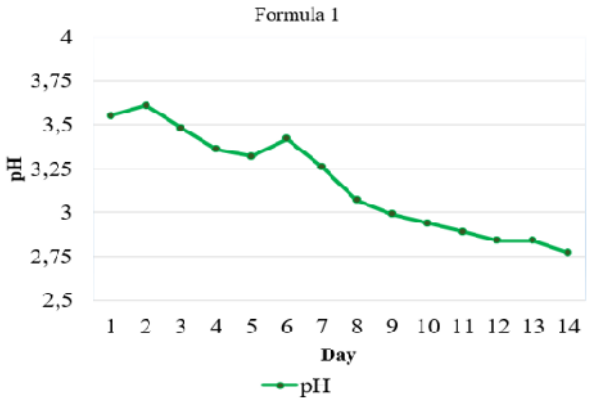

Fig. 6: pH during fermentation - Formula 1

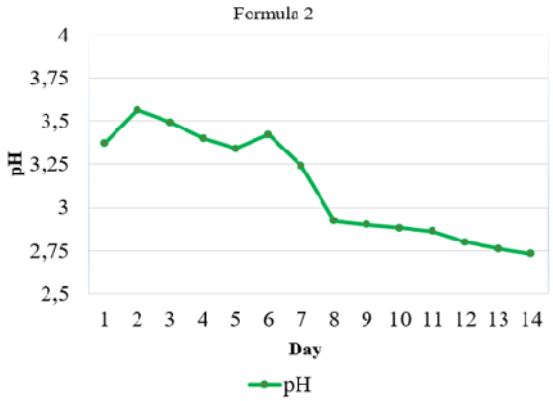

Fig. 7: pH during fermentation - Formula 2

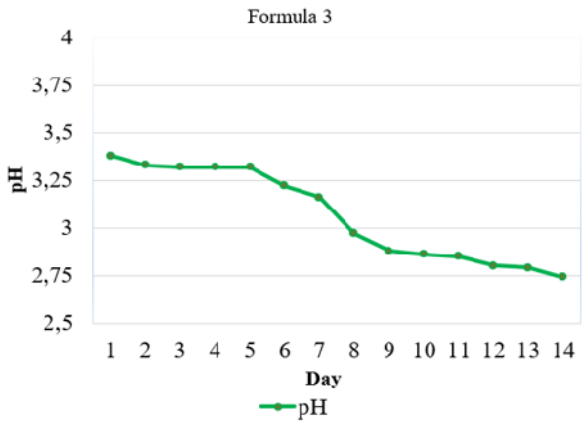

Fig. 8: pH during fermentation - Formula 3

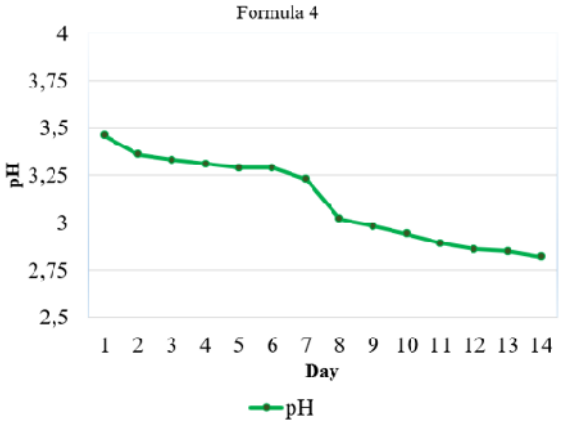

Fig. 9: pH during fermentation – Formula 4

2. The influence of different kombucha dilutions on the germination capacity of peas, cucumbers, lettuce and radishes

The fermented kombucha tea was tested in four variants of solutions with concentrations of 1%, 3%, 5%, 10% and 25%. The first count of germinated seeds was done after the 12 hours of hydration, representing "day 1" in the graphs below. At the beginning of the experiment the pH of the solutions corresponding to the studied variants was measured, the determined values are presented in Fig.1 above. Below are the graphs showing the evolution of seed germination of the studied species, along with the control, over a period of 7 days.

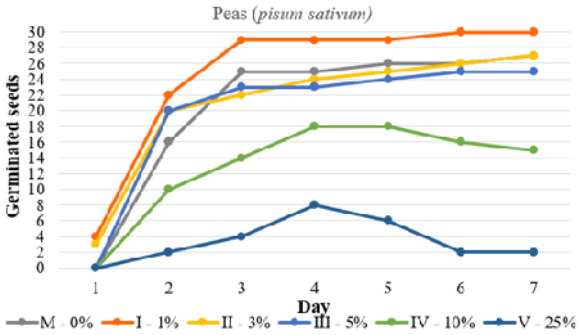

Fig. 10: seed germination – Peas

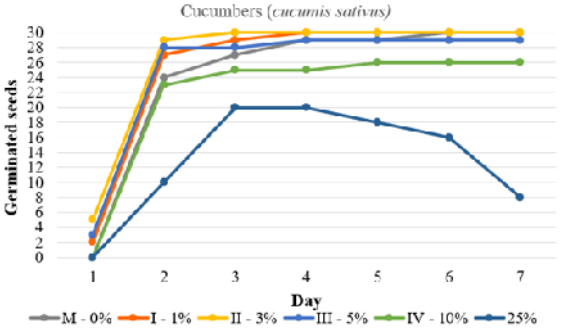

Fig. 11: seed germination – Cucumbers

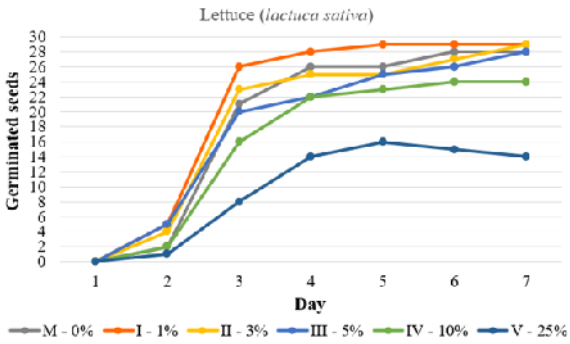

Fig. 12: seed germination – Lettuce

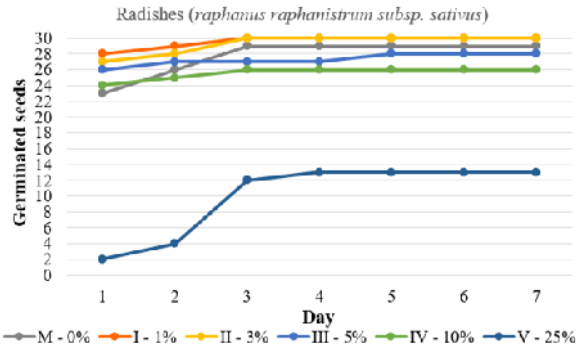

Fig. 13: seed germination – Radishes

The graphs above (fig. 10 – 13) show a clear positive influence of low-concentration kombucha solutions on germination, both by determining faster germination and by increasing the total number of germinated seeds in some cases. In the case of pea seeds the best results expressed by the germination rate and the total number of germinated seeds were obtained in the case of the variant treated with 1% kombucha solution, reaching 100% germination on day seven (30 out of 30 seeds) , compared to the control that reached a maximum of 27. Higher concentration solutions, 10% and 25% respectively had a negative effect on germination, the 25% solution causing the appearance of mold on day five, thus compromising a large part of the seeds . In the case of cucumber seeds, the same type of positive influence of low concentration solutions was observed,

causing a faster and more uniform germination. The 3% kombucha solution had the most pronounced effects on cucumber seeds, reaching a germination rate of 100% on day three. The 25% kombucha solution had negative effects on germination compared to the control, causing mold and seed compromise. In the case of lettuce and radish seeds, the same pattern was observed, the 1% and 3% solutions having beneficial effects on the germination time, as well as on the total amount of germinated seeds. In both species, the maximum germination rate was observed for variants treated with solutions of concentrations of 3% and 5% kombucha. It was observed that the solutions of high concentrations (10% and 25%) had negative effects on the germination capacity, and in the case of the 25% solution there are adverse effects that almost completely compromise the seeds. 1%, 3% and 5% solutions have been shown to be beneficial in most cases, accelerating the germination process and increasing the germination rate

Conclusions

The research described in this paper sheds light on kombucha and introduces it to the world of horticulture, demonstrating the beneficial effects of fermented tea on seed germination.
In the first stage of the study, a quality kombucha tea was obtained for the next stage, the analyzes performed confirming the correct deployment of the fermentation process.
Kombucha cultivation can be done with live material obtained from local growers, as long as it has been kept in the right conditions (at room temperature), is unpasteurized and free of pathogens.
The recommended recipe for kombucha for consumption is: 8g green / green-black tea + 70g white sugar + 6-8% starter liquid + „mother" culture / 1 liter of water. It is not recommended to exceed the threshold of 8% starter liquid to avoid the formation of vinegar taste at the end of fermentation.
In the case of cultures prepared with 90 grams of white sugar per liter of water, the formation of a biological membrane of greater thickness was observed, compared to the variants prepared with 70 grams of sugar, from which we conclude that this is the right amount of sugar for the multiplication of the culture.
The kombucha membranes formed during fermentation can be stored for months at room temperature in a dedicated container, ensuring, if necessary, supplementation with fresh sweetened tea and regular aeration.
In the second stage of the study, positive results were obtained regarding the bio-stimulatory effect of the preparation, observing the improvement of the germination capacity in the samples treated with kombucha tea.
Concentrations of 1% and 3% were beneficial for all species studied, both in terms of germination time and the total percentage of germinated seeds.
In the case of the 5% concentration, a faster onset of germination was observed in all species, but no improvement was observed in the total amount of germinated seeds.
High concentrations of 10% and 25% had negative effects on germination in all species.
In future research, in order to determine the influence of the preparation on the germination capacity, it is recommended to study kombucha concentrations between 0.5% and 10%.
For the preparation of solutions it is recommended to use a tea in the fermentation stage for consumption, with a balanced content of sugars and acids.
A possible future direction of research is the use of kombucha preparations in more advanced stages of fermentation for the prevention and foliar treatment of diseases and pests in horticultural crops. This hypothesis is based on the effects of the preparation observed during the present study and taking into account the content of organic acids, vitamins and microorganisms of fermented tea

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