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Producción de fertilizante orgánico líquido utilizando levadura (*Saccharomyces cerevisiae*) residual del proceso de elaboración de cerveza

Production of liquid organic fertilizer using residual yeast (Saccharomyces cerevisiae) from the brewing process

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RESUMEN

El objetivo del estudio fue revalorar la levadura (*Saccharomyces cerevisiae*), del proceso de elaboración de cerveza, para obtener fertilizante orgánico líquido. El experimento se realizó en dos etapas: en campo, la recolección de materia prima y en el laboratorio, la determinación del porcentaje de humedad y los análisis microbiológicos, fisicoquímicos y agronómicos. Se utilizó un diseño completo al azar (DCA) con arreglo factorial de 4x4 con tres repeticiones. La caracterización de la levadura fue pH: 6,52, CE: 3,60 dS/m, HR relativa: 84,94% y materia orgánica en solución: 66,64%. Mientras que, la caracterización del fertilizante orgánico líquido estuvo en un pH: 3,96, CE: 21,40 dS/m, sólidos totales: 183,30 g/L y materia orgánica en solución: 146,40 g/L. La fermentación láctica realizada por las bacterias ácido-lácticas del consorcio microbiano (B-lac), es una opción para la producción de fertilizante orgánico líquido utilizando levadura residual del proceso de elaboración de cerveza. Mediante la caracterización de levadura, se demostró que la concentración de materia orgánica, el contenido de macronutrientes y micronutrientes de la levadura residual es alto en determinados elementos, tales como; nitrógeno (9681 mg/L), fósforo (2203,70 mg/L) y potasio (3850 mg/L); resultando ser un producto de calidad agronómica.

Palabras clave: levadura; abono; caracterización; fermentación láctica; orgánico.

ABSTRACT

The goal of the research was to re-evaluate the yeast (*Saccharomyces cerevisiae*) from the brewing process to obtain liquid organic fertilizer. The experiment was conducted in two stages: in the field by collecting raw material; and, in the laboratory by determining the moisture percentage and the microbiological, physicochemical, and agronomic analyses. A complete randomized design (CRD) with a 4x4 factorial arrangement with three replications was performed. The yeast characterization was pH: 6.52, EC: 3.60 dS/m; relative RH: 84.94%; and, organic matter in solution: 66.64%; while, the liquid organic fertilizer characterization was pH: 3.96; EC: 21.40 dS/m; total solids: 183.30 g/L; and, organic matter in solution: 146.40 g/L. Lactic acid fermentation carried out by lactic acid bacteria of the microbial consortium (B-lac) is an option for producing liquid organic fertilizer using residual yeast from the brewing process. Through yeast characterization, it was shown that the concentration of organic matter, macronutrient and micronutrient content of the residual yeast is high in certain elements, such as nitrogen (9681 mg/L), phosphorus (2203.70 mg/L), and potassium (3850 mg/L), resulting in a product of agronomic quality.

Keywords: yeast; fertilizer; characterization; lactic acid fermentation; organic.

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INTRODUCTION

Generating waste during the production process in the brewing industry is a loss of the process, and if one adds the poor use of raw materials, the cost of the production process increases (IICA, 1999). However, non-agricultural digestates, produced from municipal and industrial waste, such as household organic waste, food processing waste, and green waste from gardens and public green areas, have received less attention in the literature (Efosa et al., 2023). On the other hand, there are numerous reports on the production of LOF (liquid organic fertilizer) from food waste and industrial water and manure. LOF is known to have been successfully implemented to grow leaf lettuce (*Lactuca sativa*) (Maya et al., 2023). In addition, waste generation has an economic impact associated with the costs of waste treatment and disposal.

Every process generates waste *per se*. However, an environmentally friendly management must be followed by implementing a kind of treatment.

Currently, non-polluting technologies are being developed as a tool to prevent pollution and help develop productive activity in an economic, sustainable, social, and environmental manner (OAS, 2006). For example, compost is the residual result of the biological activity of the soil that is beneficial for improving the physical, chemical, and biological properties of the soil. These improvements play a very important role in supporting crop growth and production (Yassi et al., 2023). Food waste refers to biodegradable organic waste derived from different sectors, such as non-tradable agricultural products, by-products from the food processing industry, hospitality, and households. Pre-cooked and leftover foods may also produce biodegradable waste. The problem of food waste has become a global concern and is recommended to emerge as a priority on political agendas globally. The Food and Agriculture Organization of the United Nations (FAO) has reported that approximately one-third of the food produced in the world ends up as food waste (Siddiqui et al., 2023). On the other hand, one of the main aspects of modern agriculture is optimizing plant nutrition and minimizing the negative impact of mineral fertilization on the environment. Organic fertilizers and biostimulants are being established as an alternative to reduce the use of mineral fertilization and receive ecologically clean forage production (Zhelyazkova et al., 2023).

It is an environmental management option to avoid alternatives that are not compatible with environmental conservation. Non-polluting technologies are aimed at both reducing and avoiding pollution by modifying processes and/or products (Arroyave & Garcés, 2006). Incorporating changes in production processes can generate a series of economic benefits for companies, such as more efficient use of resources, and reducing collection, transportation, treatment, and final disposal costs (OAS, 2006).

Consequently, clean technologies, together with other pollution prevention tools, constitute a set of concrete actions that allow for developing productive activities in an economically, socially, and environmentally sustainable manner. There are still several barriers to promoting and adopting these technologies, ranging from problems in communicating and disseminating the beneficial results of their application; resistance to change; training human resources; and access to funding to implement technological changes (Social Advisory Commission, 2004).

Several studies have been conducted on liquid organic fertilizer (Florez et al., 2020) from yeasts inoculated with effective microorganisms, efficient microorganisms (Alarcón et al., 2019; Alarcón et al., 2020), lactic acid microbial consortia (Quispe et al., 2019), among others. Fertilizers are composed of a wide variety of beneficial and non-pathogenic microorganisms produced through natural processes (EM Technology, 2012). In this way, pollution generated by the brewing industry is reduced and added value is provided. Additionally, organic fertilizers are a relevant alternative that covers the requirements that the soil needs, such as recovering some of its properties, including moisture retention, activating soil microorganisms; and improving nutrient retention capacity. Organic fertilizers are affordable and easy to produce, because when producing them, they use waste or residues and unsophisticated materials as inputs. However, it requires technical criteria to obtain results of better quality and economic efficiency.

The objective of this research was to evaluate the residual yeast from the brewing process by obtaining organic fertilizer; to characterize the residual yeast; to determine the operating parameters of the system, such as pH, electrical conductivity, and acidity for producing organic fertilizer; to characterize the organic fertilizer, and, to evaluate its efficiency in lettuce seeds.

METHODOLOGY

Raw material

The yeast was obtained from a fermentation tank of the Bebidas Naturales S.A.C. company. The sample, which consisted of 65 kg of yeast, was refrigerated at the facilities of the Food Pilot Plant of the Faculty of Food Industries of the Universidad Nacional Agraria La Molina (UNALM).

Analysis

Microbiological and physicochemical analyses were performed at the Microbial Ecology and Marine Biotechnology Laboratories – Tabusso and the Soil, Plant, Water and Fertilizer Analysis Laboratory – Universidad Nacional Agraria La Molina (UNALM), respectively.

Experimental treatments

Figure 1 shows the flow diagram that the research followed. Sixteen treatments were carried out (Table 1). The concentrations of both the molasses factor and the effective microorganisms were 0, 10, 15, and 20 %.

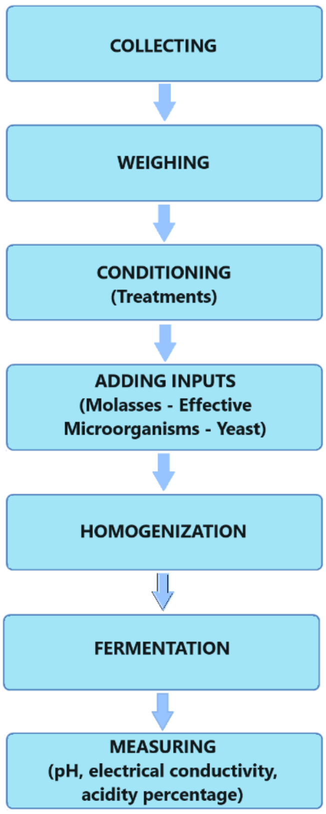

Figure 1. Experiment research flowchart.

Table 1
Treatments versus Molasses Effective Microorganisms, and Yeasts Percentage

Treatments	Percentage		
	Molasses	E.M.	Yeast
T1	0	0	100
T2	10	0	90
T3	15	0	85
T4	20	0	80
T5	0	10	90
T6	10	10	80
T7	15	10	75
T8	20	10	70
T9	0	15	85
T10	10	15	75
T11	15	15	70
T12	20	15	65
T13	0	20	80
T14	10	20	70
T15	15	20	65
T16	20	20	60

E.M. = Effective Microorganisms.

Evaluating the efficiency of organic fertilizer on lettuce seeds

The best treatment (T16) was used in the bioassay to evaluate the effect of organic fertilizer on lettuce seeds, according to the evaluation flowchart shown in Figure 2. For this purpose, we considered to use a plant with the following characteristics: sensitive to salt concentration and rapid germination (Peralta, 2010).

Evaluating the stability of organic fertilizers

Stability over time was evaluated by measuring pH, electrical conductivity, and titratable acidity percentage (Table 2) every ten days for thirty calendar days (Peralta et al., 2016).

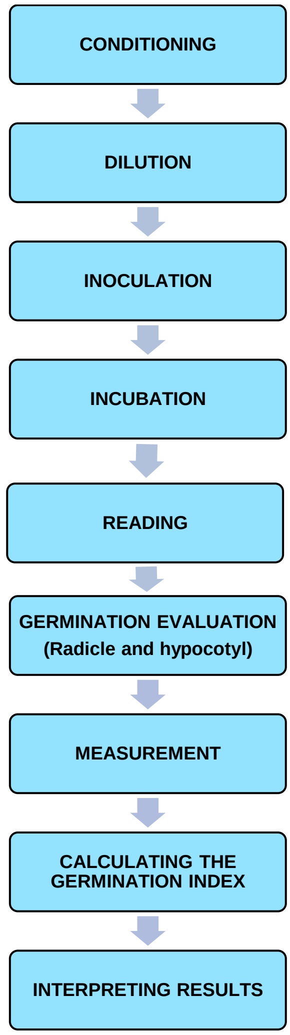

Figure 2. Flowchart: Evaluating Organic Fertilizer Efficiency in Lettuce Seeds.

Methods of analysis

pH measuring

The pH of the organic fertilizer was measured using a pH-meter. This consists of a pair of electrodes, capable of measuring small voltages, which were calibrated before measurements were made. The intermittent value of the meter was considered as a valid reading (Peralta, 2016).

Electrical conductivity

The electrical conductivity was determined using a conductivity meter. To do so, an electrode was introduced into the liquid solution of the organic fertilizer, after calibrating the equipment with the 1.433 dS / m saline buffer (Peralta, 2010).

Table 2
Methods used in the organic matter analysis

Parameters	Method
pH	Potentiometric
Acidity	Volumetry
Electrical Conductivity	Conductimetry
Organic matter in solution	Potassium dichromate or walkley and black and/or to ashify or to combust
Total Solids	Gravimetry, weight difference
Macroelements	
Nitrogen (N)	Kjeldahl
Phosphorus (P)	Molybdate vanadate yellowing
Potassium (K), Calcium (Ca), Magnesium (Mg) y Sodium (Na)	Atomic Absorption Spectrophotometry
Microelements	
Iron (Fe) Zinc (Zn) Manganese (Mn)	Atomic Absorption Spectrophotometry
Boron (B)	Colorimetry Curcumin in glacial acetic acid
Copper (Cu)	Atomic Absorption Spectrophotometry
Heavy Metals	
Cadmium (Cd), Chromium (Cr), Lead (Pb)	Atomic Absorption Spectrophotometry

Source: The Soil, Plant, Water, and Fertilizer Analysis Laboratory (LASPAF) – UNALM (2012).

Measuring the acidity percentage
The titratable acidity was determined using an assemblage, which consisted of a burette filled with NaOH, a beaker precipitated with organic fertilizer solution, and a pH meter.
10 grams of the sample were diluted in 50 ml of distilled water, then titrated with 0.1 N sodium hydroxide. The percentage of lactic acid in the samples was calculated using the following formula (Peralta, 2010):

$$\% \text{ titratable lactic acid} = \frac{G \times N \times 0.090}{m} \times 100$$

Where G: NaOH expenditure (ml); N: NaOH normality; m: sample mass (g); 0.090 Conversion factor.

Microbiological analysis
Microbiological analysis of the yeast sample and of T16 was carried out at the Tabusso Marine Microbial Ecology Laboratory, following the methodology of the International Commission on Microbiological Specifications for Foods (1983). The analysis included a list of total coliforms, a list of fecal coliforms, and a list of *Escherichia coli*.

Physicochemical analysis
The physicochemical analysis of the yeast sample and T16 was carried out at the Soil, Plant, Water, and Fertilizer Analysis Laboratory of the Faculty of Agronomy of the UNALM. The pH, electrical conductivity, macroelements (nitrogen, phosphorus, potassium, calcium, magnesium, and sodium), microelements (iron, zinc, manganese, boron, and copper) and heavy metals (cadmium, chromium, and lead) were determined.

Agronomic analysis
The analysis of the nutritional composition of T16 was carried out at the Soil, Plant, Water, and Fertilizer Analysis Laboratory of the Faculty of Agronomy of the UNALM, Lima, Peru.

Experimental design and statistical analysis liquid organic fertilizer
The statistical design used in the comparison of the data on pH, electrical conductivity, and titratable acidity percentage of the treatments according to the doses of effective microorganisms and molasses in the production of organic fertilizer was by means of a variance analysis ($p < 0.05$) in a complete randomized design (CRD) with 4x4 factorial arrangement with three replications (2 factors: molasses and microorganisms, with 4 levels each: 0%, 10%, 15% and 20%). Significant differences among treatments were determined and the Tukey grouping test was applied.

Seed germination
Comparison of the germination percentage of five dosages of the treatment compared to the blank control was performed by analysis of variance ($p < 0.05$) in a completely randomized design (CRD) with three replications. Statistical analysis was performed using *Statistical Analysis Software* (SAS), version 8.2.

RESULTS AND DISCUSSION

Raw material characteristics
Table 3 shows the results of the physicochemical analysis of the sample of unpasteurized yeast and pasteurized yeast.
Nagib et al. (2022) mention that nitrogen is an essential nutrient of plants and the main element for their intensive production. Table 3 shows the percentage of nitrogen in organic fertilizer (8.38%). The performance of this element in the division, synthesis, protoplasms, enzymes and structured mixtures such as nucleoproteins, amino acids and chlorophyll and mixtures are considerable (Abdulkhaliq, 2019).

Given the supply of available nutrients and growth-promoting substances, crop yields are maximized with the use of biofertilizers, and agricultural costs are lowered (Nagib et al., 2022). In addition to balancing the disadvantages of conventional chemical-based technology, they allow for recovering healthy agricultural practices and bioagriculture (Nagib et al., 2022).
The effect of biofertilizers is important, due to the different groups of strains that compose it, and in the same way it favors the availability of nutrients in the available forms and increases the levels of minerals in the soil (Shoeip et al., 2022).

Table 3
Physicochemical analysis of the unpasteurized yeast and pasteurized yeast sample

Physicochemical Analysis	UY Value	UY Unit	PY Value	PY Unit
pH	6.52	-	4.38	-
Electrical conductivity	3.60	dS/m	7.77	dS/m
Organic material	66.64	%	-	-
Nitrogen (N)	8.38	%	-	-
Humidity (Hd)	84.94	%	-	-
Sodium (Na)	0.04	%	-	-
Hierro (Fe)	146	ppm	13.95	mg/L
Copper (Cu)	10	ppm	1.55	mg/L
Zinc (Zn)	215	ppm	18.28	mg/L
Manganese (Mn)	11	ppm	1.65	mg/L
Boron (B)	27	ppm	0.49	mg/L
Lead (Pb)	0.53	ppm	0.41	mg/L
Cadmium (Cd)	0.00	ppm	0.33	mg/L
Chromium (Cr)	2.83	ppm	0.21	mg/L

UY= Unpasteurized yeast; PY= Pasteurized yeast.

The supply of nitrogen, phosphorus, potassium, and other plant nutrients is positively affected by the activation of bacterial strains in soil microbiological processes. For this reason, the growth of microorganisms in the soil is favored by biofertilizers, as well as an increase in organic and inorganic matter, producing organic matter when the material dies, and this serves as a food source for living microbes (Shoeip et al., 2022).
The development and productivity of crops is enriched by yeast, which is a natural biostimulant and biofertilizer (Shoeip et al., 2022). *Saccharomyces cerevisiae* is a promoter of plant development (Wenkai et al., 2021), it is also a biofertilizer for improving quality and yield, in addition to being safe for the environment and human health (Wenkai et al., 2021).
Finally, *S. cerevisiae* as a substitute for organic fertilizers is safer for humans, animals, and the environment (Omer et al., 2023). Yeast is a natural growth enhancer, due to its high density of proteins, carbohydrates, nucleic acids, lipids, vitamins, and other minerals (Omer et al., 2023), and improves the ability of plant roots to absorb phosphorus and manganese (Omer et al., 2023). Recently, there has been an opportunity to use yeast as a stimulant of plant development and a biological control agent for soil-borne diseases to plants (Omer et al., 2023).

Organic fertilizer characteristics

In general, the value of biol as a fertilizer is high (Table 4), because it contains available nutrients. It also plays an important role as a source of nutrients (especially nitrogen) for crop production, as they are more available compared to manure, so it has a greater fertilization effect in the short term (Diaz, 2017).
Table 4 shows the results of the chemical analysis of agronomic interest of organic fertilizer for evaluating the potential of this product as a liquid organic fertilizer.
There is variability in the content of macronutrients and micronutrients. According to Diaz (2017) the diversity in biols makes it difficult to establish standards for macronutrient and micronutrient content, as well as growth precursor content for the final product. It will only be possible to know the biol composition at the end of the process and analyze it. In organic farming versatility this is not a disadvantage but an opportunity, because producers during trial-and-error tests are permanently adapting their formulations, methods, and applying rates to their crops, without the need to know in detail the composition of crops (Diaz, 2017).

Table 4
Chemical analysis of agronomic interest of organic fertilizer

Parameters	Organic fertilizer					
	UY		PY			
			40 °C		Ambient T°	
	Value	Unit	Value	Unit	Value	Unit
pH	3,96	-	4,15	-	4,01	-
Electrical conductivity	21,40	dS/m	9,88	dS/m	9,95	dS/m
Total solids	183,30	g/L	161,98	g/L	158,64	g/L
Organic matter in solution	146,40	g/L	148,18	g/L	144,82	g/L
Macronutrients (Total)						
Nitrogen (N)	8974,00	mg/L	11970.00	mg/L	11200.00	mg/L
Phosphorus (P)	1619,05	mg/L	1914.81	mg/L	1914.81	mg/L
Potassium (K)	9625,00	mg/L	4200.00	mg/L	4075	mg/L
Calcium (Ca)	1472,00	mg/L	334.30	mg/L	343,80	mg/L
Magnesium (Mg)	1275,00	mg/L	355,00	mg/L	347,50	mg/L
Sodium (Na)	232,50	mg/L	300,00	mg/L	310,00	mg/L
Micronutrients (Total)						
Iron (Fe)	41,40	mg/L	18,38	mg/L	17,43	mg/L
Copper (Cu)	0,95	mg/L	1,28	mg/L	1,23	mg/L
Zinc (Zn)	13,33	mg/L	11,83	mg/L	18,85	mg/L
Manganese (Mn)	4,00	mg/L	1,50	mg/L	1,47	mg/L
Boron (B)	7,07	mg/L	1,73	mg/L	1,41	mg/L
Heavy metals (Total)						
Lead (Pb)	0,01	mg/L	0,403	mg/L	0,593	mg/L
Cadmium (CD)	1,39	mg/L	0,000	mg/L	0,000	mg/L
Chromium (Cr)	0,01	mg/L	0,440	mg/L	0,498	mg/L

UY = Unpasteurized yeast. PY = Pasteurized yeast. Ambient T° = Ambient temperature.

Diaz (2017), interested in biols containing a high content of nitrogen and phosphorus for their application, consider these two elements to be fundamental for the processing process. These nutrients, like all nutrients, will be available to bacteria only in their soluble form (Diaz, 2017). According to Table 4, the highest amount of these minerals is found in pasteurized yeast at 40 °C.

Diaz (2017) points out that studies conducted on biol as a fertilizer show a wide range of parameters and that the content in biols varies widely, depending on many variables such as: the type of manure used (from pigs, cows, or chickens); other residues; animal fodder (quality and quantity); climate (particularly temperature); and biodigester technology as such.

Diaz (2017) considers that each biol is made with different inputs and proportions and produced under different environmental conditions. Each of them has unique and different characteristics. Biol is the result of a complex and dynamic process of organic matter decomposition, where inputs, preparation, environmental conditions, and time determine unique characteristics for each final product. Which indicates that formulating yeast-based organic fertilizer is unique and will depend only on the characteristics mentioned by Diaz (2017).

Plants need several chemical elements. Their growth is affected by deficiency of any of these for normal development (Rou et al., 2022).

Nitrogen is a building block of numerous organic compounds, including amino acids, proteins, nucleic acids, enzymes, and chlorophyll. It is also essential for producing essential structural, genetic, and metabolic compounds in plant cells. It results in stunted plants and yellow leaves due to nitrogen insufficiency. Older leaves are the first to show symptoms of this deficiency, due to the movement of nitrogen in the plant (Rou et al., 2022).

Yeast is a favorable candidate for biofertilizers, due to the stability of its genome, yet it passes over bacteria and fungi because it is easier to grow than the latter (Rou et al., 2022). Sustainable agriculture has also been favoured by biofertilisers, due to their interactions with biotic and abiotic components of the rhizosphere (Rou et al., 2022).

Evaluating the effect of organic fertilizer on lettuce seed germination

The toxicity bioassay was recommended and applied by different environmental protection agencies for ecotoxicologically evaluating environmental samples and pure compounds (Sobrero & Ronco, 2004). Similarly, with the lettuce seed toxicity bioassay, the phytotoxic effects of pure compounds or complex mixtures on the seed germination process and seedling development during the first days can be evaluated (Sobrero & Ronco, 2004).

Due to the growing demand for food and to maximize agricultural production, fertilizers emerge. Although higher yields are now available, human health and the environment are affected, due to its dangerous effects (Abdel & Youssef, 2019).

Plant growth is due to increased nutrient availability; that biofertilizers, natural materials, and the use of organic matter provide. In addition to mobilizing nitrogen and phosphorus, biofertilizers produce naturally yielding quality crops, which are effective, low-cost sources of nutrients for plants (Abdel & Youssef, 2019). Its excessive use is to achieve high productivity in crops, without considering the negative environmental impact (Mahmoud et al., 2021) such as causing some ecological problems and harming the environment.

It is not only about the quality of the soil, but also about the survival of the organisms in the soil, which the impact of chemical fertilizers has on agricultural land (Alami et al., 2018). Although, chemical fertilizers cause negative impacts, these are counteracted by the application of biofertilizers (Alami et al., 2018).

Table 5 shows the germination rate expressed as a percentage. For example, for the 100 and 10^{-1} solutions it is 0% for both samples, unpasteurized yeast, and pasteurized yeast.

In the unpasteurized yeast sample, solution 10^{-2} presented a germination index of 33.12%, which means that phytotoxic substances are strongly present. In addition, solution 10^{-3} and 10^{-4}, have a germination index of 57.07% and 78.13% respectively, which means that phytotoxic substances are moderately present. As for the pasteurized yeast samples, the germination rate in 10^{-2}, 10^{-3}, and 10^{-4} dilutions, in both conditions (condition 1: 40 °C; and condition 2: ambient temperature), are 84.2%, 96.3% and 97.8%, respectively. This means that there are no toxic substances, or they are in very low concentration. Similarly, the control also presented germinated seeds. According to Buchelli (2014), research was conducted where the germination index (GI) is evaluated in liquid biofertilizers manufactured by homolactic fermentation (Table 5). At the concentration of 0.1% or less, conditions conducive to lettuce seedling growth are present.

Table 5

Comparison of germination rates of organic fertilizer versus other studies

Concentration	Germination Rates of Liquid Biofertilizers						
	Organic fertilizer			Fast Biol 20(a)	Rocoto biofertilizer (b)	Biol II G(c)	Cuyinaza Biofertilizer (d)
	UY 40 °C	PY Ambient T°	PY				
0,01/100 (10^{-4})	78,13	97,8	97,8	---	---	94,7	99,5
0,1/100 (10^{-3})	57,7	96,3	96,3	84,5	81,9	85,8	98,9
1/100 (10^{-2})	33,12	84,2	84,2	65,8	67,9	67,1	54,0
10/100 (10^{-1})	0	0	0	0,41	0	0	0
100/100 (10^{0})	0	0	0	0	0	0	0

UP = Unpasteurized yeast; PY = Pasteurized yeast.

(a) Fast Biol 20 of cattle manure, Peralta (2010). (b) Biofertilizer from rocoto residues, Ricse (2013).

(c) Biol II-G of sheep manure, Medina (2013). (d) Cuyinaza biofertilizer, Román (2012).

Table 5 shows the germination index values, whose results are like those of another liquid organic biofertilizers, which shows that the experiment has been carried out correctly regarding the germination rate. Even at a concentration of 1% (10^{-2}), values higher than 80% were obtained with the organic fertilizer based on pasteurized yeast in its two conditions (condition 1: 40 °C; and condition 2: ambient temperature).

CONCLUSIONS

The organic matter concentration, macronutrient and micronutrient content of the residual yeast was found to be high in nitrogen (9681 mg/L), phosphorus (2203.70 mg/L), and potassium (3850 mg/L).

The T16 treatment obtained the best results with pH 4.65 and 4.89. Its qualitative characteristics were optimal and did not require pasteurization.

The concentration of organic matter, macronutrients and micronutrients content of the liquid organic fertilizer is high in the following elements: nitrogen (11200.00 mg/L), phosphorus. (1914.81 mg/L), and potassium (4075 mg/L). This is a high-quality product for agriculture.

There are other types of raw materials that could be used in producing organic fertilizer, such as: coffee pulp, cachaza and by-products of the sugar manufacturing process, residues generated by the rejection of bananas and rachis, which have a high potassium content because in addition to having a high nitrogen content, they contain a good number of sugars, water, carbon sources, and adequate particle sizes. One can also experiment with organic wastes and animal excrements.

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