

Full Length Research Paper

Phytotoxicity of extracts of *Myrciaria dubia* (Kunth) McVaugh bioprocessed in vegetable crop sensitive to allelochemicals

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The present work is a contribution to the advancement of the scientific knowledge of vegetal species of the Amazon and training of professionals in the area of vegetal and related sciences in the use of alternative methods and techniques more coherent with the context of the socioeconomic and environmental reality of the region. This work aimed to evaluate the effect of extracts prepared from bioprocessed remaining (BR), seeds of fruit of *Myrciaria dubia* (caçari) from the northern Amazon, applied to biological assay with *Lycopersicon lycopersicum* (tomato) seeds. To obtain and classify the degree of phytotoxicity were utilized samples of control and different aqueous extracts, with and without treatment, assessing their physical and chemical quality via pH analysis, electrical conductivity and total of dissolved salts. Thereafter, monitoring of bioassay was held for five days in closed glass plate, with samples incubation at $25 \pm 1^\circ\text{C}$ in a dark environment. It was obtained preliminary knowledge about four extracts prepared from T1 and T2 in proportion to 1:10 and 1:100. The methodological process applied allowed the quick examination, up to 120 h, of the effect of the extracts on seed germination and root growth of tomato. Of the four extracts evaluated, those who were previously treated T2D1 and T2D2, regardless of the applied concentration, showed no phytotoxicity to the plant tested and classified according to Germination Index (GI) obtained, 90.2 and 96.6%. The results show an important information about the influence of treatment of the bioprocessed compounds on their chemical and physicochemical characteristics, which can alter their effects on the development of other plants. Therefore, there is possibility of availability of finished products and raw materials from the *M. dubia* seeds, since previously sterilized.

Key words: Caçari, germination index, methodological process, bioprocessed remaining.

INTRODUCTION

The *Myrciaria dubia* is a fruit species that originated from the Amazon, and has been the focus of different

technical-scientific studies in Brazil currently, having prospects of improvement in its production. In this context, in the course of researches, a gap was verifiably completed, referring to the possibility of development of new products after processing of the fruit, known as *caçari*, in the extreme north of Brazil, making good use only of the pulp and discarding peel and seeds as residues, general practice, widely applied in the northern Amazon yet.

In the processing of the fruits of *M. dubia* for each ton is estimated up to 270 kg, only seeds, which are not yet well exploited, in the Amazon, mainly as a product, possibly due to ignorance or lack of adequate disclosure about its potential, as well as its quality and level toxicity when bioprocessed. It is essential that interdisciplinary projects be developed aimed at increasing the knowledge about vegetal species of the Brazilian biodiversity in the chemical - and toxic-pharmacological interfaces (Campos et al., 2016).

Considering the current methods of toxicological evaluation, *in vitro* tests offer several advantages, low cost; a small amount of material required; a limited amount of toxic waste, cells and human tissues used; as well as transgenic cells carrying human genes and reduced animal testing (Araujo et al., 2014). According to Araujo and Monteiro (2005), seeds germination and plant growth bioassays are the most common techniques used to assess the phytotoxic compounds. According to Trautmann and Krasny (1997), it enables the determination of whether there are, in the compound or in the raw material, substances which may inhibit the germination of seeds, the root growth or development of plants.

Simoneto and Cruz-Silva (2010) reported that the use of seeds of cultivated species and good quality is advisable, including the tomato, easily found and quite sensitive to many allelochemicals (chemical compounds), secondary metabolic products of plants according to Ribeiro et al. (2012).

Researches have shown that certain plants, such as alfalfa, contain water soluble phytotoxic compounds which are released into the soil environment using fresh leaves, stems and crown tissues, as well as dry material, roots in decomposition and seeds (Hall and Henderlong, 1989; Dias de Almeida et al., 2008). In fact, they are according to Weir et al. (2004), who emphasized that allelopathic substances released by the plant, can affect the growth, damage the normal development and even inhibit the germination of seeds of other plant species.

According to Souza Filho et al. (2010), the allelochemicals are found in different parts of the plant including leaves, flowers, roots, stalks, fruits, peels, seeds, and pollen grains. These secondary metabolites

may be released directly into the environment by root exudation, volatilization, leaching or decomposition of plant material (Moreno, 1989; Cipollini et al., 2012).

Furthermore, plant secondary metabolites can act in the recipient plant by altering the structure of cell membranes, including the receptors and also there are present flags, capable of causing interference in the cell cycle, modify the action of several hormones, alter the conformation of enzymes and process of transcription and translation (Habermann et al., 2015).

Previous studies demonstrated the phytotoxic potential of aqueous leaf extracts of *Blepharocalyx salicifolius* on the early development of bio-indicator species such as onions, tomatoes and lettuce (Mairesse et al., 2007; Imatomi et al., 2013). In a study conducted by Sausen et al. (2009) the aqueous leaf extracts of *Eugenia involucrata* (Myrtaceae) and *Acca sellowiana* (Myrtaceae) were phytotoxic to the germination and growth of tomato and onion seedlings growth.

However, work on the phytotoxicity of *M. dubia* (Myrtaceae) on these bioindicators using extracts obtained from seeds, remnants of the pulping of fruits of this species were not found in literature.

The vegetables germination test is a widely used model to assess the potential of the plant extracts (allelochemicals or isolated compounds). One of the purposes set out when certain compounds interfere with cell function is the change in the germination rate of seeds, revealing their toxic and/or cytotoxic action (Luz et al., 2012).

However, according to Faria et al. (2009), further studies are needed regarding the forms of extraction, types of extractors, extraction time and application rates, in addition to part of the plants to be used as low phytotoxic effect may occur by low concentrations of compounds inhibitors present in the extracts tested.

According to Noldin et al. (2003), the use of vegetable biological assays to monitor bioactivity of extracts, fractions and compounds isolated from plants is one of the alternatives that have often been incorporated into the identification and monitoring of potentially toxic substances.

In this study, evaluation of the effect of extracts with agrifood potential made from the bioprocessed remaining seeds of the fruit of *M. dubia* was purposively taken from the northern Amazon, applied to biological assay with seeds of *Lycopersicon lycopersicum* (L.) H. Karst. (tomato) in the laboratory to obtain and classify the degree of phytotoxicity with a view to providing raw materials and new process methodological/biotech product for Brazilian society.

This study is a contribution to the advancement of the scientific knowledge of vegetal species of the Amazon

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Table 1. Results obtained on visual inspection absence (-) or presence (+) of microorganisms in different dosages of BR sand (S), sandy soil (SS) and clay soil (CS).

Treatments	Identification	Visual inspection	
		3rd day	7th day
1	100% S	-	-
2	75% S + 25% BR	+	+
3	50% S + 50% BR	+	+
4	25% S + 75% BR	+	+
5	100% SS	-	-
6	75% SS + 25% BR	+	+
7	50% SS + 25% BR	+	+
8	25% SS + 75% BR	+	+
9	100% CS	-	-
10	75% CS+ 25% BR	+	+
11	50% CS + 50% BR	+	+
12	25% CS + 75% BR	+	+
13	100% BR	+	+

and training of professionals in the area of vegetal and related sciences in the use of alternative methods and techniques more coherent with the context of the socioeconomic and environmental reality of the region.

MATERIALS AND METHODS

Study site and provenance of the plant material

The study was conducted at the Brazilian Agricultural Research Corporation, 02°45'28 "N 60°43'54" W, located in the state of Roraima, in June, in the year 2015, with bioprocessed remaining (BR), seeds of fruit of *M. dubia*, from technological prospection, related to the years 2012, 2013 and 2014 in the northern Amazon.

Samples of the plant material – BR

The BR samples were obtained from 10 materials that were stored and preserved in a freezer since the collection period, 2012-2014.

Samples preparation

The BR samples were prepared from the formation of a series of sub-samples (1 kg) prepared from materials that were stored in the freezer, intending to obtain at least 500 g of processed sample according to granulometric specifications usually used for organic fertilizers. They were pre-dried in air circulating stove, calibrated in the range of $60 \pm 5^\circ\text{C}$, for 48 h. After this period, they were weighted and processed using a Willye type mill with a 1 mm mesh, having previously calculated the efficiency of crushing and time required for processing.

From the processed material (suitably uniformed by granulometry), there were selected two samples, each containing 100 g to compose the biological assay in the laboratory, according to methodology principles of Zucconi et al. (1981) and Wong et al. (2001), with some adjustments.

Bioassay preparation

Here, a witness control (WC) (only deionized water) and aqueous extracts was prepared, including liquid substrates made from BR with and without treatment, known as T1 and T2, respectively. To obtain T2, it was placed 100 g of BR in a calibrated stove at a temperature of $200 \pm 10^\circ\text{C}$ for 2 h, in order to control the microbial population.

The experiment was conducted from preliminary results obtained in qualitative microbiological test conducted in a greenhouse with BR samples. In 76.9% treatments designed (solid substrates with different dosages in sand, sandy soil and clay soil), shown in a simplified way, treatment and identification in Table 1 revealed the presence of microorganisms in visual inspection from the third until the end, the seventh day of implementation of the experiment in all treatments containing the BR-intensive growth.

From the established treatments (T1 and T2), the biological assay was performed in specialized laboratory using sterilized materials, deionized water, ionic contaminants free and BR aqueous extracts, five repetitions for each witness control (WC) and treatments, obtained by means of two dilutions (D1 and D2) respectively defined by the ratio m: v, 1:10 normally used in the assessment of organic compounds and 1: 100 was performed as Sousa et al. (2015).

Once prepared, the extracts were shaken up manually for 20 s each, with a glass rod, repeating the action for three more times, in order to dissolve the material satisfactorily. Therefore, each extract was filtered through Whatman filter paper #1. Thereafter, simplified physicochemical evaluation, pH, electrical conductivity (EC) and total dissolved salts (TDS) was realized to obtain preliminary knowledge of the elaborated extracts. The measurement of pH, TDS and EC was determined by pH meter and microprocessor conductivity meter, after calibration with standard solution, according to the producer's instructions.

A model scheme was developed (Table 2) for simplified facilitation and identification of the experiment and extracts, used in the tabulation and presentation of results, as follows: RBST originated from untreated bioprocessed remaining (identified as T1) and RBCT originated from treated bioprocessed remaining (identified as T2), added to their respective dilution: T1D1 = 1:10; T1D2 = 1:100; T2D1 = 1:10; T2D2 = 1:100.

Table 2. Schematic model for simplified identification of the experiment and extracts made from bioprocessed remaining (BR).

Identification	Treatment	Dilution	Name simplified
RBST	T1	D1	T1D1
		D2	T1D2
RBCT	T2	D1	T2D1
		D2	T2D2

Bioassay installation

In laboratory bench, for each established treatment (Table 2) was added a qualitative analysis filter paper (Whatman #2) into five glass petri plate 9 cm in diameter, wetting with 5 ml of extract, each. For the WC, the same procedure was used, adding, in this case, 5 ml of deionized water per plate. Then, in each Petri dish were placed 10 *L. lycopersicum* seeds uniformly distributed.

The tomato seeds were acquired from agricultural store, after lifting of the most cultivated varieties in Roraima, also taking into consideration the characteristics of the "cultivar IPA 6". According to information contained in the package, the IPA 6 has determined growth, vigorous and productive plant, firm (globular) fruit, with excellent internal and external color, as well as being resistant to disease such as *Fusarium*, *Verticillium*, nematode and Cracking. The germination takes place from 5 to 14 days after sowing.

Assessment of the bioassay

To obtain the results in the laboratory, visual inspection of the presence or absence of microorganisms and monitoring of the germination process of the seeds of the tomato proposed treatments was conducted for 120 h (five days), in a closed plate, by incubating the samples at $25 \pm 1^\circ\text{C}$ in a dark environment. At the first 120 h were recorded through photographic images and spreadsheet on the number of germinated seeds (NSG) and also by measuring with a caliper, root length (RL) of germinated seeds in each Petri dish. The seeds were considered to be germinated when they had rootlets and/or developed a little stem.

Germination index (GI)

The value for the germination index (GI) was obtained by quantifying the RSG and RRG, as proposed by Zucconi et al. (1981). Thus, the calculation of the RSGs made by the equation:

$$\text{RSG (\%)} = (\text{NSG}_T / \text{NSG}_C) * 100$$

Where NSG_T is the arithmetic mean of the number of germinated seeds in each extract (treatment) and NSG_C is the arithmetic mean of the number of germinated seeds in witness control (WC).

The relative percentage of root length, RRG was obtained by the equation:

$$\text{RRG (\%)} = (\text{LR}_T / \text{LR}_B) * 100$$

Where LR_T is the mean length of the roots on the aqueous extract and LR_B is the length mean of the roots in the witness control (WC).

As was proposed by Zucconi et al. (1981), the germination index (GI), was computed using the equation:

Table 3. Methodological model proposed to quantify the degree or phytotoxicity level of the product from the tests with *Lycopersicon lycopersicum*.

GI (%)	Product rating under analysis
80-100	Non-phytotoxic
60-80	Moderately phytotoxic
30-60	Phytotoxic
< 30	Very phytotoxic

Source: Adapted from Trautmann and Krasny (1997) and WERL (2000).

$$\text{GI (\%)} = (\text{RSG (\%)} * (\text{RRG (\%)} / 100)$$

Statistical analysis

The results were submitted to analysis of variance and means compared by Tukey test at 5% probability ($p < 0.05$) with the help of statistical program SISVAR® (Ferreira, 2011).

Degree qualification or phytotoxicity level of bioprocessed remaining, *M. dubia* seeds

The proposed methodological model is used (Table 3), from the GI (%), obtained after the use of the aqueous extracts with different treatments applied to biological assay with tomato was selected one that varies slightly among others found in the literature, adding small adjustments.

RESULTS AND DISCUSSION

In this study, preliminary knowledge were obtained related to the physical and chemical quality, presence of microorganisms in BR extracts of *M. dubia* seeds of northern Amazonia, also germination rate and degree of phytotoxicity (Table 4) of these from bioassay with *L. lycopersicum* (tomato), containing a control sample and four elaborated extracts from T1 treatments (RBST) and T2 (RBCT) in the proportion to 1:10 (D1) and 1:100 (D2).

In Table 4, it can be seen that all extracts (T1D1, T1D2, T2D1 and T2D2) tested showed pH values ranging from 4.73 to 5.18, below the obtained (5.83) with the witness control (WC), clearly indicating the influence of BR. Irrespective of treatment and applied dilution, T1D1 and T2D1 showed no significant difference related to pH, while T1D2 and T2D2 suffered directly influences in the applied dilution, differing from each other, and between WC and the other treatments performed with BR.

Analyzing the pH values obtained in the treated and untreated samples (Table 4), it is possible that pre-drying may have influenced the results, causing decay in pH values when compared to WC (Zanatta et al., 2010). Minor pH occurred in extracts where BR was more concentrated (T1D1 and T2D1) (Table 4).

The results show important information about the influence of treatment of the bioprocessed compounds on

Table 4. Mean results obtained in the simplified physicochemical characterization, visual inspection of absence (-) or presence (+) of microorganisms, germination index and degree of phytotoxicity in the treatment with different doses of RBST and RBCT concentrated (10%) and diluted (1%).

Analyzed parameters	Treatment/Concentration				Attestation
	T1D1	T1D2	T2D1	T2D2	WC
pH	4.75±0.010 ^d	5.15±0.010 ^c	4.73±0.008 ^d	5.18±0.017 ^b	5.83±0.018 ^a
EC (dS m ⁻¹)	1.73±5.513 ^a	0.25±5.642 ^c	1.35±5.748 ^b	0.18±5.518 ^d	0.003±0.007 ^e
TDS (mg L ⁻¹)	951.50±3.390 ^a	135.63±3.470 ^c	742.17±3.535 ^b	100.49±3.393 ^d	2.05±0.046 ^e
Visual inspection	+	+	-	-	-
GI (%)	3.57	0.08	90.24	96.57	97.82
Phytotoxicity degree	Very phytotoxic	Very phytotoxic	Non-phytotoxic	Non-phytotoxic	Non-phytotoxic

Means followed by the same letters on the lines do not differ among them by Tukey test, at 5% probability.

Table 5. Average number of germinated seeds (NSG) (%) and average roots length (RL) (mm) of *Lycopersicon lycopersicum* (tomato), to five days after sowing (DAS) in different treatments (WC, T1D1, T1D2, T2D1 and T2D2).

Treatments/Parameters	WC	T1D1	T1D2	T2D1	T2D2
NSG (%)	92 ^a	84 ^a	24 ^b	88 ^a	90 ^a
RL (mm)	36.59 ^a	2.32 ^b	0.22 ^b	33.37 ^a	38.02 ^a

Means followed by the same letters on the lines do not differ among them by Tukey test, at 5% probability.

their chemical and physicochemical characteristics, which can alter their effects on the development of other plants. According to Chaves et al. (2004), the pH directly influences the development of microorganisms and temperature of sterilization, among other factors, corroborating to the data obtained, concerning to the classification of the degree of phytotoxicity found for the four treatments performed (Table 4).

During the visual inspection for the absence (-) or presence (+) of microorganisms in different doses of RBST and RBCT, after 3 days of seeding, it was observed on plates containing aqueous extract without treatment/sterilization, in the proportion to 1:10 to 1:100, a low rate of germination and visible presence of microorganism colonies, demonstrating the phytotoxicity. Dampened plates with deionized water (WC) and treated extracts already presented in this period, even on the third day, acceptable germination rate, values above 75%.

Generally, the germination capacity is often affected by the presence of pathogens inside or on the surface of the seeds (Souza et al., 2013). In this case, in T1D1, the most concentrated extract (proportion to 1:10), it was observed that the microorganisms present on the fifth day, not affect the germination process.

On other hand, in T1D2, the germination capacity of the seeds was affected (Table 5). In this case, the extract had become more diluted, in ratio to 1:100 as well as the BR used for the preparation of both extracts had not undergone any treatment, in addition to washing and pre

drying stove. In T2D1 and T2D2 extracts, where there was a previous treatment, sterilization, it was observed that the germination of the seeds was not affected (Table 5).

Evaluation of the health of seeds enables development, more precisely, treatments to promote the elimination of present pathogens provide the restoration of health quality (Souza et al., 2013). Among the treatments, the phytotoxicity sources induced different effects regarding to the size and appearance of sunn hemp seedlings (Nunes et al., 2009). The same effect was observed, regarding to the appearance of roots of *L. lycopersicum*.

In Table 5 are shown results for the average number of germinated seeds (NSG) and average root length (RL) which led to the germination index (GI) of *L. lycopersicum* and its classification of phytotoxicity degree of the tested extracts (Table 4), obtained on the 5th day after sowing (DAS) in different treatments.

Of the four extracts evaluated among those who were previously treated, T2D1 and T2D2, regardless of the applied concentration, showed no phytotoxicity to the tested plant, being classified according to the germination index obtained (Figure 1).

The said extracts, T2D1 and T2D2 (Figure 1), when applied to substrates did not inhibit germination of the seeds of *L. lycopersicum* (Table 5) nor the growth of their roots. This may have occurred due to pH values presented (from 4.73 to 5.18) (Table 4) respectively acids to all the treatments, as well as the ionic behavior of the extracts (Figure 2).

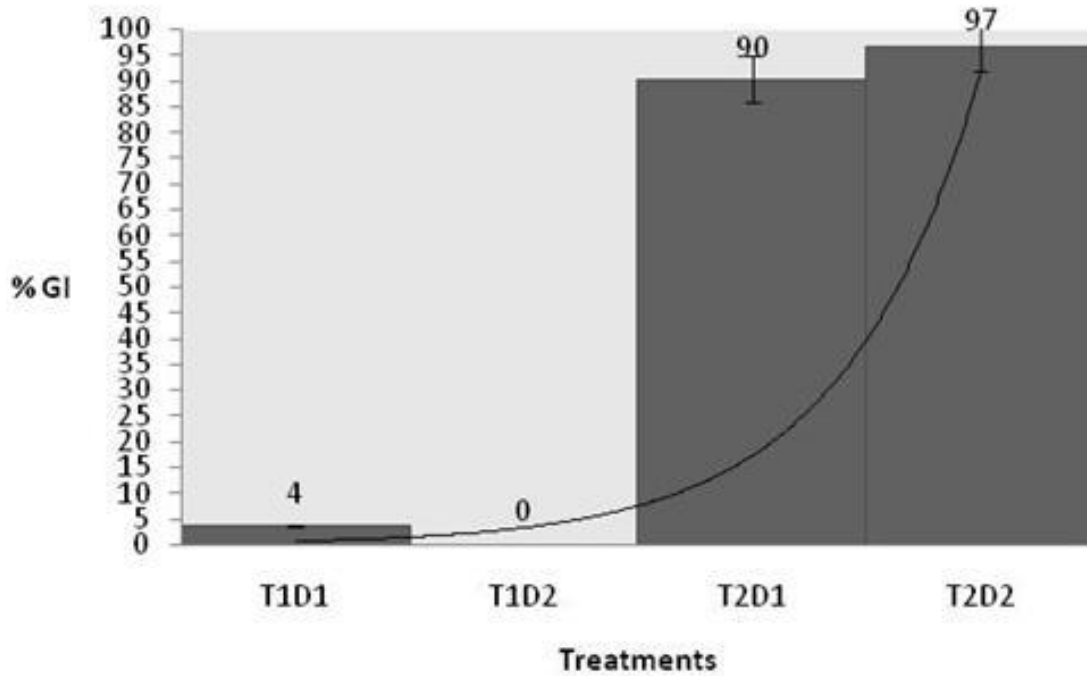


Figure 1. Germination Index (GI) of *L. lycopersicum* (tomato) obtained at 5 days after sowing (DAS) with *Myrciaria dubia* extracts of different treatments, T1D1, T1D2, T2D1 and T2D2 of BR.

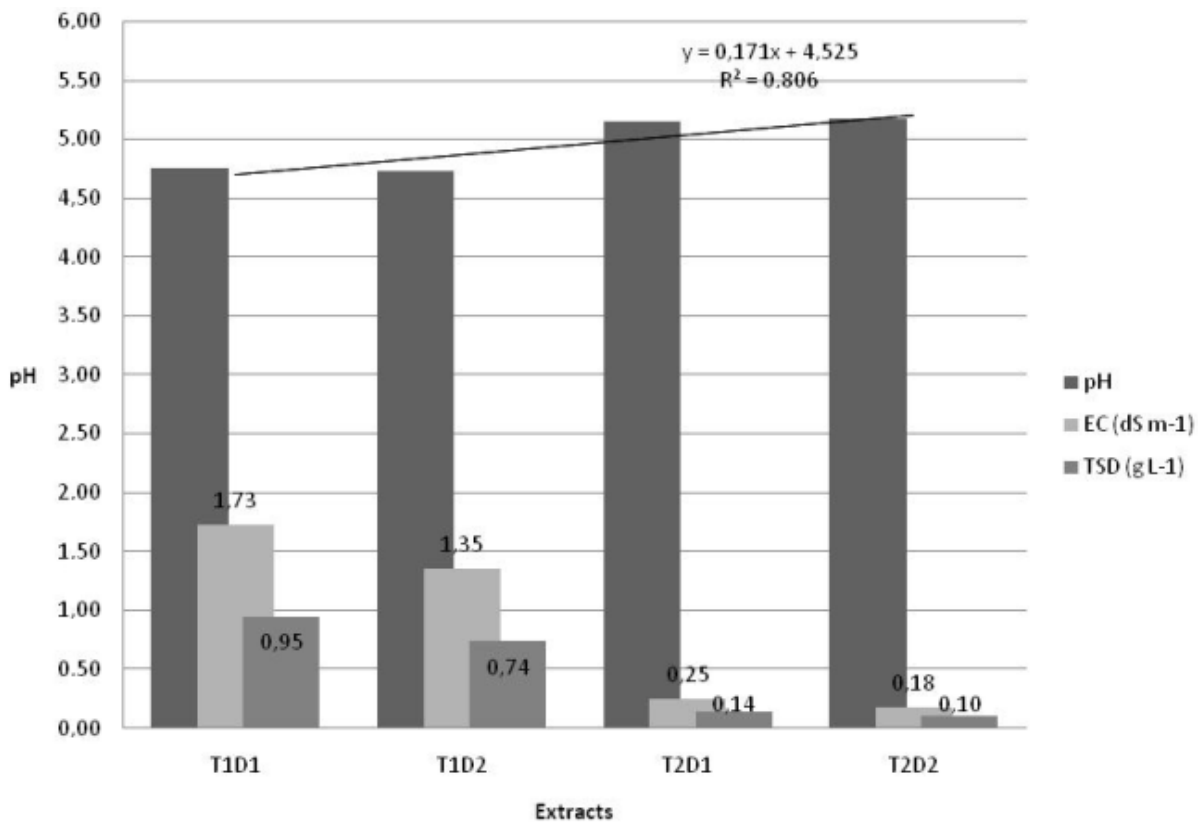


Figure 2. Demonstration related to pH trends, interrelated to electrical conductivity (EC) and total dissolved salts contents (TDS) of the four types extracts (T1D1, T1D2, T2D1 and T2D2) of bioprocessed remaining of *Myrciaria dubia* evaluated.

Figure 2 shows the ionic behavior of the extracts analyzed, T1D1 and T1D2 untreated and T2D1 and T2D2 with treatment when the pH values obtained are interrelated to electrical conductivity (EC) and total dissolved salts contents (TDSC).

The acidity and pH are considered important antimicrobial factors, providing greater stability to the product and to the microorganism's development (Souza et al., 2009). Despite of the presence of this natural barrier, the physiology of many yeasts and molds allows its adaptation to these adverse conditions, growing on substrates with intolerable sugar concentrations for bacteria, because they are not so sensitive to the high osmotic pressure. For these characteristics, Souza et al. (2006, 2009) found pH values ranging between 3.15 and 4.66. When evaluating the effect of particle size suspended in mango juice on the electrical conductivity, Vieira and Cartapatti-Stuchi (2006), concluded that the larger the particles on suspension, the lower will be the electrical conductivity, due to reduced ion mobility. This fact can be observed for EC results obtained in T1D1 and T2D1 (Figure 2).

From the mentioned results (Figure 2), it can be verified from data obtained by other authors, that the electric conductivity can be influenced by many parameters, such as temperature, electrolyte concentration, contents of chemical components, viscosity, solids in suspension, electrolytic strength and the presence of cellular structures (Min et al., 2007).

According to Lewicki (2004), it can be inferred that the electrical conductivity occurs in media containing electrically charged molecules. Thus, as was mentioned by Min et al. (2007), *in situ* measurement of the electrical conductivity makes it possible to check the physical and chemical changes during the plant products transformation process.

The electrical conductivity (EC) reflects the salinity degree, which may indicate possible phytotoxic effects on the germination and growth of plants (Lin, 2008). Therefore, it can be a factor with a determining effect, mainly in the germination stage. For this study, it was found that the same trend, only with extracts which have not undergone any heat treatment. These showed relatively high electrical conductivity values (Figure 2), making possible its influence on the GI obtained; 3.6 and 0.1% for T1D1 T1D2 (Figure 1).

In this context, the results obtained from the analysis of electrical conductivity, total dissolved salts of aqueous extract at the beginning of the experiment and further visual inspection of the presence or absence of microorganisms, as well as the pH, facilitated the testing and evaluation of the phytotoxicity of bioprocessed remaining of *M. dubia* seeds.

Extracts derived from bioprocessed remaining, *M. dubia* seeds from the northern Amazon, including only those treated with thermally sterilized (T2D1 and T2D2) independent of the applied concentration, were not

phytotoxic to the production of tomato.

Fracassetti et al. (2013) performed an evaluation of polyphenols, vitamin C content and antioxidant capacity of the dehydrated pulp, dry powder, the flour obtained from the peel and seeds, remaining residue after processing the *M. dubia* fruits. As result, fifty-three different phenolic compounds were determined, which were characterized by cutting-edge instruments. However, the content of phenolic compounds of remaining residue flour was higher than the pulp powder (4007.95 mg/100 g vs. 48.54 mg/100 g).

The flour is a rich source of bioactive compounds with potential health-promoting properties such as antioxidant, anti-inflammatory activity and hypocholesterolemic which have been linked to vitamin C and phenolic compounds such as flavonoids and ellagitannins. Nevertheless, according to the researchers, works *in vivo* and intervention studies are needed to assess the nutritional and functional potential of this product (Fracassetti et al., 2013).

According to Akter et al. (2011), Yuyama (2011) and Chagas et al. (2015), fruit of *M. dubia* (Caçari) is promising sources of various bioactive compounds like vitamin C, carotenoids and phenolic compounds. They are also good sources of potassium, iron, calcium, phosphorus and various types of amino acids, such as serine, valine and leucine. In this way, these evidences enable the following inferences, the presence of different bioactive compounds in this fruit can be used to retard or prevent various diseases such as cardiovascular disease and cancer (Akter et al., 2011).

The methodological process applied allowed a quick assessment, within 120 h, the aqueous extracts of *M. dubia* effect on seed germination and root growth of *L. lycopersicum*. From originated extracts of BR, those who were treated independent of the applied concentration, were not phytotoxic in the initial production of tomatoes.

Conclusions

The results show important information about the influence of treatment of the bioprocessed compounds on their chemical and physicochemical characteristics, which can alter their effects on the development of other plants. Therefore, there is possibility of availability of finished products and raw materials from the *M. dubia* seeds, since previously sterilized.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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ABBREVIATIONS

BR, Bioprocessed remaining; **S**, sand; **SS**, sandy soil; **CS**, clay soil; **EC**, electrical conductivity; **TDS**, total dissolved salts; **RBST**, untreated bioprocessed remaining; **RBCT**, treated bioprocessed remaining; **NSG**, number of germinated seeds; **RL**, root length; **GI**, germination index; **RSG**, relative percentage of germination; **DAS**, day after sowing.

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