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Biodegradation of plantain rachis using phytopathogenic fungi for composting

Biodegradación del raquis de banano usando hongos fitopatógenos para compostaje

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Abstract

The phytopathogenic fungi such as: *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* were isolated from banana and tomatoes respectively, 3 treatments were used: with spores *Colletotrichum gloeosporioides* (B1), with spores *Rhizopus stolonifer* (B2) and a mixture of spores *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3). Using the treatment (B3) was obtained the highest degradation of the plantain rachis being of 42.91%, and the leachates production was of 80 mL with pH of 7.96 on the 30th day of the experimentation. The banana rachis degraded by the mixture of spores of *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3) showed the lowest organic carbon content being of 29.52% and the highest organic nitrogen content being of 2.30%, generating a Carbon/Nitrogen (C/N) ratio of 12.84%. The results evidenced that the treatment composed by the mixture of *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3) can be used to increase the degradation of the plantain rachis encouraging the use of this biomass in the composting process.

Keywords: *Colletotrichum gloeosporioides*, phytopathogenic fungi, rachis, *Rhizopus stolonifer*.

Resumen

Los hongos fitopatógenos como: *Colletotrichum gloeosporioides* y *Rhizopus stolonifer* se aislaron de bananos y tomates respectivamente, se utilizaron 3 tratamientos: con esporas de *Colletotrichum gloeosporioides* (B1), con esporas de *Rhizopus stolonifer* (B2) y una mezcla de esporas de *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3). Usando el tratamiento (B3) se obtuvo la mayor degradación del raquis de banano siendo del 42.91%, y la producción de lixiviados fue de 80 mL con un pH de 7.96 en el día 30 de la experimentación. El banano degradado por la mezcla de esporas de *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3) presentó el menor contenido de carbono orgánico siendo del 29.52% y mayor contenido de nitrógeno orgánico siendo del 2.30%, generando una relación Carbono/Nitrógeno (C/N) de 12.84%. Los resultados evidenciaron que el tratamiento compuesto por la mezcla de esporas de *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3) puede ser utilizado para aumentar la degradación del raquis de banano fomentado el uso de esta biomasa en el proceso de compostaje.

Palabras clave: *Colletotrichum gloeosporioides*, hongos fitopatógenos, raquis, *Rhizopus stolonifer*.

1 Introduction

The production of plantain (*Musa paradisiaca* (L.) AAB) in Ecuador represents an important sector for the economy, the land area of the country uses for banana cultivation is nearly 0.6% mainly on the alluvial plains of the coastal lowlands in the provinces of Los Rios, Guayas and El Oro (SICA, 2004). Instead, 80% of the total export production comes from growers maintaining areas smaller than 30 ha (Jimenez *et al*., 2007). In 2014 the production was 634341 tons, the main varieties of plantain are: Dominico used for domestic consumption and Barraganete used for export purposes (Ulloa-Cortazar *et al*., 2017).

The most common substrates used for farmers for the production of plantain is the compost of different kind of biomasses degraded such as: grape pomace, lees, stalk, dewatered sludge and also the plantain rachis (Ruggiero *et al*., 2009). The banana rachis is a crop residue that accumulates in the farms and in the concentrating markets of agricultural products, and is considered a pollutant of environment (Ayala *et al*., 2016), the biodegradation of the agricultural wastes is the greatest problem for farmers for the time uses in the degradation process and the space

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needs to keep the volume of the organic material until the total degradation (Mena-Nevarez *et al*., 2012). New technologies have been proved like the use of alkaline subcritical-water treatment and alkaline heat to increase the biodegradability of the agricultural wastes (Fox *et al*., 2003). On the other hand, with the use of these chemical methods is possible that the organic wastes can lose important properties for being used in the production of plantain because these techniques only have the finality of the reduction of the agricultural wastes volume. Studies have presented that the use of phytopatogenic fungi such as: (*Colletotrichum* sp., *Penicillium* sp. and *Rhizopus* sp.) in the biodegradation of organic wastes like: mangoes and oranges for being used in the composting process without cause biologic hazards or several damage to crops (Mena-Nevarez *et al*., 2012).

Composting is an aerobic process, during which organic waste is biologically degraded by micro-organisms to humus-like material, the final product is an important source of nutrients and carbon (Epstein, 1997). The compost method is an effective method that eliminate nematodes, bacteria, viruses, and pathogenic thermophilic fungi, but needs conditions to inactivate the intense microbial competition that cause diseases in plants, animals and humans (Farrell, 1993; Gerba *et al*., 1995; Bollen and Volker, 1996; Suárez-Estrella *et al*., 2003; Erickson *et al*., 2009). The end product should not contain pathogens or viable seeds, and it should be stable and suitable for use as a soil amendment. Many factors such as oxygen content, moisture, composition of the feed, pH, and temperature, affect the composting process and ultimately the end product (Partanen *et al*., 2010). There are not studies about the use of phytopathogenic fungi in banana rachis to the point of complete degradation to make this agricultural waste available for composting.

The aim of this research was determined the degradation, the leachate production, the microbiological parameters and physical-chemical composition of the plantain rachis after using the 3 treatments composed by phytopatogenic fungi *Colletotrichum* and *Rhizopus*.

2 Materials and methods

2.1 Plant material

The plantain “Barraganete" and tomatoes were purchased at the Central de Abasto “Montebello" in Guayaquil, Ecuador.

2.2 Culture media

The culture media was prepared by dissolving 39 g of potato dextrose agar (PDA) in 1 L of distilled water using an Erlenmeyer flask. The flask was sterilized in autoclave at 15 psi (121 °C) for 15 min, subsequent, 10 mL of sterile medium were poured into Petri dishes. The dishes with the medium solidified were put in plastic bags and incubated at 28 °C for 24 h to check the sterility. Then, the Petri dishes without contamination were used for propagation of the mycelium of phytopathogenic fungi (Eger *et al*., 1976; Coello-Loor *et al*., 2017).

2.3 Propagation solution

The solution of propagation was prepared by dissolving in 1 L of distilled water with 40% glucose and 15% of guar gum. After that, 150 mL were poured into jars and sterilized at 15 psi (121 °C) for 30 min. The jars with the propagation solution were incubated at 28 °C for 24 h to check the sterility.

2.4 Isolation of phytopathogenic fungi

The strains of *Colletotrichum* were isolated from plantain that showed symptoms of anthracnose or green mold on PDA dishes, while the strains of *Rhizopus* were isolated from tomatoes that showed symptoms of *Rhizopus* rot on dishes with PDA. The isolation of the phytopathogenic fungi was realized at Research and Development Laboratory of Ecuahidrolizados Industry localized in Guayaquil (Ecuador).

2.5 Morphological determination of the fungi

For morphological characterization, mycelia description was carried out taking into account particular features of mycelium (Schipper, 1984). Isolated strains (0.5 cm diam) were grown on PDA dishes at 30°C, the mycelial growth was measured

every day until the total colonization. Shape of conidia was recorded from the colonies grown on PDA plates at room temperature 30°C. Conidia were taken from actively growing colonies mounted in lactic acid, and examined for shape (Photita *et al*., 2005; Hernández-Lauzardo *et al*., 2006).

2.6 Obtention of the spores

The mycelium on the PDA dishes was washed with 100 mL of distilled water, obtaining in the washing the spores of the phytopathogenic fungi. The washing was put in an Erlenmeyer flask with 1 L of propagation solution until obtain a concentration of 3.50×10^6 spores/mL.

2.7 Treatments

Treatment 1 (B1): 1 L of propagation solution with a concentration of 3.50×10^6 spores/mL (*Colletotrichum* sp.) + 1 L of sugar cane sterilized.

Treatment 2 (B2): 1 L of propagation solution with a concentration of 3.50×10^6 spores/mL (*Rhizopus* sp.) + 1 L of sugar cane sterilized.

Treatment 3 (B3): 1 L of propagation solution with a concentration of 3.50×10^6 spores/mL (1.75×10^6 *Colletotrichum* sp. + 1.75×10^6 *Rhizopus* sp.) + 1 L of sugar cane sterilized.

2.8 Tests of biodegradation

The plantain rachis was cut in a piece of 2 kg (wet basis) and was inoculated using the 3 treatments composed by phytopatogenic fungi. The inoculated plantain rachis piece was placed in plastic containers (3 kg of capacity) and incubated at 23°C and 95 to 100% relative humidity (RH) for 30 days. In the experiment was used a piece of 2 kg of plantain rachis without treatment. The biodegradation test was evaluated daily for 30 days and the experimentation was realized at the Farm “Sofía" localized in Milagro (Ecuador).

2.9 Degradation of plantain rachis

The plantain rachis (with or without treatment) was weighed daily using a digital capacity scale. The degradation was determined based on the initial and final weight of the rachis and was presented as a percentage of the initial weight.

2.10 Leachate production

The plantain rachis (with or without treatment) was put in a box strainer, and then it was placed over a plastic container where the leachate was collected. The volume of leachates produced daily was determined with a graduated glass test tube.

2.11 pH of leachates

A pH meter was used to determine pH of the leachates produced on the 30th day of the plantain rachis (with or without treatment).

2.12 Microbiological parameters and chemical composition of the plantain rachis

The microbiological parameters of the banana rachis (with or without treatment) evaluated were: aerobic mesophilic bacteria, salmonella, yeasts and molds (AOAC, 2005; Valenzuela-Cobos and Vargas-Farías, 2020). The humidity, ash, organic carbon, organic nitrogen, crude fiber were determined using the methodologies of (Arango-Osorno *et al*., 2016; Valencia del Toro *et al*., 2018; Valenzuela-Cobos, 2018; Valenzuela-Cobos *et al*., 2019).

2.13 Amino acids determination

The amino acids were determined using the methodology of (Valenzuela-Cobos and Vargas-Farías, 2020) only to the banana rachis with the highest degradation.

2.14 Phytotoxicity test

The phytotoxicity test was determined according to the percentage of viable seeds (germination index) only to the banana rachis with the highest degradation. In the experimentation was used seeds of lettuce and turnip and were tested in 5 mL of water-soluble extracts of compost “C1" (from 10 g of fresh sample in 50 mL of distilled water) and only in 5 mL of distilled water “C2", using conditions of darkness at 25 ºC for 72 h (Fels *et al*., 2014). To determine the germination index (GI) was necessary the number of germinated seeds (tests 72 h), and growth of roots (tests 72 h), see Eq. (1)

$$GI\% = \frac{NGext \times LRext}{NGwater \times LRwater} \times 100 \qquad (1)$$

where:

Table 1. Characteristics of plantain raquis after using different treatments.

Treatments	Degradation (%)	Leachate production (mL)	pH of leachates
Control	19.18 ± 0.48^{d}	31.00 ± 0.42^{d}	6.44 ± 0.16^{c}
B1	28.35 ± 0.64^{c}	49.00 ± 0.91^{c}	6.84 ± 0.37^{b}
B2	35.58 ± 1.93^{b}	57.00 ± 0.37^{b}	7.19 ± 0.75^{b}
B3	42.91 ± 1.15^{a}	80.00 ± 0.46^{a}	7.96 ± 0.28^{a}

*Control: The plantain rachis without treatment, B1: Propagation solution with a concentration of 3.50 $\times10^{6}$ spores/mL (*Colletotrichum gloeosporioides*) + sugar cane sterilized, B2: Propagation solution with a concentration of 3.50 $\times10^{6}$ spores/mL (*Rhizopus stolonifera*) + sugar cane sterilized, B3: Propagation solution with a concentration of 3.50 $\times10^{6}$ spores/mL (*Colletotrichum gloeosporioides* + *Rhizopus stolonifera*) + sugar cane sterilized.

*The degradation of the raquis was calculated according to the following equation: %Degradation = 100-(%Final weight of the raquis*100/% Initial weight of the raquis).

*Different letters in each column indicated significant difference among the characteristics of the plantain rachis degraded at level p<0.05, according to Duncan's test, n = 3.

NGext, NGwater= number of seeds germinated in water-soluble extracts and distilled water, respectively; and LRext, LRwater= the length of rootlets in soluble extracts and distilled water, respectively.

2.15 Statistical analysis

In all analyzes, a completely randomized design and the results were studied using one-way analysis of variance (ANOVA) to determine the significance of individual differences at p<0.05 level, the growth rates of the phytopatogenic fungi, the degradation, the leachate production, the microbiological parameters and physical-chemical composition of the plantain rachis (with or without treatment) after of the biodegradation test, when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. The analyses were carried out using statistical software (Statgraphic ver. 16).

3 Results and discussion

3.1 Morphological identification

Colletotrichum isolated from banana were grown on PDA dishes at 30ºC presented growth rate of 0.87 cm.day^{-1}. The culture media presented sparse, cottony, white to pale grey mycelium with abundant mycelia containing bright orange conidial masses produced in concentric rings on the colonies. The conidia showed cylindrical shape and rounded at ends. These morphological characteristics led to the identification of fungus as *Colletotrichum gloeosporioides*. Photita *et al*. (2005) reported growth rates ranged from 0.82 to 1.16 cm.day^{-1} for 27 strains of *Colletotrichum gloeosporioides* isolated from banana, while (Lu *et al*., 2000) informed aerial mycelia where white to grey and grey to darkish green on the reverse side of the plate, and cylindrical conidia for one strain of *Colletotrichum* isolated from older stems of *A. annua*.

Rhizopus isolated from tomatoes were grown on PDA plates at temperature room (30ºC) presented growth rate of 5.10 cm.day^{-1}, and mycelium aerial and cottony. These morphological characteristics are the most representative to the identification of fungus as *Rhizopus stolonifer*. Hernández-Lauzardo *et al*. (2006) reported growth rates between 5.20 and 5.56 cm.day^{-1} for 3 strains of *Rhizopus stolonifer* isolated from infected tomatoes, while (Romero-Cova, 1988) informed that *R. stolonifer* showed an adequate sporulation and the formation of particular structures after 48 h incubation.

3.2 Characteristics of banana rachis after of the degradation test

Table 1 shows the characteristics of banana rachis after using the different treatments for 30 days.

By using the treatment (B3) composed by the monosporics of the mixture of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* on banana rachis piece exhibited the highest degradation being of 42.91%, while using the treatment (B1) of spores of *Colletotrichum gloeosporioides* showed the lowest biodegradation being of 28.35%. On the other hand, the banana rachis using the treatment (B2) composed by spores of *Rhizopus stolonifer* presented

degradation of 35.58%, whereas the banana rachis without treatment (control) showed biodegradation of 19.18%. Similar results have been reported, Mena-Nevarez *et al*. (2012) showed degradation since 28 to 33% on mangoes using different mixtures of spores of *Colletotrichum* sp. and *Rhizopus* sp., whereas (Velázquez del Valle *et al*., 2008) reported important economic losses for soft rot in most fruits and vegetables caused by *Rhizopus stolonifer*. Specific fungus attack fruits, flowers and vegetables that generate large postharvest losses (Vidales, 1997). The treatment (B3) can used in agricultural industries and in farms to descompose banana wastes.

The highest leachates production (80 mL) during the 30 days of the experimentation was showed by the banana rachis degraded with the treatment (B3), while the lowest leachates production (31 mL) was presented by the banana rachis without treatment (control). The use of leachate of banana rachis as a fungicide reduced the powdery mildew of roses caused by *Sphaerotheca pannosa* on the plants (Álvarez *et al*., 2001; Muñoz and Molina, 2005). The pH of the leachates on the 30th day was between 6.44 (from banana rachis without treatment) and 7.96 (from banana rachis degraded with the treatment B3), the rise in pH can be explained by the production of ammonia from the degradation of amines which can release bases already existing in the organic waste (Ouatmane *et al*., 2000), or can correspond to the degradation of organic acids and to the release of exchangeable bases (Fels *et al*., 2014).

3.3 Microbiological and chemical composition of the plantain rachis degraded

The microbiological parameters of the plantain rachis after using the different treatments during 30 days are presented in the Table 2.

The plantain rachis degraded with the treatment (B1) presented aerobic mesophilic values of 4.10×10^3 UFC/g, yeasts and molds values $< 1.00 \times 10^3$ UP/g, and salmonella was not detected. For otherwise, plantain rachis degraded with the treatments (B2 and B3) presented aerobic mesophilic values of 5.30×10^8 UFC/g, yeasts and molds values $< 1.00 \times 10^3$ UP/g, and salmonella was not detected. The plantain degraded without treatment (control) showed aerobic mesophilic values of 2.10×10^2 UFC/g, yeasts and molds values $< 1.00 \times 10^1$ UP/g, and salmonella was not detected. Arango-Osorno *et al*. (2016) presented for compost of legume waste and bovine rumen: aerobic mesophilic values between 1.30×10^9 and 8.00×10^{10} UFC/g, yeasts and molds values ranged from 0.00 to 2.4×10^4 UFC/g, and salmonella was not detected.

The chemical composition of the plantain rachis using the different treatments during 30 days are shown in the Table 3.

The lowest content of ash (10.81%) and the highest humidity content (82.98%) was presented by plantain rachis degraded without treatment (control). On the other hand, the plantain rachis degraded with the treatment (B3) showed the lowest organic carbon content (29.54%) and highest organic nitrogen content (2.30%) presenting a Carbon/Nitrogen ratio (C/N) of 12.84%, whereas the plantain rachis degraded with the

Table 2. Microbiological parameters of the plantain rachis after using different treatments.

Treatments	Aerobic mesophilic (UFC/g)	Salmonella (25/g)	Yeasts and molds (UP/g)
Control	2.10×10^{2c}	Nd	$<1.00 \times 10^{1b}$
B1	4.10×10^{7b}	Nd	$<1.00 \times 10^{3a}$
B2	5.30×10^{8a}	Nd	$<1.00 \times 10^{3a}$
B3	5.30×10^{8a}	Nd	$<1.00 \times 10^{3a}$

*Control: The plantain rachis without treatment, B1: Propagation solution with a concentration of 3.50×10^6 spores/mL (*Colletotrichum gloeosporioides*) + sugar cane sterilized, B2: Propagation solution with a concentration of 3.50×10^6 spores/mL (*Rhizopus stolonifer*) + sugar cane sterilized, B3: Propagation solution with a concentration of 3.50×10^6 spores/mL (*Colletotrichum gloeosporioides* + *Rhizopus stolonifer*) + sugar cane sterilized.

*Different letters in each column indicated significant difference among the microbiological parameters of the plantain rachis degraded at level $p<0.05$, according to Duncan's test, n = 3.

* Nd= Not detected.

Table 3. Physical-chemical composition of the plantain rachis after using different treatments.

Treatments	Humidity (%)	Ash (%)	Organic carbon (%)	Organic nitrogen (%)	C/N (%)	Crude Fiber (%)
Control	82.98±0.09^a	10.81±0.54^b	42.09±1.01^a	1.69±0.84^c	24.91±1.03^a	20.12±0.92
B1	82.08±0.12^a	10.15±0.94^b	38.12±0.97^a	1.82±0.75^b	20.94±1.54^b	24.31±1.02^c
B2	80.12±0.45^b	11.97±1.38^a	32.96±0.18^b	1.97±0.13^b	16.73±0.95^c	26.93±0.95^b
B3	81.08±0.21^b	12.10±0.42^a	29.54±0.52^c	2.30±0.42^a	12.84±0.71^d	28.07±1.21^a

*Control: The plantain rachis without treatment, B1: Propagation solution with a concentration of 3.50 $\times 10^6$ spores/mL (*Colletotrichum gloeosporioides*) + sugar cane sterilized, B2: Propagation solution with a concentration of 3.50 $\times 10^6$ spores/mL (*Rhizopus stolonifer*) + sugar cane sterilized, B3: Propagation solution with a concentration of 3.50 $\times 10^6$ spores/mL (*Colletotrichum gloeosporioides* + *Rhizopus stolonifer*) + sugar cane sterilized.

*Different letters in each column indicated significant difference among the physical-chemical composition of the plantain rachis degraded at level $p<0.05$, according to Duncan's test, n = 3.

treatments (B1 and B2) presented C/N ratio values between 16.73 and 20.94%, and the plantain degraded without treatment (control) showed C/N ratio of 24.91%. The carbon/nitrogen mass ratio (C/N) is very important to determine the quality of compost, since it influences the bacterial biomass (Negro *et al*., 2001). The C/N ratio values between 10 and 15% indicates the maturity of composting (Dorfman and Batsch, 1985; El Hajjouji *et al*., 2007; Pfirter *et al*., 1982). The plantain rachis degraded with the treatment (B3) exhibited microbiological and chemical characteristics for being used for composting process.

3.4 Phytotoxicity testing

The germination index (GI) of the turnip species using water-soluble extracts of compost "C1" (10 g of banana rachis degraded with treatment B3 in 50 mL of distilled water) was of 16%, while using 5 mL of distilled water "C2" was of 10%. For otherwise, the germination index of the lettuce species using water-soluble extracts of compost "C1" was of 10%, whereas using 5 mL of distilled water "C2" was of 4%. Fels *et al*. (2014) presented germination index value for turnip species ranged from 16 and 58% using two different kind of compost during the thermophilic phase, and for lettuce species presented a germination index of 0% using two composts during the stabilization phase. The germination index value is related to the inhibitory effect of short-chain organic acids, phenols, alkaloids, aldehydes, ketones, amino acids, lipids, ammonia, heavy metals, phenolic compounds especially tannins, and the values of initial pH (Ait Baddi *et al*., 2004; Boopathy and Melancon, 2004; Hachicha *et al*., 2009; Keeling *et al*., 1994 Novoa-Munoz *et al*., 2008; Piotrowska *et al*., 2006; Solbraa, 1979).

3.5 Amino acids composition of the plantain rachis degraded

Table 4 indicates the amino acids composition of plantain rachis degraded with the treatment B3. In the study were detected 9 amino acids after pre-column derivatization with DEEMM within 45 min.

The amino acid with more presence in the plantain rachis was the leucine with value of 0.91%. Other important amino acids were detected: valine, isoleucine and phenylalanine with values of 0.32, 0.54, and 0.63% respectively. Yields of flowers, fruit and mushrooms are directly related with compost rich in amino acids such as: leucine, isoleucine, valine and phenylalanine (Sinden and Schisler, 1962; Neeraja *et al*., 2005; Royse and Sánchez, 2008). The plantain rachis degraded with the treatment (B3) due to its amino acids content can be used as compost to increase the yields of different kind of plants.

Table 4. Amino acids composition of the plantain rachis degraded with the treatment B3.

Amino acids	%
Proline	0.65
Cysteine	0.12
Tyrosine	0.41
Valine	0.32
Methionine	0.21
Lysine	0.50
Isoleucine	0.54
Leucine	0.91
Phenylalanine	0.63

Conclusions

The plantain rachis degraded with the treatment (B3) composed by spores of *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* showed the highest biodegradation, leachate production and organic nitrogen in comparison with the plantain rachis degradaded with the other treatments.

The plantain raquis degraded with the treatment (B3) showed the highest germination index, the amino acid with more presence in the raquis using the treatment (B3) was the leucine.

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