

Microbial Load and Innocuousness of the Extract of Plants Obtained from Banana Rachis Leachate Produced in Cuba

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ABSTRACT

The objective of this research was to determine the microbiological composition and innocuousness of banana leachate, obtained at the Research Institute of Tropical Roots and Tuber Crops (INIVIT) in Santo Domingo, Villa Clara, Cuba. The determination of the microbial load and innocuousness of the leachate analyzed was carried out through established procedures at the Microbiology Laboratory of the Center for Genetic Engineering and Biotechnology (CIGB). The results obtained in the research are the scientific basis for encouraging the use of banana rachis leachate, as a high value-added product. The microbial load determined in this study 4.4×10^6 CFU/mL from bacteria of the genus *Bacillus* sp. and its innocuousness as it is free of pathogenic microorganisms, such as *Escherichia coli* and *Salmonella* sp., were findings of great importance. The research helps to encourage the use of a crop residue, underused in the production process of plantains and bananas in Cuba.

Keywords: Leachates; Musaceae; Residues; Microbial Composition

Short Communication

In the production of plantains and bananas, as in most of agricultural production systems, large amounts of residues or waste are generated, such as reject fruits, pseudo-stems, leaf litter and rachis, mainly. In this regard, investigations carried out in Cuba, considering an average value into the percentage of rachis weight (11%) and its representativeness at the annual level production of plantains and bananas in the country (675,405.1 t), can mean values of 74,294.6 t of cluster rachis. These data reaffirm that the production of plantains and bananas in Cuba, causes a considerable amount of organic waste, which are not properly used and they could be reused [1]. The Food and Agriculture Organization of the United Nations (FAO) promotes experiences in Latin America and the Caribbean, focused at reducing food loss and waste, and the use of by-products or residues, which contribute to the improvement of environmental health conditions in the context of integrated

and efficient productive systems [2]. Leachates from edible musaceae (plantains and bananas) are plant extracts, obtained by decomposition of harvest residues [3,4]. The rachis leachate (flower peduncle, also known as pinzote or stem, has a helical shape and it is the responsible for the support of clusters), as it is a product of the same plant, it has essential nutrients (N, K, Fe, Mn, Na, Cu) that can be reused for the crop itself [5-7]. These plant extracts applied to banana seedlings under nursery conditions, have proven to be effective in the growth of them, with a significant increase in the variables (height, number of leaves, as well as the total length and fresh and dry weight of the roots) [8,9]. Besides the nutritional value of this extract of plants, its potential as a pest and pathogen controller has been studied. This is attributed to the large number of microorganisms present in them [10,11]. Special interest has aroused the use of leachates to reduce the infective process of the

most destructive diseases that threaten the production of plantains and bananas worldwide: *Mycosphaerella fijiensis* Morelet (black Sigatoka) and *Ralstonia solanacearum* Smith (moko) [4,12-15].

Other existing studies support the rachis use for other purposes, such as: ingredient for the production of flours, paper, ferments, livestock fodder, and even as a seed germination mechanism [16-18]. At the Research Institute of Tropical Roots and Tuber Crops (INIVIT), a constant production volume of 200 L of extract of plants was obtained by the rachis decomposition of Musa AAA, subgroup Cavendish, cultivar 'Gran enano', from 700 kg of rachis, for an efficiency of 30 % by the end of 2019 and beginning of 2020 [1]. The authors propose the rachis leachate as a way to take advantage of an invaluable residue in the current economic situation of the Cuban agriculture. The INIVIT devotes efforts through its research-development programs and collaboration with scientific centers, to the study of rachis leachate production processes, its components, nutritional effect and disease managements. Nowadays, investigations aimed at the chemical characterization of rachis leachates of plantains and bananas, have received more attention than the studies dedicated to the detection of microorganisms or bioload present in them [8,9,13]. This research carried out in collaboration with the Center for Genetic Engineering and Biotechnology (CIGB) aims to determine the microbiological composition and innocuousness of banana leachate, obtained at the INIVIT, to understand the action mechanism as a biological controller, improve the production efficiency and encourage its use in organic and sustainable production systems.

Materials and Methods

The determination of the microbial load and innocuousness of the extract of plants, obtained from the banana rachis leachate (Musa AAA, subgroup Cavendish, cultivar 'Gran enano') was carried out at the Microbiology Laboratory, which belongs to the Center for Genetic Engineering and Biotechnology (CIGB) in Havana, Cuba, in March 2020. The banana rachis leachate analyzed in the research was obtained at the Research Institute of Tropical Roots and Tuber Crops (INIVIT). The sample for the study corresponds to a new leachate (phase less than one production year), taken from a plastic recipient that contained the leachate kept, under curing conditions, for more than 30 days. The rachis used to obtain the leachate were collected at the processing and packaging factory of the Agricultural Enterprise Quemado de Güines, located in Villa Clara province. The sample received at the Microbiology Laboratory of the CIGB was kept in refrigeration at a temperature of 2-8°C until its processing in March 2020. The sample analysis was performed following the procedure 4.09.290.91. (Microbiological analyses of non-sterile products).

Procedure Performed for Bioload Determination

Ten mL of the sample were taken and added in 90 mL of Peptone Salt Solution. From this first dilution, serial dilutions were made up

to 10⁻⁵. Later, 1 mL was filtered in duplicate of the dilutions 10⁻³, 10⁻⁴ and 10⁻⁵. The membranes were placed in plates, containing Triptone Soy Agar and incubated from 30-35°C, for a period of 72 hours.

Procedure Performed for the Determination of *Escherichia Coli*

Ten mL of the first dilution described above were taken and transferred to 100 mL of Triptone Soy Broth and incubated for 24 hours, at temperatures from 30-35°C. After this period, 1 mL was inoculated into 100 mL of Mac Conkey Broth and incubated at 42°C for 24 hours. Then, it was moved to Mac Conkey Agar plates and incubated from 30-35°C for 48 hours.

Procedure Performed for the Determination of *Salmonella* sp.

It was taken 0.1 mL of the previous enrichment in Triptone Soy Broth and transferred to a tube containing 10 mL of Rappaport Vassiliadis Broth. It was incubated from 30-35°C for 24 hours and moved to Xylose Lysine Deoxycholate (XLD) Agar plates, incubating from 30-35°C for 24 hours.

Results and Discussion

After the incubation period of each one of the determinations, it was obtained as a relative result to the Bioload, that in the dilution 10⁻⁵ grew 41 and 46 Colony Forming Units (CFU), respectively on each replica plate, so the concentration in the analyzed leachate was 4.4 x 10⁶ CFU/mL. The contaminants that predominated were mainly sporulated gram-positive bacilli, identified as *Bacillus* sp. Among the characteristics of the genus *Bacillus*, its aerobic or sometimes facultative anaerobic growth, Gram-positive, bacilar morphology, flagellar mobility and variable size (0.5 to 10 µm) stand out. Its optimal growth occurs at neutral pH, presenting a wide range of growth temperatures, although most species are mesophilic (temperature between 30 and 45°C). Investigations carried out to determine the microbiological composition and innocuousness of banana leachates, conducted by the National Institute of Agricultural Research (INIA) and the National Center for Agricultural Research (INIA-CENIAP) in Venezuela, indicate the bacteria identification of the genus *Bacillus*, as the microorganisms involved in the decomposition of fresh matter [19]. The chemical and microbiological characterization of an organic fertilizer, based on the use of residues from the harvest of bananas, showed that the greatest presence of microorganisms found were bacteria, although the genus was not identified [20]. The finding in this research is very important, due to the role of bacteria of the genus *Bacillus*, associated with the promotion of plant growth and control of pathogens [21-25]. A relevant aspect that distinguishes the bacteria of this genus is the ability to produce endospores, as a resistance mechanism to various types of stress [24,26,27].

The role of bacteria of the genus *Bacillus* as producers of growth phytohormones, like cytokinins and gibberellins [28] could help to

interpret the effect of these extracts on the growth of plantain and banana vitroplants, reported by several authors. This reaffirms the importance of studying the composition of banana rachis leachates that determines its effectiveness in the control of diseases and growth promotion to improve its production. On the other hand, the microbial load determined in this study is above 1×10^6 CFU/ml, considered as the necessary application dose, for these bacteria to exert their effect [29]. It is inferred that in the leachate obtained at the INIVIT, there are sufficient amounts of *Bacillus* to produce a possible response in the plants. Currently, our working group is conducting other leachate evaluations, obtained with a view to better understand its use and potential, as a biological controller for its integration into disease management programs in Musaceae. Biological control strategies have got importance in recent years and at the present time, they are main links in pest management programs in Cuba. They are an alternative to the use of chemical synthesis products, in the management of harmful organisms or pest causal agents. Unfortunately, the increase in food demand and the resistance of pests have forced farmers to increase agrochemical and pesticide inputs, as the main control method. The indiscriminate use of these ones may cause damage to health and the environment [30,31].

The results related to the determination of *Escherichia coli* and *Salmonella* sp. are reflected in Table 1. In relation to the leachate innocuousness, the results show the absence of *Salmonella* sp. and *E. coli* (Table 1), which is of most importance, since the international health organizations demand, as an indispensable control requirement for the use of these products, that these microorganisms must be at very low populations or absent to consider the product innocuous. The results obtained in terms of innocuousness and bacteria presence of the genus *Bacillus*, could encourage the use of rachis leachate in agro-ecological production systems and minimize the use of chemical substances that contaminate the environment, in addition to lower production costs, advantages that promote the use of this practice in the Cuban agro-ecosystems.

Table 1: Determination of *Escherichia coli* and *Salmonella* sp. in an analyzed sample.

Determination of <i>Escherichia coli</i>	Determination of <i>Salmonella</i> sp.
Absence	Absence

Conclusion

The results obtained in this research are the scientific basis to support the use of rachis leachates of bananas, obtained at the INIVIT, as a high value-added product. The microbial load determined in this study 4.4×10^6 CFU/mL from bacteria of the genus *Bacillus* sp. and its innocuousness as it is free of pathogenic microorganisms, which can harm plants or human beings are findings of great value that contribute to encourage the use of a

crop residue, underused in the production process of plantains and bananas. It is recommended to continue studies to isolate, identify, purify and lyophilize these bacteria and evaluate the possibility of their use as biological controllers.

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