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REVIEW

Viability of using organic substrates according to toxicity tests and the antioxidant activities of tomato seeds and seedlings

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Abstract

T.R. Marcon, A. Rafagnin-da-Silva, R.O. Meira, L.P.C. Guedes, J.M. Corsato, and A.M.T. Fortes. 2020. Viability of using organic substrates according to toxicity tests and the antioxidant activities of tomato seeds and seedlings. Int. J. Agric. Nat. Resour. Plant growth can be directly influenced by the physical, chemical, and biological characteristics of their substrates. We tested the viability of using alternative substrates derived from agroindustrial residues by evaluating their effects on tomato (*Solanum lycopersicum* L.) germination and growth as well as the antioxidant activities in seeds and seedlings. The extracts were classified based on their principal carbon source: cotton waste (SA); sugarcane waste (SB); napier grass (SN); tree prunings (SP); and sawdust (SS). The chemical attributes of the substrates were analyzed, the physiological characteristics of tomato seed germination were determined, and the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were measured in tomato seeds and seedlings. The extracts from tree prunings and sawdust showed the highest germination indices and root lengths, while the cotton and sugarcane waste extracts showed the lowest values for the same variables, with high pH and electric conductivity values indicating possible toxicity; increased activities of antioxidant enzymes needed to correct physiological imbalances were detected. The substrates deemed most suitable for seedling production were those derived from tree prunings and sawdust.

Key words: antioxidant enzyme, composting, germination, organic residues, oxidative stress, *Solanum lycopersicum*.

Introduction

The oxidative protection systems of plant cells are responsible for ensuring metabolic balance and avoiding damage during plant development caused by excess production of reactive oxygen species (ROS) (Silveira *et al.*, 2010).

Among the mechanisms responsible for oxidative protection are antioxidant enzymes that function to eliminate ROS through the degradation of free radicals (Curvêlo *et al.*, 2013). The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Silveira *et al.*, 2010).

SOD is a key enzyme involved in the catalysis of singlet oxygen molecules ($O_2^{\bullet-}$) into atmospheric

oxygen (O₂) and hydrogen peroxide (H₂O₂) (Alscher *et al.*, 2002). CAT and POD enzymes, in turn, protect the cells from damage caused by excessive H₂O₂ by converting it into H₂O and O₂ (Silveira *et al.*, 2010; Bhatt & Tripathi, 2011).

Many factors can contribute to increases in cell ROS levels, such as extreme temperatures, water stress, and high substrate concentrations of heavy metals and salts (Chagas *et al.*, 2013). Excess substrate salts reduce the osmotic potential of plants and cause water stress; excessive accumulation of salts in plant tissues eventually result in toxicity, causing cell membrane damage and delays in seed germination – thus reducing commercial yields (Silveira *et al.*, 2010).

Metabolic imbalances in plant cells caused by substrate toxicity will result in increased ROS production, resulting in severe damage to cell membrane permeability as well as DNA and protein synthesis (Silveira *et al.*, 2010) and leading to saline stress (Chagas *et al.*, 2013).

Plant growth is directly influenced by the physical, chemical, and biological characteristics of the substrate (Silva & Queiroz, 2014). Tomatoes (*Solanum lycopersicum* L.) are one of the most widely consumed vegetables globally and are widely produced in Brazil (tomatoes are often employed as bioindicators in substrate toxicity tests (Santos *et al.*, 2011)).

Given the influence that substrates can have on seedling quality and plant enzymatic activities (Wathier *et al.*, 2016), it is of the utmost importance to test different substrates using bioindicator species (such as tomatoes) to judge their suitability for commercial use. We sought to evaluate the qualities of alternative substrates derived from agroindustrial residues and to evaluate any toxicity in relation to seed germination and the antioxidant activities of tomato seeds and seedlings.

Materials and Methods

Our analyses were conducted between August and November/2015 at the Plant Physiology Laboratory and the Laboratory of the Analysis of Agroindustrial Residues at the Universidade Estadual do Oeste do Paraná, Campus Cascavel, Paraná State, Brazil.

The substrates tested were extracts of composted organic wastes: cotton waste (SA); sugarcane waste (SB); Napier grass (SN); tree prunings (SP); and sawdust (SS). The controls contained only distilled water (T)

The chemical attributes of the extracts of the different substrates (Tab. 1) were determined by measuring their electrical conductivity (EC), pH (Fermino, 2014), and their concentrations of nitrogen (N), phosphorus (P) (Malavolta, Vitti & Oliveira, 2009), potassium (K) (Embrapa, 2009), and total carbon (C) (Cunha-Queda *et al.*, 2003).

To obtain the extracts, 5 g of the dry substrate was crushed and homogenized in 50 mL of distilled water (at 60 °C) on a vibratory table for 20 minutes and then filtered (Zucconi *et al.*, 1981). The tomato seeds were then sown to germinate in Petri dishes lined with filter paper moistened with 3 mL of the extract, with 25 tomato seeds per plate. The Petri dishes were incubated in a BOD germination chamber at 25 °C under a 12-hour photoperiod; germination was evaluated daily. At the end of seven days, we calculated the germination percentage (%) (Brasil, 2009), synchronization index, mean germination time (days) (Labouriau, 1983), and germination speed index (Edmond & Drapala, 1958). The germination index (%) (Zucconi *et al.*, 1981) was calculated using the following formula:

$$IG(\%) = \frac{S_t \times Co_t}{S_c \times Co_c} \times 100$$

where

S_t represents the percentage of germinated seeds in the treatment,

Co_t represents the root length in the treatment (cm),

S_c represents the percentage of control seeds germinated, and

Co_c represents the mean root length of the control seedlings (cm).

The physiological variables of tomato seedlings were evaluated in a one-factorial experiment employing a completely randomized design, considering the six treatments mentioned above (extracts from the organic substrates - SA, SB, SN, SP, SS, and the control - distilled water), each with four replicates.

The activities of the antioxidant enzymes of the tomato seeds and seedlings were examined in a 6×3 two-factor arrangement, with the substrates designated as the first factor, while the times (2, 12, and 24 hours) when seeds were collected for analysis were designated as the second factor.

On the fourteenth day of evaluation, destructive analyses of tomato seedlings were conducted to measure the root (cm) and shoot length (cm) with extraction (Kar & Mishra, 1976), and characterization of the total soluble proteins (Bradford, 1976) was performed to determine the activities of the antioxidant enzymes superoxide dismutase (SOD) (Beauchamp & Fridovich, 1971), peroxidase (POD) (Teisseire & Guy, 2000), and catalase (CAT) (Azevedo *et al.*, 1998).

Statistical methods

The data from the one-factor and two-factor experiments were tested for normality (Shapiro-Wilk) and homogeneity of variance (Bartlett), and whenever necessary, transformations were performed. Analysis of variance (ANOVA) was then performed, and the means of the treatments

were compared using the Tukey test. The treatments were also compared to the control using Dunnett's test. The statistical analyses were performed using R statistical software (R Development Core Team, 2017) at a 5% level of significance.

Results and Discussion

Analyses of the specific activities of the antioxidant enzymes (SOD, CAT and POD) in the first 24 hours of germination (Tab. 1) showed increased SOD and CAT activities after 12 hours of imbibition in the Control (T - distilled water) treatment group (Fig. 1A).

Thus, activation of the antioxidant defense systems occurs soon after seed hydration and reactivation of their quiescent metabolism, generating free radicals. Enzymes then act to minimize cell damage and maintain a balance between the production and elimination of ROS, thus ensuring seed germination (Barbosa *et al.*, 2014).

The seeds exposed to the cotton waste extract (SA) (Fig. 1B) demonstrated the same SOD enzyme pattern observed in the Control, with increased specific activity 12 hours after imbibition. CAT, however, showed greater activity within 2 hours of imbibition (similar to the activity shown by the control at 12 hours) and reduced activity after 12 and 24 hours of imbibition.

It could therefore be observed that CAT enzyme activity was required at the beginning of the germination process of seeds exposed to the cotton waste extract, presumably to eliminate hydrogen peroxide radicals.

The CAT enzyme acts as an effective catalyst for hydrogen peroxide (H_2O_2) decomposition into H_2O and O_2 (Dubey, 2011), and the increasing CAT activity observed within the first two hours of evaluation appeared relevant to repairing damage resulting from lipid peroxidation that mainly occurred during Phase I of seed germination (the

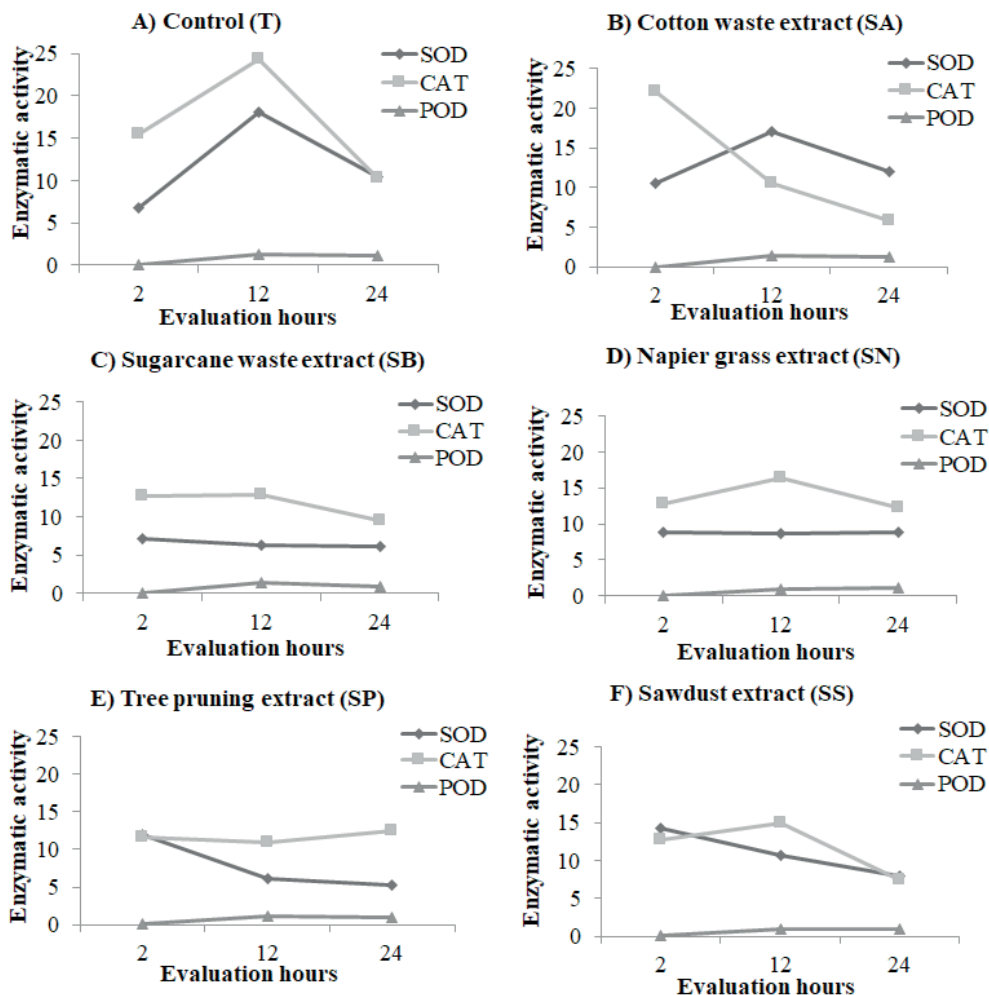


Figure 1. Graphs demonstrating enzyme behaviors: superoxide dismutase – SOD (U l mg^{-1} protein), catalase – CAT ($\text{H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$ of protein), and peroxidase – POD ($\mu\text{mol min}^{-1} \text{ mg}^{-1}$ protein) in tomato seeds in the first hours after germination (2, 12 and 24 hours) when exposed to different organic substrate extracts.

water absorption stage); these repairs are facilitated by the increased catalase activity that ensures the elimination of free radicals (Umair *et al.*, 2012).

Tomato seeds exposed to cotton waste extract (Fig. 1B) showed reduced CAT activity in the first two hours after imbibition, but increased SOD activity, indicating compensatory effects of their activities in repairing oxidative damage (Barbosa *et al.*, 2014).

Tomato seeds exposed to sugarcane waste (Fig. 1C) and Napier grass (Fig. 1D) extracts showed

almost constant SOD activity, with slight increases in CAT activity at 12 hours.

Seeds exposed to the tree prunings (Fig. 1E) and sawdust (Fig. 1F) extracts showed reduced SOD activity with increasing imbibition time. SOD is the first enzyme in the antioxidation defense system, a key enzyme that catalyzes the transformation of singlet oxygen ($\text{O}_2^{\bullet-}$) into atmospheric oxygen (O_2) and hydrogen peroxide (H_2O_2) (Alscher, Erturk & Heath, 2002).

Independent of the use (or not) of any type of organic substrate extract, POD activity remained

practically constant during tomato seed metabolic reactivation.

POD enzyme activity can vary greatly, as it depends on numerous factors, such as the type of inducer, the timing of plant exposure, and the concentration of the inducer, so that standard behaviors are not generally observed (Bhatt & Tripathi, 2011).

CAT-mediated repair was apparently efficient in protecting the cells from oxidative damage due

to excessive accumulation of H_2O_2 (Silveira *et al.*, 2010), as there were significant reductions in its activity within 24 hours of germination.

It is therefore possible to conclude that the chemical, physical, and biological characteristics of the extracts will affect the seedlings in terms of their antioxidant pathways, the enzyme activities of plant cell metabolism (Watthier, 2016) (Fig. 1), and the physiological processes related to tomato seed germination (Fig. 2).

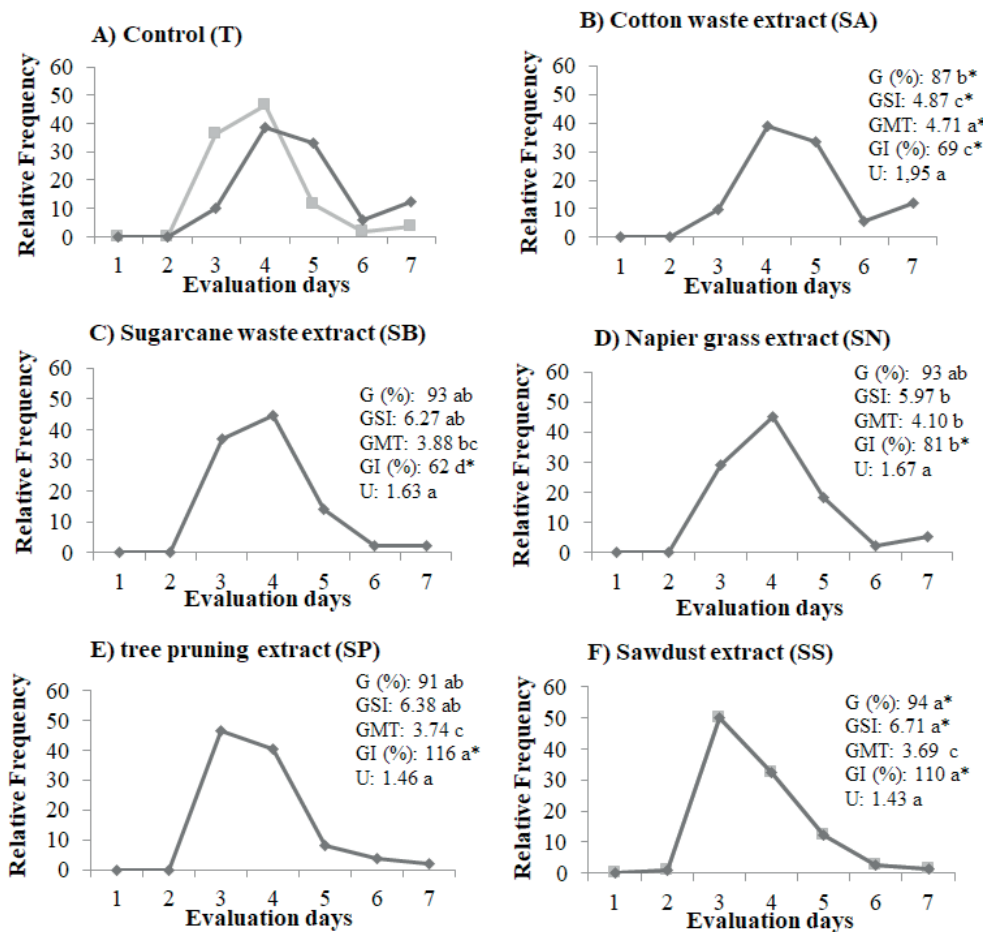


Figure 2. Graphs of the relative germination frequencies and average values of the following variables: germination percentage (G%), germination speed index (GSI), mean germination time (GMT), synchronization index (U), and germination index (GI%) of tomato seeds germinated on different organic extracts.

*Averages marked with asterisks represent statistically significant differences compared with the Control (T) by the Dunnett test (at a 5% level of significance). Similar letters indicate that the results do not differ from each other by the Tukey test (at a 5% level of significance).

The seeds exposed to the sawdust extract (SS) demonstrated mean germination rates of 94% (G%), the best result for any of the extracts tested (Fig. 2F), although they were not significantly different from seeds exposed to sugarcane waste (SB), Napier grass (SN), and tree pruning (SP) extracts (Figs. 2C to 2E). They were, however, significantly different from the control (T) (Fig. 2A) and the cotton extract (SA) (Fig. 2B), both of which showed 87% germination (considering a 5% level of significance).

The use of sawdust together with other agro-industrial wastes in composting processes has been shown to produce substrates that are amenable to lettuce and wild cabbage seedling production, with similar or better results compared to those obtained from commercial substrates, indicating its potential use in vegetable cultivation and corroborating our results for the sawdust-based substrate (which demonstrated a higher germination percentage than the control).

In addition to the sawdust extract substrate, the extracts of Napier grass, sugarcane waste, and tree prunings showed high average tomato seed germination percentage values, corroborating the results obtained for the germination speed index (GSI), with the highest mean values observed in seeds sown on the sawdust (Fig. 2F), tree pruning (Fig. 2E) and sugarcane waste extracts (Fig. 2C) (with germination speed indices of 6.71, 6.38 and 6.27, respectively).

Satisfactory results were similarly reported when using sugarcane waste extracts mixed

with sand and peanut husks in the cultivation of cherry tomatoes (Sindy cultivar) (Gonçalves *et al.*, 2014).

The lowest mean germination speed index was observed with seeds sown onto the cotton waste extract (SA) (4.87) (Fig. 2B); this treatment also showed the highest mean germination time (4.71), differing statistically from all the other treatments (Fig. 2).

Tomato seeds sown onto the tree pruning (SP) (Fig. 2E) and sawdust (SS) extracts (Fig. 2F) also had low mean germination times (GMT) (approximately three and a half days), with no significant difference between them, the sugarcane waste extract (SB) (Fig. 2C) or the control (Fig. 2A); the tomato seeds sown onto the SP extract demonstrated the greatest ability to germinate in the shortest time interval.

These results are apparently related to extract characteristics such as pH, electrical conductivity (Tab. 1), as their chemical properties are indicative of the concentrations of salt ions, which are linked to toxicity (Silveira *et al.*, 2010).

The electrical conductivity of an extract can be defined as very low (between 0 and 0.75 dS m⁻¹), low (between 0.76 and 2.0 dS m⁻¹), adequate for vegetable production (between 2.0 and 3.5 dS m⁻¹), or unsuitable for vegetable production (above 3.5 dS m⁻¹) (Gruszynski, 2002).

The sawdust (SS) and tree pruning (SP) extracts demonstrated electrical conductivity values

Table 1. Chemical attributes of the extracts of the substrates derived from different carbon sources.

Extracts of the substrates derived from different carbon sources	EC (ms Cma ⁻¹ 25 °C)	pH	N (%)	P (g kg ⁻¹)	K (g kg ⁻¹)	C (%)
Cotton waste extract (SA)	4.76	7.41	3.17	7.48	21.86	34
Sugarcane waste extract (SB)	4.09	6.99	2.25	6.05	9.25	32
Napier grass extract (SN)	4.62	7.92	2.51	5.25	18.88	30.6
Tree pruning extract (SP)	2.82	7.73	2.55	5.38	11.39	29.7
Sawdust extract (SS)	2.34	7.32	1.65	5.62	5.2	23.8

between 2.34 and 2.82 dS m⁻¹ at 25 °C (Tab. 1), which are considered acceptable in terms of the germination physiology of tomato seeds and vegetable cultivation in general (Gruszynski, 2002).

The cotton (SA) and sugarcane (SB) wastes and Napier grass (SN) demonstrated electrical conductivity values above 3.5 dS m⁻¹ (Tab. 1) and are therefore not recommended for cultivating vegetables due to osmotic-saline stress caused by excess salts (Gruszynski, 2002).

As the tree pruning (SP) and sawdust (SS) extracts showed the lowest electrical conductivity values, pH, and nutrient concentrations (N, P, K, Ca and Mg) when compared to the other extracts evaluated, our results corroborate another study that likewise observed lower values of those factors in a 100% sawdust extract (Silveira *et al.*, 2010).

The germination index (GI%) is one of the most sensitive parameters for indicating substrate toxicity, and it is widely used to classify substrate quality, as it takes into account both root growth (responsive to even low substrate toxicity) and seed germination (responsive to high degrees of toxicity) (Zucconi *et al.*, 1981).

On average, the lowest germination index values were observed in seeds sown onto substrate extracts based on sugarcane (62%) (Fig. 2C) and cotton (69%) wastes (Fig. 2B); their values differed statistically from the control (100%) and from the other treatments (Fig. 2) – indicating the probable presence of organic compounds with moderate toxicity, as substrates having germination index values between 60 and 80% will generally show moderate inhibition of seedling growth (Silva & Villas-Bôas, 2007).

Seeds exposed to the tree pruning (Fig. 2E) and sawdust extracts (Fig. 2F) demonstrated average germination index values of 116% and 110%, respectively, which were significantly greater than the control (100%) and those obtained from the other treatments. The germination index values

above 100% observed in the pruning and sawdust treatments indicated the stimulation of seed germination, with beneficial effects on seedling development (Delgado *et al.*, 2010).

No statistically significant differences were detected in terms of the synchronization indices (U) of the different treatments or the control.

The activities of SOD, CAT and POD enzymes in tomato seedlings exposed to the aqueous extracts of the different organic substrates are illustrated in Fig. 3.

The tomato seedlings showed similar enzyme activities in response to all the treatments in terms of SOD (Fig. 3A) and POD (Fig. 3C) activities (with no significant difference between them), indicating no stress response to the extracts during that phase of seedling development.

Catalase activity (CAT) (Fig. 3B), however, showed higher than average activity in tomato seedlings exposed to sugarcane waste (SB) and sawdust (SS) extracts, and they differed significantly from the other extracts and the control.

Although treatment with the sawdust extract (SS) was not significantly different in terms of catalase activity, it was apparently effective in controlling the oxidative imbalance in the cellular medium, as the seedlings exposed to that treatment showed the longest root length values and highest shoot growth values (Fig. 4), indicating that their development was not impaired.

Catalase activity apparently was not sufficiently effective in overcoming the stress induced in tomato seedlings by the sugarcane waste extract (SB), with no significant differences seen in regards to the other treatments (Fig. 3B).

Plants exposed to stress conditions concentrate their energy on antioxidant defense to overcome these stress (Sunaina & Singh, 2014). Such redirection of metabolic energy can impair full plant

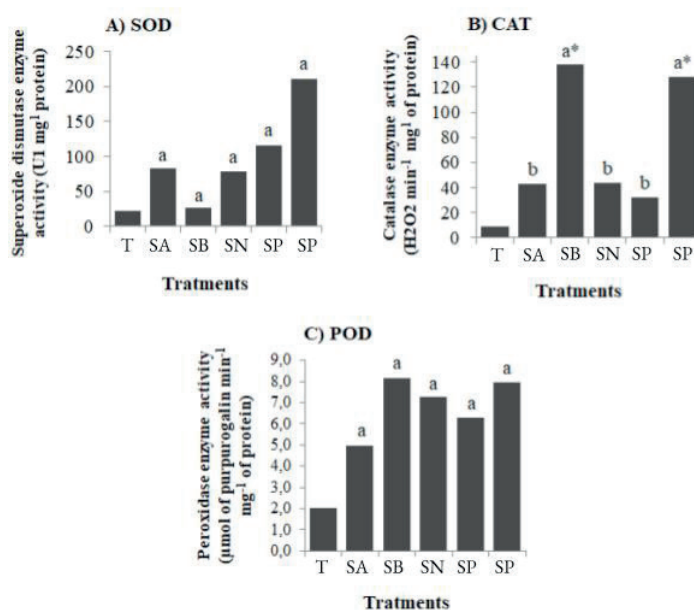


Figure 3. Graphs of the mean specific enzyme activities of a) superoxide dismutase (SOD); b) catalase (CAT); and, c) peroxidase (POD) in tomato seedlings 168 hours after germination in the following: distilled water, Control – (T); cotton waste extract (SA); sugarcane waste extract (SB); Napier grass extract (SN); tree pruning extract (SP); sawdust extract (SS).

*The means all demonstrated statistically significant differences from that of the Control (T) according to the Dunnett test (at a 5% level of significance). The same letters above the columns indicate no significant differences from each other by the Tukey test (at a 5% level of significance).

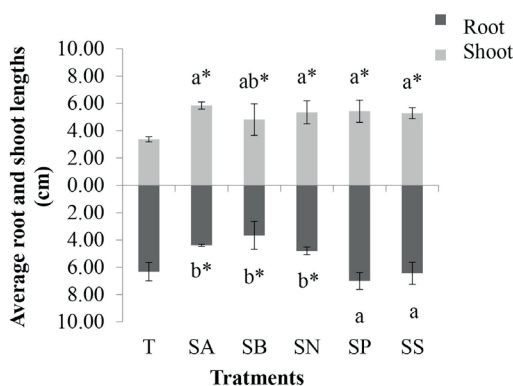


Figure 4. The columns in the graph indicate the average root and shoot lengths of tomato seedlings germinated on each of the following substrates: distilled water (Control – T); cotton waste extract (SA); sugarcane waste extract (SB); Napier grass extract (SN); tree pruning extract (SP); sawdust extract (SS).

*Averages demonstrating significant differences from that of the Control (T) according to the Dunnett test (considering a 5% level of significance). The same letters above or below the columns indicate that the substrates did not differ one from each other by the Tukey test (considering a 5% level of significance).

development and have effects such as reducing the lengths of their roots. Similar results have been observed with the roots of seedlings germinated on sugarcane waste extracts (SB), indicating a possible toxic oxidative-stress effect of that substrate.

Upon examining the shoot and root lengths of the tomato seedlings, it was observed that all the treatments resulted in average shoot lengths near 5 cm, which statistically differed from the control (3.36 cm) (Fig. 4).

The lower shoot lengths of tomato seedlings grown under control (distilled water) conditions can be explained by the lack of nutrients in that substrate.

The mean root lengths of the tomato seedlings were lower in the cotton (SA), sugarcane (SB) waste and Napier grass (SN) treatments (4.39, 3.66 and 4.81 cm, respectively), and they differed

statistically from the control (6.32 cm) as well as from the other extracts tested (Fig. 4); the high electrical conductivities (Tab. 1) of those extracts could explain these results. Substrates must have adequate pH and EC values (Kratz *et al.*, 2013) – or their high concentrations of soluble salts can cause “burning”, necrosis, or poor root development [which apparently occurred with the use of extracts of cotton (SA), sugarcane wastes (SB) and Napier grass (SN)].

The substrate produced from composting 100% Napier grass was found to demonstrate the poorest result in terms of the heights of the tomato plants (2.3 cm) when compared to a commercial substrate (8.4 cm) (Leal *et al.*, 2007), corroborating the results of the present study in which the Napier grass extract resulted in the smallest root lengths.

A combination of 33% Napier grass with 66% crotalaria was found, however, to yield high height values in tomatoes, lettuce, and beet plants (Leal *et al.*, 2007), enabling the use of Napier grass in mixtures with other organic materials.

The greatest root lengths of tomato seedlings were observed in the extracts derived from tree prunings (SP) and sawdust (SS) (7.01 and 6.44 cm, respectively), although they did not statistically differ from those of the control (6.32 cm); these values indicate the absence of toxic components in those substrates.

Waste material resulting from the pruning of urban trees could therefore provide material to form high-quality organic composts that are useful for seedling production while simultaneously minimizing environmental impacts and production costs.

Our results show that the chemical, physical, and biological characteristics of organic substrates can affect seedling quality (Wattier *et al.*, 2016), in terms of the physiological processes of seed germination (Fig. 2), seedling shoot and root lengths (Fig. 4), and antioxidant pathways of plant cell metabolism (through changes in superoxide dismutase, catalase, and peroxidase activities) (Fig. 1 and 3).

The main conclusions are the following. Extracts derived from tree prunings and sawdust showed the greatest positive effects on seed germination, potentially enhancing the growth and development of tomato seedlings in substrates that are free of toxicity and oxidative stress and, therefore, appear to be the most suitable for tomato seedling production.

Extracts derived from cotton and sugarcane waste, however, showed negative impacts on the tomato seed germination index and tomato seedling lengths, indicating their moderate toxicity. Under those conditions, the antioxidant enzyme defense systems (CAT, SOD and POD) of the seeds and seedlings were not effective in reversing stress-induced damage.

Resumen

T.R. Marcon, A. Rafagnin-da-Silva, R.O. Meira, L.P.C. Guedes, J.M. Corsato, y A.M.T. Fortes. 2020. Viabilidad del uso de sustratos orgánicos según las pruebas de toxicidad y las actividades antioxidantes de las semillas y plántulas de tomate. Int. J. Agric. Nat. Resour. El crecimiento de las plantas puede estar directamente influenciado por las características físicas, químicas y biológicas de sus sustratos. Probamos la viabilidad de utilizar sustratos alternativos derivados de residuos agroindustriales mediante la evaluación de sus efectos sobre la germinación y el crecimiento del tomate (*Solanum lycopersicum* L.), así como sobre las actividades antioxidantes de sus semillas y plántulas. Los extractos se clasificaron en función de su principal fuente de carbono: residuos de algodón (SA); desechos de caña de azúcar (SB); napier grass (SN); podas de árboles (SP); y aserrín (SS). Se analizaron los atributos químicos

de los sustratos, se determinaron las características fisiológicas de la germinación de la semilla de tomate y se midieron las actividades de las enzimas antioxidantes SOD, CAT y POD en semillas de tomate y plántulas. Los extractos de poda de árboles y aserrín mostraron los índices de germinación y longitud de raíz más altos, mientras que los extractos de algodón y residuos de caña de azúcar mostraron los valores más bajos de esas mismas variables, con altos valores de pH y conductividad eléctrica que apuntan a una posible toxicidad; se detectaron actividades incrementadas de enzimas antioxidantes para corregir los desequilibrios fisiológicos. Los sustratos considerados más adecuados para la producción de plántulas fueron los basados en poda de árboles y aserrín.

Palabras clave: compostaje, enzima antioxidante, estrés oxidativo, germinación, residuos orgánicos, *Solanum lycopersicum*.

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