APPLICATION OF BIOSURFACTANT- BASED FORMULATION FOR BIOSTIMULANT

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ABSTRACT

The agricultural industry has been breaking productivity records annually, but with the exponential increase in population and a limited area of land for planting, the agricultural industry has invested in new techniques and methods to increase productivity, one of these techniques is the application of biostimulants that have been gaining ground from the beginning of the 20th century, one of the substances that began to be used as biostimulants were biosurfactants, which are biodegradable chemical compounds that are expanding on the global market due to their diversity of applications. Therefore, this work aimed to evaluate the application of formulations based on biosurfactant from *Starmerella bombicola* ATCC® 22214™ as biostimulants in gherkin seeds (*Cucumis anguria*). To verify the efficiency as biostimulants, the germination test was applied in seedbeds, where 3 gherkin seeds were placed in each cell of the seedbed and the biostimulants were applied then, after 7 days the germination index was checked, the test was carried out in triplicate and the Tukey test was applied. All formulations had excellent results for biostimulants, where the formulation of biosurfactant + Oleic acid + distilled water performed the best result.

Keywords: Biosurfactant 1. Biostimulant 2. Oleic Acid 3. Sustainable Agriculture 4. Eco Friendly 5.

1 INTRODUCTION

The agricultural industry is one of the fastest growing sectors in the world, in Brazil agriculture grew 15.1% in 2023, with a total income of R$677.6 billion[1]. Even though this increase in food production is quite significant, in the same year, the world surpassed the mark of 8 billion people, according to the German Foundation for World Population (DSW)[2], In the UN(United Nations Organization) SOFI(State of Food Security and Nutrition) 2023 report, it was found that there had been 735 million people were still hungry, approximately 9% of the world population[3]. To have more food, the agricultural industry applied techniques and methods to improve food and productivity. With the use of transgenic foods, which are foods genetically modified to have greater productivity and/or resistance to pathogens[4] and biostimulants, which are substances or microorganisms when applied to seeds, plants or in the rhizosphere, stimulates natural processes to increase or benefit the absorption of nutrients, nutrient use efficiency, abiotic stress tolerance, or crop quality and yield[5].

The application of biostimulants is relatively new, being introduced at the beginning of the 20th century, but there is already work with relevant results[6], such as the application of seaweed extract in a grape plantation where it generated a 14.7% higher yield[7], in application of *Ascophyllum nodosum* extract in the cultivation of tomatoes and peppers[8] or in the application of green surfactants to increase nutrients in the soil[9]. Green surfactants are substances that have the characteristic of reducing surface tension, as they have a hydrophilic part and a hydrophobic part in their molecular structure, in addition to being biodegradable, low toxicity, fungicides[10]. They are subdivided into 2 classes, biosurfactants (surfactants of microbial or vegetable origin) and natural-based surfactants (biosurfactants that have undergone some type of chemical modification)[11]. Green surfactants have several applications in the literature, such as removal of petroderivatives[10], soil bioremediation[12], bioremediation of marine fouling[13] in application as agricultural pesticides and biostimulants[14] and among others.

Having said that, the present work aimed to evaluate the application of formulations based on biosurfactant from *Starmerella bombicola* ATCC® 22214™ as biostimulants in gherkin seeds (*Cucumis anguria*).

2 MATERIAL & METHODS

Obtaining assets: The yeast *Starmerella bombicola* ATCC® 22214™ obtained from the American Type Culture Collection (ATCC) was the strain used to produce the biosurfactant. The maintenance and production medium were as follows Hipólito et al[15]. The essential oil of Lemongrass (*Cymbopogon flexuosus*) and Tea Tree (*Melaleuca alternifolia*) were obtained from the company Bioessência from Brazil LTDA, the castor oil (*Ricinus communis*) was obtained from the company Dinâmica from Brazil LTDA and Oleic Acid was obtained from company Dinâmica from Brazil LTDA.

Formulation of biostimulants: The composition of the test formulas is described in Table 1. All liquid formulations were prepared without heating, with continuous stirring for more than 5 minutes using a Vortex-type magnetic stirrer until they formed a single phase. To maintain a reduced number of treatments, only the biosurfactant concentration at 0.1% v/v was tested in all formulations.

Table 1 Composition of biostimulants used in research.

Formulations	Composition
C	Distilled water
B	Biosurfactant + distilled water.
BO	Biosurfactant + Oleic Acid + distilled water.
BL	Biosurfactant + Lemon Grass oil + distilled water.
BT	Biosurfactant + Tea Tree oil + distilled water.
BC	Biosurfactant + Castor oil + distilled water.

Analysis of the effect of biostimulants on sowing: The germination and root growth method were carried out using Gherkin (*Cucumis anguria*) seeds. In this analysis, biostimulants were tested, as shown in Table 1. Cultivation trays with up to 15 cells were filled with a balanced substrate, according to the manufacturer, coconut powder or fiber, ground, and composted pine bark, Biokashi (organic additive with Macro and Micronutrients), organic compound Biomix formula. The treatments were placed separately in seedbeds with 3 seeds per cell. The seeds were incubated for 7 days with artificial light in a closed environment with a light cycle of 12/12 h on/off. After this period, the number of germinated seeds was then counted, and the length of the roots measured from the hypocotyl transition point to the tip of the root. The germination index (GI), was used to identify the biostimulant effect of formulations, was calculated according to Equation 1:

$$\%GI = ((\%\ seed\ germination) \times (\%\ root\ growth): 100 \quad (1)$$

In which % seed germination = (% germination of formulation) / (% germination in control); and % root growth = (average growth in formulation) / (average growth in control). Data were statistically analyzed using the Tukey test with a factor of 5%.

3 RESULTS & DISCUSSION

This test aimed to identify which of the biostimulants were effective. In Figure 1 we have the visual growth of the seeds. We can see that all the seeds germinated and entered the first follicular phase within 7 days of the experiment.

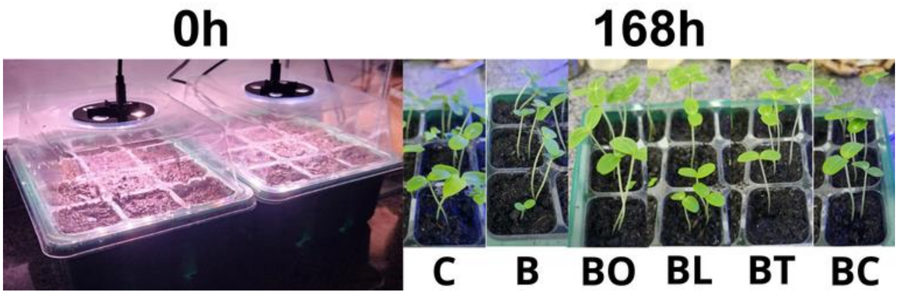

Figure 1 Assessment of germination and growth of Maxixe (Cucumis anguria), when sown between 0 and 168h in soil and in the presence of a light source for the biostimulants tested (C (Distilled water), B (Biosurfactant + distilled water), BO (Biosurfactant + Oleic Acid + distilled water), BL (Biosurfactant + Lemongrass Oil + distilled water), BT (Biosurfactant + Tea Tree Oil + distilled water), BC (Biosurfactant + Castor Oil + Distilled water).

However, it is only possible to identify a biostimulant activity by measuring the length of the roots. After carrying out the calculations using equation 1 where these results are found in Figure 2, it was possible to identify differences between the formulations.

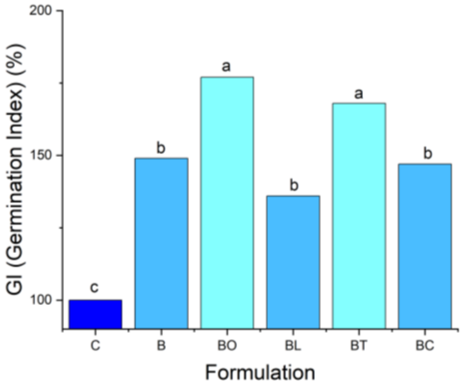

Figure 2 Maxixe (*Cucumis anguria*) germination index, when sown between 0 and 168h in soil and in the presence of a light source for the biostimulants tested (C (Distilled water), B (Biosurfactant + distilled water), BO (Biosurfactant + Oleic Acid + distilled water), BL (Biosurfactant + Lemongrass Oil + distilled water), BT (Biosurfactant + Tea Tree Oil + distilled water), BC (Biosurfactant + Castor Oil + Distilled water).

The equal letters located above the columns are the results of Tukey's statistical test, in which equal letters mean that statistically those values are similar. With this we can see that all formulations had excellent results, as they are all above C (distilled water), the application of B (Biosurfactant + distilled water) had a very satisfactory result of approximately 50% above C, as for the formulations BL (Biosurfactant + Lemongrass oil + distilled water) and BC (Biosurfactant + Castor oil + distilled water) which had results statistically equal to formulation B would not be viable, as the formulation with only biosurfactant would already be completely effective. If a result of 50% improvement in relation to C would already be a satisfactory result, the formulations BO (Biosurfactant + Oleic Acid + distilled water) and BT (Biosurfactant + Tea Tree oil + distilled water) had exceptional results of approximately 80% germination index above C. According to current literature data[14,15], biosurfactants can improve soil quality through the mobilization of nutrients from the environment and, in addition to biostimulants and biofertilizers, they have applications against pathogens and insect control.

4 CONCLUSION

The biosurfactant produced by the yeast *Starmerella bombicola* ATCC® 22214™ is highly effective in application as biostimulants, so that of the formulations created that had the best result was BO (Biosurfactant + Oleic Acid + distilled water), opening the possibility of using the raw production medium, as oleic acid is part of the biosurfactant production ingredients and there are other metabolic residues that can be very advantageous nutrients for plants and vegetables, thus reducing the biosurfactant processing time and jointly having a lower cost of production.

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