

In Vitro Activation of Microbial Growth in Low Frequency Electromagnetic Fields

Ricardo Bemfica Steffen¹, Gerusa Pauli Kist Steffen² & Joseila Maldaner²

Abstract

The advances in productivity and product quality observed in world agriculture lead to the use of new technologies capable of meeting current demands. The use of low frequency electromagnetic fields has shown significant results. In this context, the objective of this study was determine the effects of PENERGETIC technology on the direct stimulation of beneficial soil microorganisms under in vitro conditions. Mycelial growth and conidial formation of *Trichoderma asperelloides*, mycelial growth of ectomycorrhizal fungi *Pisolithus microcarpus* and *Suillus* sp. and the multiplication of bacterial cells of *Bradyrhizobium japonicum* species in culture medium with and without the addition of PENERGETIC. Significant increases in fungal and bacterial growth were observed when using the commercial biostimulator in culture medium. The use of sustainable technological tools represents a viable path because it consists of a solid and efficient management alternative capable of increasing the stimulus to biological development in the soil and, consequently, to agricultural productivity in an environmentally and sustainable safety.

Keywords: Biostimulation; microbial activity; resonance; ambience; harmonization.

1. Introduction

One of the greatest challenges facing today's agriculture in the world is to develop sustainable agricultural systems that can produce food and fiber in sufficient amount and quality with little impact on environmental resources. However, successful agriculture is not the result of a simple formula based on additional fertilizer use and pest and disease control. Sustainable production requires more.

Most technologies currently used in agriculture depend on synthetic molecules, whether to control weeds, pests or phytopathogenic microorganisms. Each time a synthetic molecule is used to control a particular group of insects, fungi, bacteria or plants, the environment suffers the consequences and tends to seek balance restoration. The introduction of pesticides and mineral fertilizers in agrosystems alters the chemical, physical, microbiological and energetic balance in the soil, directly affecting all life forms directly. Although nature seeks to restore the state of equilibrium through a feature called resilience over time and as the number of interventions increases, it is virtually impossible to restore this equilibrium and return to the original stage. In this sense, the use of technological and innovative products may represent a viable strategy for producers who are seeking to have more sustainable production systems, without reducing crop productivity.

Increasingly, technological innovation is moving away from usual concepts such as mechanical physics and using concepts from quantum physics. The transmission of information through wi-fi networks, georeferencing systems, semiconductors used in computers and even the operation of digital equipment use concepts of quantum physics. In agriculture could not be different. Research groups are demonstrating that plants communicate through a network of electromagnetic frequencies and that this energy flow is critical to the balance of biological systems. Some research results show that exposure of microorganisms to different intensities of electromagnetic fields can result in significant increases in the development and activity of these microorganisms (Potenza et al. 2004; Gao et al. 2005; Buchachenko and Kuznetsov 2014; Pospíšilová et al. 2015; Buchachenko 2016). PENERGETIC technology is one of the products based on technological innovations with effect on restoring balance in agricultural systems that has drawn attention among farmers and the scientific community.

Although the use of technology in agriculture is recent, research has already shown the effect of the product on wheat crop (Kadziuliene et al. 2005; Perkarskas et al. 2011; Pekarskas 2012a; Pekarskas and

¹ PhD in Biodynamic Soil Management. Rua dos Salsos, 410, 97030-770 Santa Maria, RS, Brazil.

² Researcher of Centro de Pesquisa em Florestas – Departamento de Diagnóstico e Pesquisa Agropecuária da Secretaria da Agricultura Pecuária e Desenvolvimento Rural do Rio Grande do Sul. Rodovia BR 287, Acesso VCR 830, km 4,5, 97001-970 Santa Maria, RS, Brazil.

Sinkevičienė 2015; Pekarskaset al. 2017), soybean (Souza et al. 2017), cucumber and tomato (Jankauskiene and Surviliene 2009), coffee (Franco Júnior et al. 2018; Franco Júnior et al. 2019), bean (Brito et al. 2012; Cobucci et al. 2015), potato (Jakiene et al. 2008) and barley (Pekarskas, 2012b). Steffen et al. (2016) observed an increase in the biological activity of Penergetic treated soils in soybean and wheat crops. The authors observed that the feeding activity of fauna and microorganisms in the sub superficial layer was intensified with the use of Penergetic technology with or without combination with mineral fertilization. All the study carried out have a common aspect: the quality of plants. The authors describe the increase in plant development in addition to finding an increase in productivity. The root volume stands out among the observed factors.

The connection between root development and water and nutrient absorption is known to have a direct correlation. It is also known that the greater the microbial activity in the rhizosphere, the greater the absorption and translocation of nutrients, thus increasing plant development. Thus, it is assumed that the increases in plant quality and crop productivity may be related to the stimulus of the microbial community in the rhizosphere of the studied crops. However, under field conditions it is difficult to isolate the direct effects of the benefits of a given technology on the diverse soil microbiota under field conditions. Thus, the objective of this study was to determine whether Penergetic technology has a direct stimulating effect on one group of bacteria and two groups of beneficial soil fungi under “in vitro” conditions.

2. Material and methods

The direct effect of the technology was evaluated on two groups of fungi and one group of bacteria, all naturally occurring in the soil and widely distributed worldwide. We used the fungus *Trichoderma asperelloides* (biological control agent and plant growth promoter), two species of ectomycorrhizal fungi *Pisolithus microcarpus* and *Suillus* sp. (symbiotic fungi of forest species) and the bacterium *Bradyrhizobium japonicum* (biological nitrogen fixing bacteria). Four treatments were evaluated, the control treatment (using unchanged culture media), silicon dioxide treatment (addition of commercial SiO₂ in the culture medium) and two doses of Penergetic Plant (1 and 2 grams of Penergetic per liter of medium).

Mycelium discs (9 mm diameter) from a pure culture maintained in BDA (potato dextrose-agar) culture medium for 15 days were transferred to the Petri dish center (90 mm diameter) containing GY culture medium (Bradley-Sylvester et al. 1982) according to the modified methodology of Katznelson and Bose (1959) to evaluate the effect of Penergetic (PNG) on stimulating *T. asperelloides* development. Penergetic Plant was added to GY medium prior to the autoclave sterilization process (25 minutes at 121 °C and 1 ATM) at dosages of 1 and 2 grams of Penergetic per liter of culture medium. Eight repetitions per treatment were used. The experimental units were randomly distributed inside the climatized chamber (temperature of 25 ± 2 °C and photoperiod of 12 hours). The plates were analyzed for mycelial growth area calculated by the growth radius in the vertical and horizontal directions after 120 hours of incubation. The plates were washed with saline solution (0.85% NaCl – v/v) to determine the mean number of conidium per plate in a hematimetric chamber (Neubauer chamber) after measurements. Both isolates were maintained in MNM modified solid Melin-Norkrans (Marx 1969) culture medium at pH 5.8 in 90 mm diameter Petri dishes and kept in a greenhouse at 26 °C to evaluate the effect of Penergetic on stimulating mycelial growth of ectomycorrhizal fungi.

Penergetic Plant was added to the MNM medium prior to the autoclave sterilization process at dosages of 1 and 2 grams of Penergetic per liter of culture medium. After sterilization of the MNM medium (25 minutes at 121 °C and 1 ATM) and after solidification of the culture medium in the Petri dishes, the transfer of the fungal mycelium discs from *Pisolithus microcarpus* and *Suillus* sp. to the center of the plate, which are sealed with plastic film. Eight repetitions per treatment were used. The experimental units were randomly distributed inside the climatized chamber (temperature of 25 ± 2 °C and photoperiod of 12 hours). The plates were analyzed for growth of fungal isolates, by evaluating the perimeter of the mycelial growth edge and for the average diameter of the hyphae, by photo documentation of the hyphae under a 300x magnification after 720 hours of incubation. The images obtained were enlarged using the GIMP 2.4.5 software (GNU Image Manipulation Program), which evaluated the average diameter of the hyphae. The bacterial colonies were incubated in an Erlenmeyer containing 250 mL of YM medium (yeast mannitol) with pH 6.0, adjusted with 2 mol L⁻¹ HCl solution and grown under orbital agitation at 110 rpm at 28 °C to evaluate the effect of Penergetic in stimulating the development of *B. japonicum* bacteria. Penergetic Plant was added to YM medium prior to the autoclave sterilization process at dosages of 1 and 2 grams of Penergetic per liter of culture medium. One mL aliquots were taken from each treatment to determine the number of colony forming units (CFU) by counting in a hematometric chamber after 96 h of growth.

The microbial stimulus data obtained in the evaluations with the four species of microorganisms were submitted to analysis of variance and Tukey test at 5% probability of error by SISVAR software (Ferreira 2011), based on significance levels greater than 95% (p ≤ 0.05).

3. Results and discussion

The results obtained in controlled environment microbial stimulus assays demonstrated the incremental effect on *T. asperelloides* (biocontrol agent and plant growth promoter), *P. microcarpus* and *Suillus* sp. (ectomycorrhizal fungi) and the bacterium *B. japonicum*. These results demonstrate that the use of Penegetic Plant product has direct benefit on the growth and biological activity of the evaluated microorganisms.

Silicon dioxide, a substrate used by Penegetic technology, did not result in a significant increase in fungal development without the industrial process. Stimulus effect resulting from the presence of silicon dioxide has already been described by Kumawat et al. (2019). The authors observed SiO₂ stimulation potential on biological activity. However, when electromagnetic bioprogrammed SiO₂ (Penegetic Plant) was used, sporulation was anticipated and the number of conidia per plate increased. The addition of Penegetic to the culture medium resulted in an increase in mycelium growth of 33.6% at 1 gram per liter and 72.6% at 2 grams per liter. For the production of *T. asperelloides* conidium, the addition of Penegetic at a dosage of 1 gram per liter increased 1376.4% and at a dosage of 2 grams per liter increased 2017.6% (Figure 1 and 2).

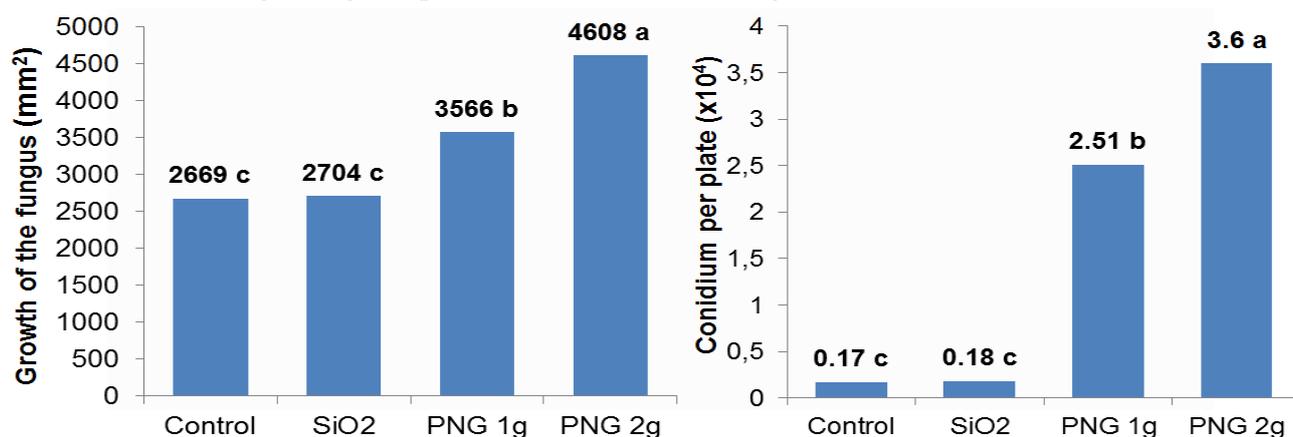


Figure 1: *Trichoderma asperelloides* growth determined by sporulation border diameter and number of conidium per plate. *Means followed by the same letter do not differ by the Tukey test at 5% probability.

In evaluations using ectomycorrhizal fungi, the response pattern was similar to that obtained with the tests with *Trichoderma*. There was an increase in fungal mycelial development in the presence of silicon dioxide, but not significant. However, in the presence of Penegetic, in both dosages, there was a significant increase of the fungus “in vitro”. The addition of Penegetic to the culture medium resulted in an increase in *P. microcarpus* mycelium growth of 154.2% at 1 gram per liter and 216.3% at 2 grams per liter. For the species *Suillus* sp. the addition of Penegetic at a dosage of 1 gram per liter increased 236.3% and at a dosage of 2 grams per liter increased 300.7% (Figure 3).



Figure 2: Mycelial growth and sporulation intensity of *Trichoderma asperelloides* in the treatments evaluated after 120 hours of incubation.

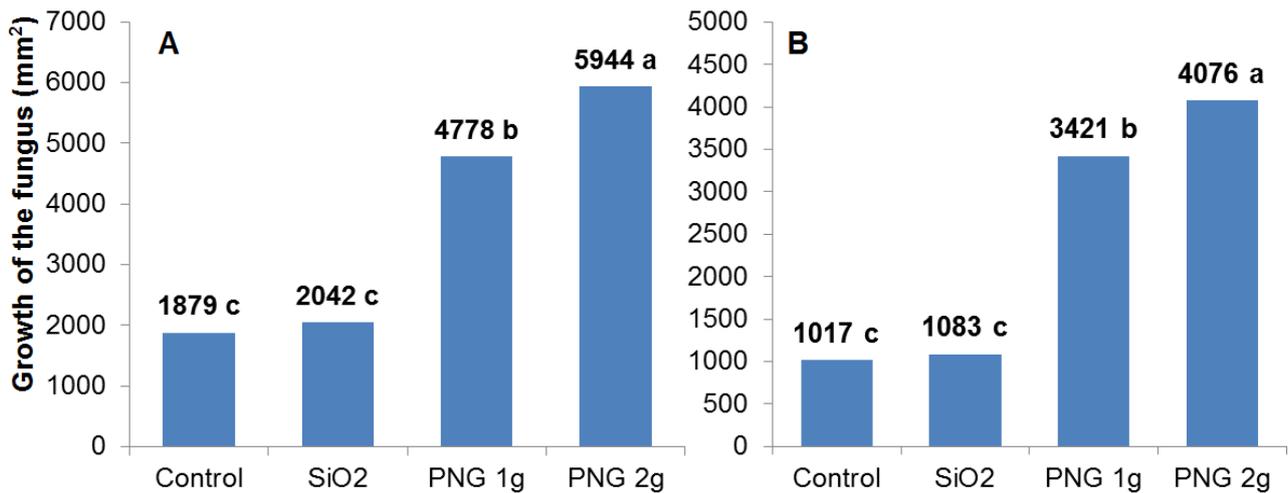


Figure 3: Fungal growth of *Pisolithus microcarpus* (A) and *Suillus* sp. (B) determined by border diameter. *Means followed by the same letter do not differ by the Tukey test at 5% probability.

The addition of Penergetic in the culture medium resulted in higher fungal mycelium density, evidenced by the smaller distance between the branch points, the larger hyphae diameter and the greater number of lateral hyphae from the same branch point (Figure 4). According to Denny and Wilkins (1987), this higher mycelium density gives the isolated greater degree of field survival and greater efficiency in the association and maintenance of symbiosis with symbiotic plants. According to Hestrin et al. (2019) stimulation of the development of mycorrhizal fungi depends on environmental and nutritional factors, but mainly on photoassimilates. According to the author, these fungi are strongly dependent on interaction with plants to maintain activity and development in the ecosystem.

The results obtained in the assay using the bacterium *B. japonicum* followed the patterns observed in the evaluations with *Trichoderma* and ectomycorrhizal fungi species. The addition of 1 and 2 grams of Penergetic to the culture medium increased the concentration of bacterial cells by 107.2 and 174.2%, respectively (Figure 6).

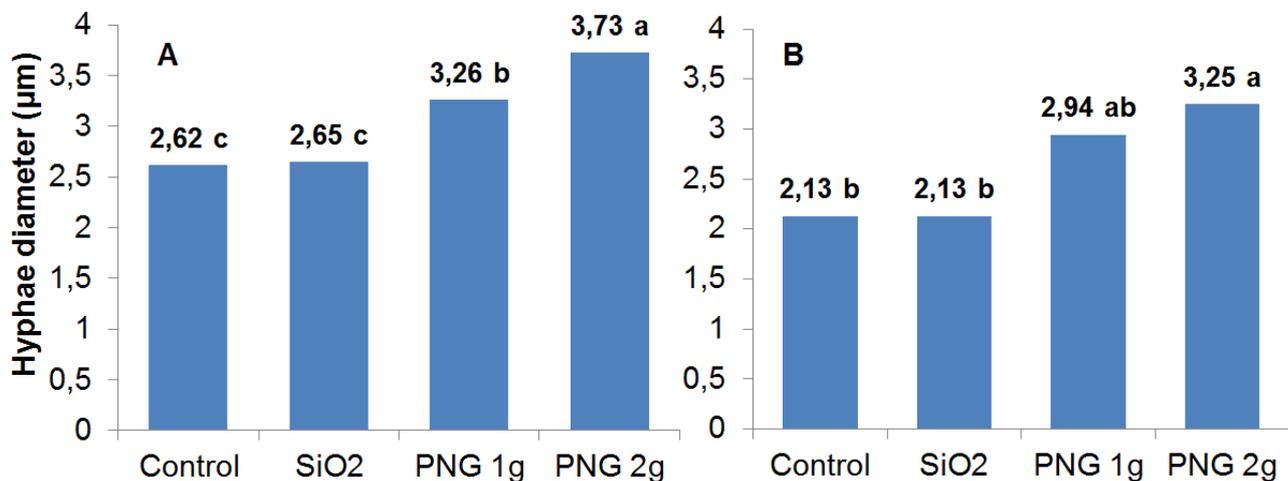


Figure 4: Average hyphae diameter (µm) of ectomycorrhizal fungi (A) *Pisolithus microcarpus* and (B) *Suillus* sp. in the different treatments. *Means followed by the same letter do not differ by the Tukey test at 5% probability.

According to Pintom et al. (2001) and Ahmad et al. (2019), microbial stimulation is related to the addition of carbon chains and amino compounds, sugars and nutritional complexes, which increase biological cycles in ecosystems in most cases. According to technical information, the Penergetic product is manufactured using the bioprogramming process, where extremely low electromagnetic fields (EMF) are generated to create energy potentials (Prade 2009). Among these is the oxidation-reduction potential, stimulating various biological processes in plants, animals, soils and water. The consequence is the ability of microbial activation and regulation of biological processes, resulting in improved quality of the agricultural environment and, consequently, of crops.

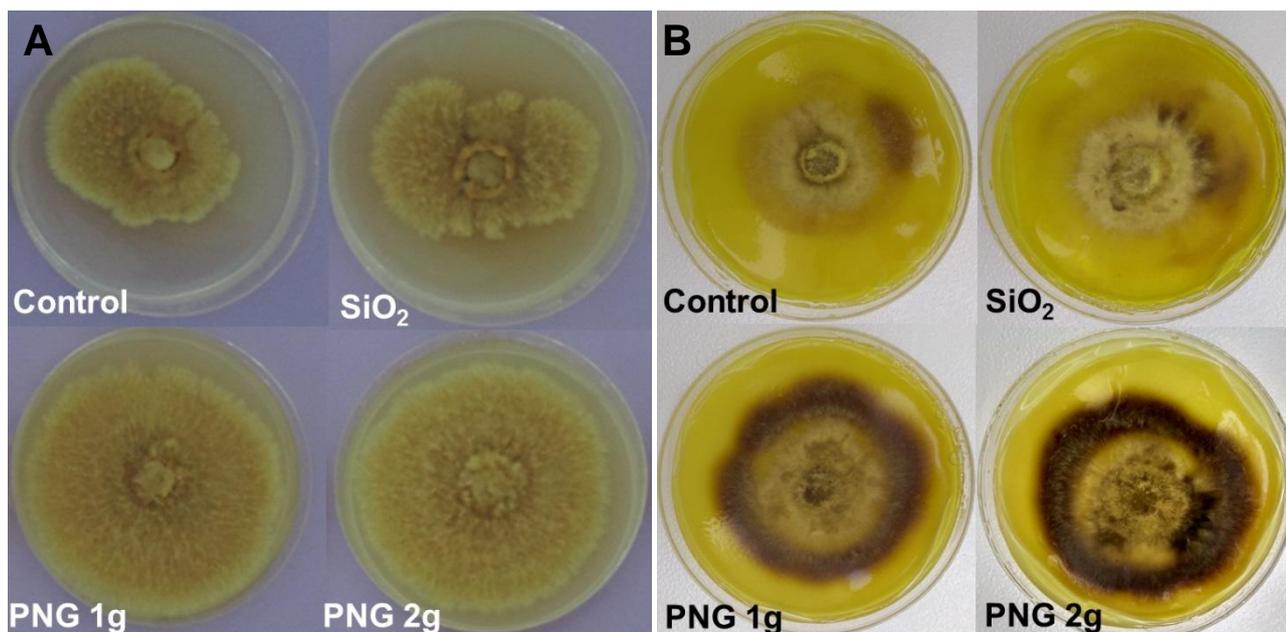


Figure 5: Mycelial growth of *Pisolithus microcarpus* (A) and *Suillus* sp. (B) in the treatments evaluated after 120 hours of incubation.

For some years agricultural scientific research has been studying energy flows in productive environments. More intensely from 2015, groups of physics scientists have been developing studies with soil microbiologists, using concepts and theories of quantum physics. Based on the knowledge that each atom, each molecule, each compound and each substance has its own energy measured by electromagnetic frequencies, science is demonstrating the effects of using these energies in different biological cycles.

Evaluating the development of plant tissue in tissue culture with the presence of so-called “biofields” or regulated electromagnetic energy, Nayak et al. (2018) observed an increase in plant development in the order of 68% when using different frequencies of biofields. Similar results were also observed by Mihaela et al. (2006), Hozayn and Qados (2010), Rashmi et al. (2014), Nayak and Altekar (2015) and Nyakane et al. (2019).

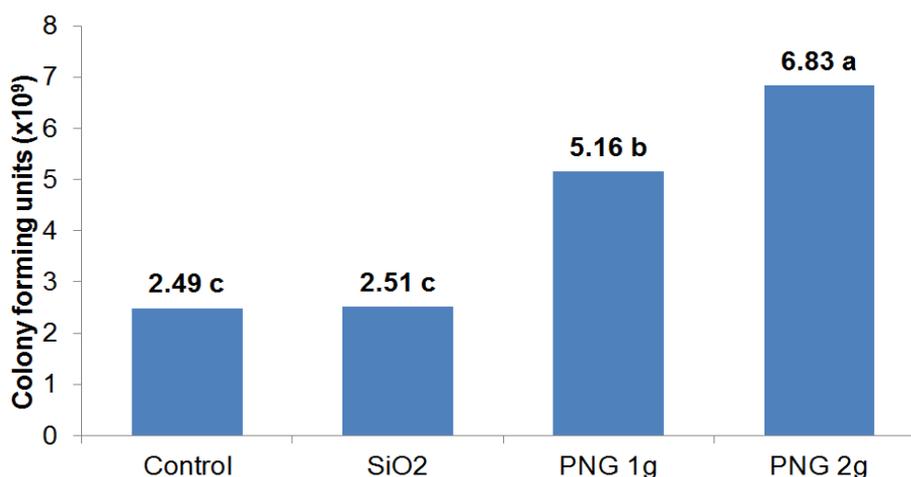


Figure 6: Colony Forming Units (CFU) of the *Bradyrhizobium japonicum* per milliliter of suspension. *Means followed by the same letter do not differ by the Tukey test at 5% probability.

Increases in microbial growth have also been reported, demonstrating effects on acceleration of substrate consumption kinetics, increased resistance to soil pollutants, microbial biomass, metabolic activity or specific enzymes, increased metabolic activity or respiration rate, variation ATP concentration, alterations in cellular proteome, alterations in DNA / RNA synthesis and related activities, variations in transposition and production of secondary metabolites, increased cell hydrophobicity and adhesion between bacterial cells (Alvarez et al. 2006; Cellini et al. 2008; Aslanimehr et al. 2013; Fijalkowski et al. 2013; Ahmed et al. 2015; Beretta et al. 2019). According to Bereta et al. (2019), although evaluations regarding the effects of the use of different electromagnetic scales on microbial activity are scarce, the known results support the possibility of using this technology on a large

scale. From the above, it is observed that studies on edaphic microbiota stimulation and its consequent response in plant productivity are relevant and fundamental for understanding the biogeochemical processes involved in plant cultivation. Maintaining soil biological fertility through sustainable technological tools represents a viable path because it consists of a solid and efficient management alternative capable of broadening the stimulus to biological development in the soil and, consequently, to sustainable agricultural productivity.

4. Conclusions

The use of low frequency electromagnetic fields (Penergetic technology) has a direct stimulating effect on the microbial development of *Trichoderma asperelloides*, *Pisolithus microcarpus*, *Suillus* sp. and *Bradyrhizobium japonicum*.

The dosage of 2 grams per liter of culture medium provides higher growth increase in microorganisms when compared to the dosage of 1 gram per liter.

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