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Evaluation of a prototype of soil thermal solarizer for control of gall nematode in the production of coffee seedlings

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ABSTRACT

In coffee crops at Rondônia State of Brazil, there is an increase in phytonematode epidemics.Since most local coffe nurseries at Rondônia use soil as substrate for seedling production, this work aimed to test the minimum exposure time at 60 ºC to control of root-knot nematodes *Meloidogyne incognita* in soil by means of solar heating, using a solarizer, in an adapted version for better heating perfomance. The used solarizer prototype was built of a wooden box covered with metal sheets, thermal blankets, painted black, with aluminum pipes of 0.5 cm in diameter at the bottom of the box that circulates the water heated by the collector box. The soil was inoculated with a suspension of 1000 eggs + J2 of *M. incognita* per liter and placed in equipment with a capacity of 150 liters. Exposure times at a minimum temperature of 60 °C were evaluated, with 14 treatments: C1 (non-inoculated and not autoclaved), C2 (inoculated), C3 (non-inoculated and autoclaved) T0', T15', T30', T45', T60', T180', T360', T720', T1440', T2880' and T4320' . The temperature was measured every 15 minutes using a skewer-type digital thermometer. The soil was removed according to the exposure time, and placed in 8-liter pots, in which clone 125 and BRS 2357 coffee seedlings, susceptible to *M. incognita* , were transplanted and growing in a greenhouse for 180 days. The experimental design used was DIC, with 6 replications, each seedling as na experimental unit. Reproduction factor (FR), number of eggs per plant (NOGR) and number of galls per gram of root (NGGR) were evaluated. The FR, NOGR and NGGR variables reduced with exposure time. All treatments showed an effect to control the population of *M. incognita* in infected soils, with 100% efficacy of pathogen eradication from the T45' treatment, 60 minutes of exposure is recommended for the production of *Coffea canephora* seedlings. Taken together, thir work showed the high potential of thr solorizer to contribute in the root-knot nematode control to seedling production.

Key words: Thermal control; *Meloidogyne incognita; Coffea canephora.*

1 INTRODUCTION

Brazilian coffee production is expected to reach 46.9 million bags benefited in 2021, according to the third crop survey carried out by the National Supply Company - CONAB. These data represent a reduction of 25.7% compared to the 2020 total Brazilian coffee yield. At the same time, the production area is estimated at 1.8 million hectares, a reduction of 4.4% compared to the previous crop (Companhia Nacional de Aastecimento - CONAB, 2021).

The *Coffea canephora* culture have great economic and social importance in Rondônia; activity more concentrated in the Zona da Mata in the southwest of Rondônia, however, there are municipalities in the north and central region that also stand out as major producer (Santos; Da Costa Silva, 2017).

In Rondônia, the harvest stood at around 2,167.7 thousand bags of processed conilon coffee, in an area of 63.6 thousand hectares. Even with the use of new areas that were recovered for production, using materials of great production capacity, the 2021 weather conditions influenced the progress of the crop, causing a reduction of 11.3% when compared to the volume of the previous season (CONAB, 2021). Additionally, the occurrence of pests and diseases is among the factors that contribute negativelly in crop productivity. The root-knot nematodes, of the genus Meloidogyne are among the most importante soil pathogens in coffee crops, where production is estimated to fall by 20 to 40% in infested areas (Rosso et al., 2011; Oliveira et al., 2015).

The Root-knot nematodes have a great economic impact on coffee production in several coffee-producing countries. (Fatobene et al., 2018). There are many genera and species of phytonematodes linked to coffee production, causing significant damage and even plant mortality, in addition to economic damage, affecting the economy at the local level and expanding according to the spread of the pathogen (Villain; Salgado; Trinh, 2018).

Infected seeds and seedlings can introduce a new pathogen or increase its occurrence in areas of incidence (Bedendo; Massola-Junior; Amorin, 2018), these organisms live in the soil and parasitize the roots, forming feeding sites.

Their structures, such as eggs and cysts, remain in the soil in the absence of a host, which makes disinfestation difficult (Lima et al., 2019), in addition to being polyphagous, they produce resistance structures for survival for long periods in edaphoclimatic conditions (Oliveira et al., 2017).

To survive in the soil, some characteristics are necessary, for example the temperature, for its development it must remain from 15 to 30 °C, being able to become inactive between 5 and 15 °C and 30 to 40 °C, temperatures outside these limits can be fatal from exposure and time. Moisture, where the presence of water is essential for survival and movement, considering the type of soil, which can influence movement, so nematodes are found more frequently in sandy or clayey soils (Ferraz; Brown, 2016).

M. incognita is among the species that attack the coffee tree that cause the most damage, due to its great dissemination and ability to damage the root system, (Goulart et al., 2019), making it difficult to insert areas for cultivation, conservation of areas already infested and causing the death of the plant (De Oliveira et al., 2019). They cause peeling, necrosis, lesions and reduction of the root system and the growth of seedlings and young plants, yellowing and leaf fall and chlorosis (Vieira Júnior; Fernandes, 2015).

Studies carried out in the State of Rondônia between 2004 and 2008 (Vieira Junior et al., 2008) and between 2011 and 2014 (Vieira Junior et al., 2015) on the propagation of root-knot nematode in coffee producing areas, demonstrated the dissemination in the municipalities of the state. In these surveys, the *M. incognita* was verified, especially in the coffee zone of Ji-Paraná. The Meloidogyne ssp. was recently found in nurseries and coffee plantations in 16 municipalities, which have the largest coffee production in Rondônia; the data demonstrate that *M. exigua* and *M. incognita* are the main species that parasitize *C. canephora*. The occurrence of *M. incognita* was higher, being found in most municipalities, accounting for 30% of detections in assessments.

Ordinance no. 558/2016 of the Agrosilvopastoral Health Defense Agency of the State of Rondônia - IDARON, came to regulate phytosanitary processes from production to storage and use of coffee seedlings produced in nurseries. Among the changes is the requirement of laboratory analysis for the presence of nematode in the seedlings, the main objective of the ordinance is the control of the root-knot nematode (Brasil, 2016).

For the production of coffee seedlings, substrates can be commercial and soil types. In terms of substrate and container, seedlings do not suffer significant influences on their development. But there are considerations to be observed depending on the mode of production. When in soils, for example, with the propagation of pathogenic microorganisms (Fonseca et al., 2017).

Chemical disinfestation through methyl bromide was the most used method for the production of coffee seedlings in the country until 1980. However, its use was prohibited due to risks to environmental and human health. There are currently few products on the market registered for the control of phytonematodes in coffee trees. The action of these products is able to reduce the presence of the nematode for about 65 days after application, with an increase in the population after this period (Lima et al., 2019).

The use of heat treatment on substrates using solar energy is the one that stands out method has proven efficient for the treatment against soil pathogens and invasive plants. (Rocha; Carneiro, 2016). Solarization is the process of increasing temperature in the soil, where solar radiation crosses a transparent surface and a greenhouse effect occurs, causing the evaporation of water in the environment and raising the temperature in values that can reach 50 °C or more; this temperature eliminates microorganisms in the soil such as viruses, bacteria, fungi and nematodes that need a temperature below 35 °C to survive; the greenhouse effect caused by the ground cover causes a rise in temperature, which causes the weakening and death of these phytopathogens (Vieira Junior et al., 2016).

With the objective of producing soil for root-knot nematode-free substrate, the present work evaluated different exposure times of contaminated soil, at a minimum lethal temperature of 60 ºC, to verify the shortest exposure time necessary for the eradication of *M. incognita.*

2 MATERIAL AND METHODS

The experiment was carried out at the Brazilian Agricultural Research Corporation/Agroforestry Research Center of Rondônia-Embrapa Rondônia, Rodovia BR 364 Km 5.5, RO, 76815-800 in the phytopathology laboratory and Greenhouse.

The nematodes used in the tests were obtained from the Embrapa experimental field in Ouro Preto do Oeste, RO. These populations were identified by means of electrophoresis, by the method of Carneiro and Almeida (2001) and, multiplied in tomato plants, cultivar “Santa Clara” in a greenhouse, from eggs collected from a single female. For the extraction of eggs, the method of Hussey and Barker (1973), modified by Bonetti and Ferraz (1981) was used. The roots were ground in a blender in a 0.5% hypochlorite solution dissolved in water and the material obtained was sieved through 20, 325 and 500 mesh sieves. From the solution retained on the last sieve, maintaining a volume of 30ml, a suspension of eggs and second-stage juveniles (J2) was obtained.

The equipment used for substrate solarization was developed according to the principles of Ghini (2004) and Vieira Junior et al. (2016). This model aims to continue evaluation off the effects of the solarizer on the nematode with

a Minimum Lethal Temperature - TLM of 55ºc in equipment with a capacity of 100 liters of soil. It was observed that the TLM at 55 ºC influenced the reduction of the number of eggs in the treatments, however, it was not enough to eradicate the presence of *M. Incognita*, even in longer treatments. The eradication of the nematode is the main objective of this work, so the TLM chosen for its accomplishment was 60 ºC. Another factor observed and adapted was the substrate production capacity, seeking to increase the volume of soil, the equipment used in this work has a production capacity of 150 l of soil in the exchanger box (the one that receives the soil to be treated).

A model was built, whose exchanger box can treat soil according to the daily needs of a nursery with dimensions of 1.0 x 2.0 x 0.08m. All the boxes were made of wood and, internally, were coated with metal sheets of galvanized steel number 20. Thermal blankets were placed between the metal sheets and the wooden box. The boxes were painted with black asphalt paint number 2.

Between the insulating lining and the metal cover on the bottom of the exchanger box, 0.5 cm aluminum pipes were installed, which were distributed in the bottom of the boxes in a sinusoidal shape. At the outlet of the tubing of the exchanger box, vehicular-type hoses were connected, for high temperatures, made of rubber and coated with insulating thermal protection. These were connected to the collection box, which receives the cooled water. At the bottom of the collection box, a register was installed and, after this, a 10W and 12V water pump, with a pumping capacity of 250 l/h. This pump drives the water through the hose to the inlet of the heating system of the hot box, which is also made of wood and had the interior coated with an insulating thermal blanket and, on top of this, a steel sheet metal coating nº 20, painted black. Over this structure, aluminum piping was installed, also painted black, through which the heated water circulates. The same hose was installed at the outlet of this box, which is connected to the inlet of the aluminum tubing of the exchanger box. (Vieira Junior et al., 2016).

The closed circuit allows the water to be heated in the collection box and its temperature raised by approximately 80 °C, and by the action of the water pump this heated water is taken to the exchanger box, closing the heating-cooling cycle. Both boxes were closed with 3 mm acrylic sheet, the exchanger box was made in the form of a door, for supply and removal of the soil. The acrylic cover of the collection box was sealed with silicone to increase the water heating efficiency and reduce the heat loss in the system.

The soil was heated from above with the direct incidence of light by the acrylic and below by means of heated water, in a “heat sandwich”. The electric pump is driven by electrical energy produced by transforming sunlight into electrical energy, in a 150 W photovoltaic plate.

The water in the container was kept warm by means of an electric heating resistor, also at 12 V, powered by the photovoltaic plate, intensifying, and accelerating the heating-heat transfer process to the soil. As described and, for the purpose of differentiating the traditional model of solarizer, this new equipment was called “Solarizador-Type-Sandwiched” (STS) (Figure 1), since the soil receives heating in two regions, being between them and going through a faster process of heat exchange, this process is important to reduce the time of heating the soil, to reach the TLM of 60 ºC in less time and increase the efficiency of the equipment.

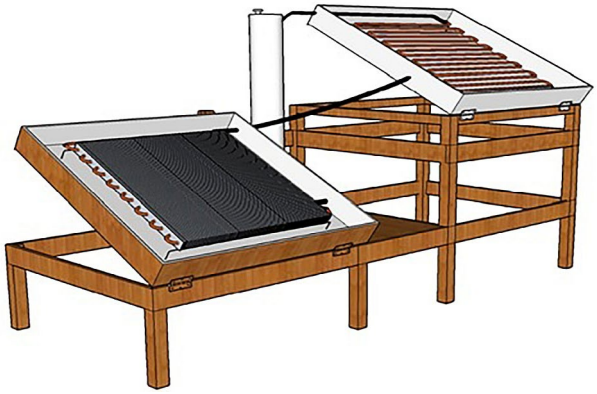

Figure 1: Schematic representation of the Sandwich Solarizer (STS) model. Source: José Roberto Vieira Junior – Embrapa Rondônia.

The test that was carried out in the prototic “STS”, used 150 l of soil per treatment, which was previously solarized in the traditional model adapted by Vieira Júnior et. al. (2016), for a period of one day to eliminate any nematodes that could be contained in the substrate. This soil was stored in impermeable bags until its use in tests with the pathogen.

Then, the previously treated soil was infested with 1000 eggs+J2 (second stage juveniles infective of *Meloidogyne* spp.) per liter of soil for use in the equipment, to determine the population of nematodes present at the beginning of the treatments.

Fourteen treatments were carried out, at 0’, 15’, 30’, 45’, 60’, 180’, 360’, 720’, 1440’, 2880 and 4320 minutes after the temperature reached TLM. The time count was interrupted every time the soil temperature drops one degree Celsius from the TLM, the soil is immediately removed from the solarizer after reaching the exposure time established by its treatment. A part of the substrate treated in the traditional solarizer was used for the controls, being the first (C1) without heating in the STS and without contamination with nematodes, the second (C2) infested with nematode and the third (C3) heated and without infestation with nematodes. nematode.

Coffee seedlings *Coffea canephora* clones C125 and BRS 2357, susceptible to nematodes (Santos et al., 2017; 2018)

were produced in pathogen-free substrate and, at 120 days of age, they were planted in 8l pots containing the substrates treated in the STS, according the treatments.

The seedlings were kept in a greenhouse, receiving all cultural treatments for 180 days. After this period, the following variables were collected for evaluation: mass of shoot and root matter, number of galls and number of eggs, for which the counting were performed in a Peters chamber and the fator of reproduction was obtained by the ratio between the final population, obtained by the total number of eggs and J2 produced by root system, and the population initial used for substrate infestation. For the nematological evaluations, the method of Bonetti and Ferraz (1981) of extraction and counting was used.

In the evaluations, the roots were separated from the shoot, washed, weighed, and later processed with a solution containing 0.5% hypochlorite in water, according to Hussey and Barker (1973). The resultant is sieved through 20, 325 and 500 mesh sieves. What was retained on the last sieve of 500 Mesh was used as a suspension for eggs and second stage juveniles (j2).

The suspension containing eggs + *M. incognita J2* was transferred to a beaker and made up to 30 mL with water. The counting of the number of eggs and J2 was performed with the aid of an optical microscope and Peters camera, obtaining the final population estimates (Pf) of M. *incognita* for each plant or repetition. The nematode reproduction factor in the different treatments was calculated according to the equation: FR= Final population/Initial population).

The number of galls/grams of root, number of eggs/ grams of root, the total number of eggs, and the reproduction factor were evaluated. The weight of fresh and dry matter of shoots and roots was also evaluated and correlated with greater or lesser severity.

The experimental design to be used was completely randomized in a 2 x 4 x 4 factorial (treatments x time x cultivars), with 6 repetitions, each one composed of one plant growing in a plot. The comparison between the means was made using the standard deviation of the mean over time. Statistical analyzes were conducted using the one-way ANOVA program and Student's t - test of the SPSS software for Windows (Kinnear and Gray., 2000).

3 RESULTS

The water temperature graphs (Figure 2 and 3) show that the tests in the solarizer showed similar heating behavior and temperature drop between different periods within the same year, verifying that it is possible to carry out heating by the STS system over the course of year.

The temperatures inside the solarizer were similar when compared in two different periods of the year (Figure 4 and 5), follow the same trend presented in the water temperatures of the solarizer heating system, that is, there is influence of the water temperature in the heating system on the heating of the soil inside the box.

In the shoot dry matter weight of Experiments 1 (Figure 6) carried out on BRS 2357 coffee and Experiment 2 (Figure 7) carried out on Clone 125 coffee, the weight decreased with increasing soil exposure to TLM, starting with the highest weights in the Control treatments, and reaching the lowest weight in the treatments with longer TLM time, being this weight difference between the treatments more accentuated in Experiment 2.

In the root dry matter weight of Experiments 1 (Figure 8), and Experiment 2 (Figure 9), the weight decreased with increasing exposure to TLM, the relationship of weight loss with increasing TLM is more uniform in Experiment 2.

The number of root grass-knots decreased with the increase of TLM, in Experiment 1 (Figure 10) it becomes stable from Treatment T60', with the exception of Treatment

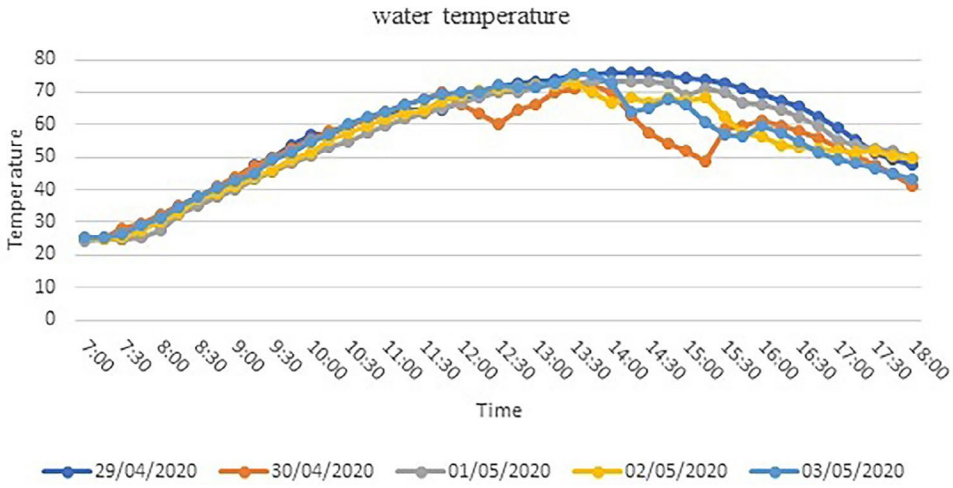

Figure 2: Water temperature in the solarizer heating system from April 29, 2020 to May 3, 2020.

T30' which showed the same result as the other treatments with higher TLM. In Experiment 2 (Figure 11), the decrease in the number of galls is more uniform when compared to Experiment 1 in relation to its treatments, also observing that there is no presence of galls from the treatment T720'.

In the number of eggs per gram of root in experiment 1 (Figure 12), from the TLM of the T15' treatment, there is no presence of nematode eggs, with the lowest TLM being necessary to eradicate the presence of the pest. In Experiment 2 (Figure 13), the lowest TLM that eradicated the presence of the nematode was the T45' treatment, noting that the previous treatments had already caused a significant reduction in the number of eggs, when compared to the control treatment 2 (inoculated).

The reproduction factor of experiments 1 (Figure 14) and 2 (Figure 15) in coffee seedlings, decrease with increasing soil exposure to TLM, reaching zero with 45 minutes of exposure. It is possible to observe that in the previous treatments, with shorter exposure time, the Reproduction Factor dropped considerably, as observed in the treatments of 0, 15 and 30 minutes of exposure to TLM, compared to the C2 treatment.

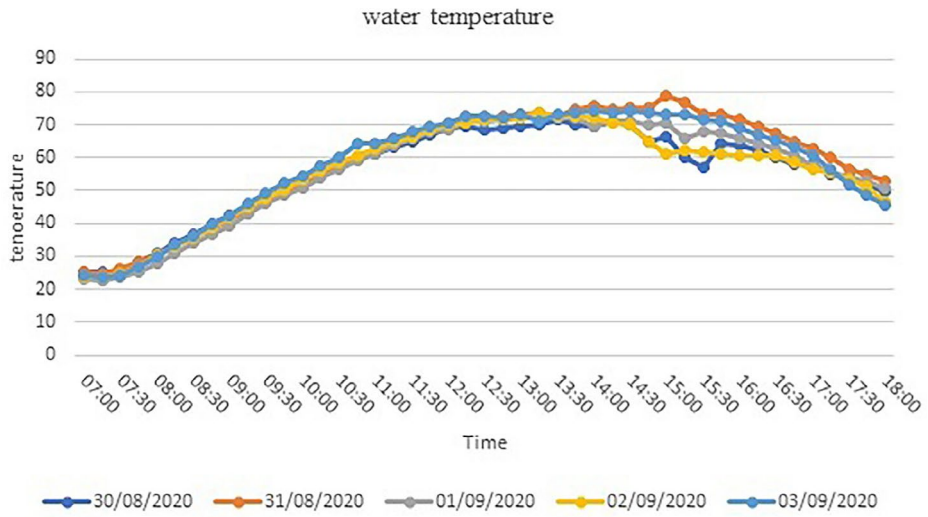

Figure 3: Water temperature in the solarizer heating system from August 30, 2020 to September 3, 2020.

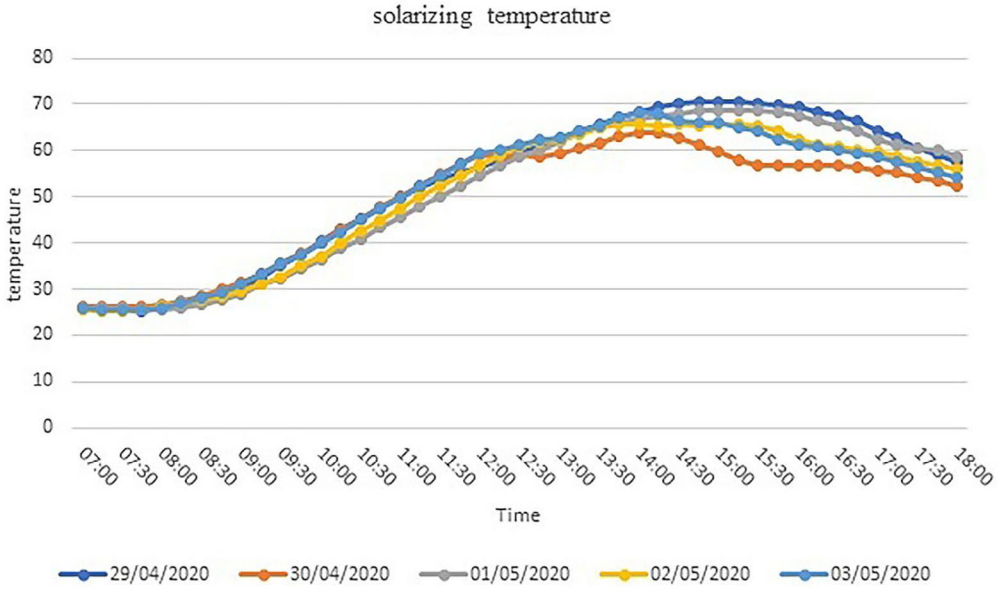

Figure 4: Substrate temperature in the solarizer from April 29, 2020 to May 3, 2020.

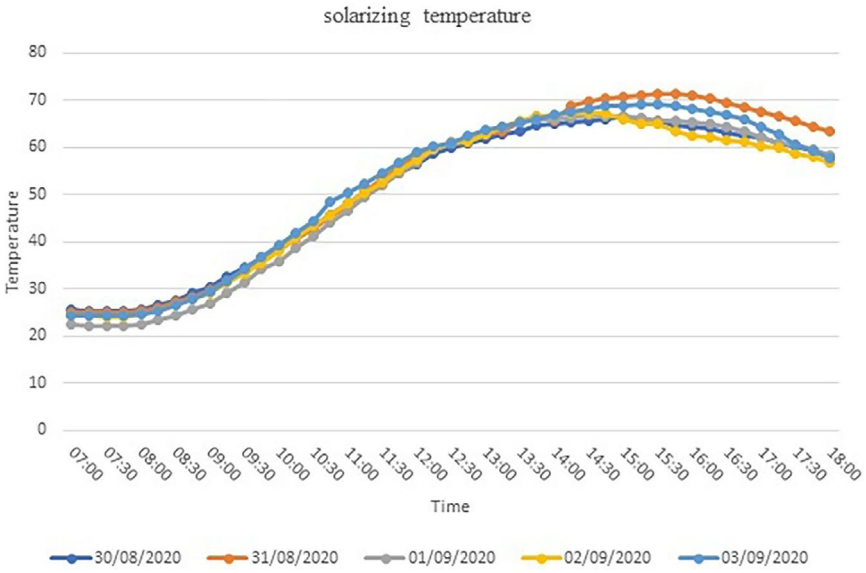

Figure 5: Substrate temperature in the solarizer from August 30, 2020 to September 3, 2020.

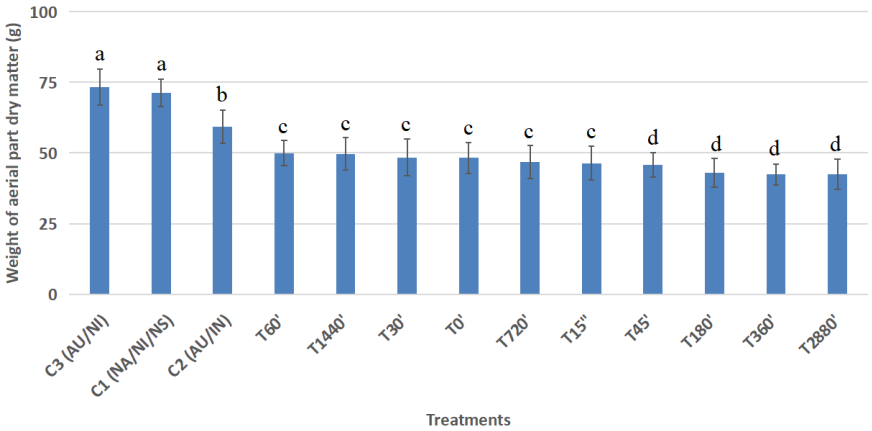

Figure 6: Variation in the Dry Matter Weight of the Aerial Part by treatment in experiment 1 of the variety BRS 2357. Mean followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (NA= not autoclaved, NI= not inoculated, NS= not solarized, AU= autoclaved, IN= inoculated, T= time, ‘ = minutes).

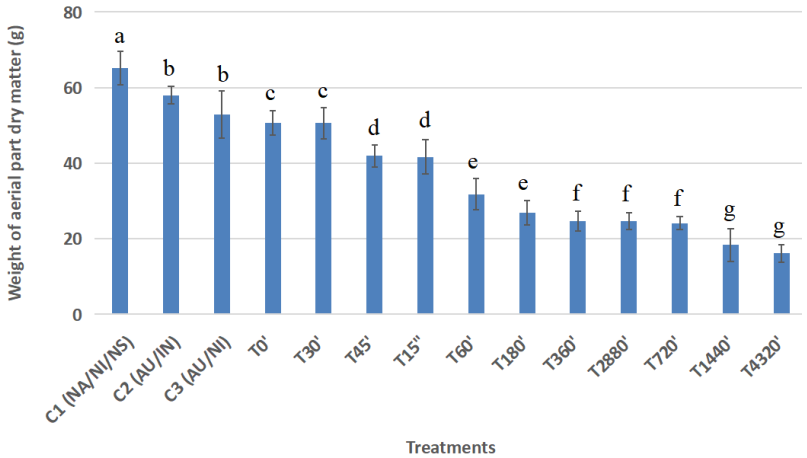

Figure 7: Variation in the Dry Matter Weight of the Aerial Part by Treatment in Experiment 2 of the Clone 125 variety. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (NA= not autoclaved, NI= not inoculated, NS= not solarized, AU= autoclaved, IN= inoculated, T= time, ‘ = minutes).

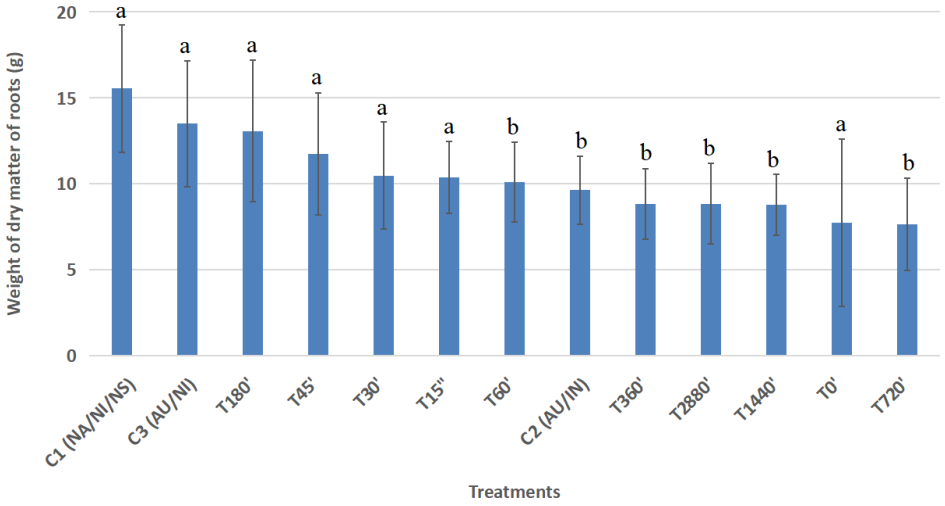

Figure 8: Variation of Dry Matter Weight of roots by treatment in experiment 1 of the variety BRS 2357. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (NA= not autoclaved, NI= not inoculated, NS= not solarized, AU= autoclaved, IN= inoculated, T= time, ' = minutes).

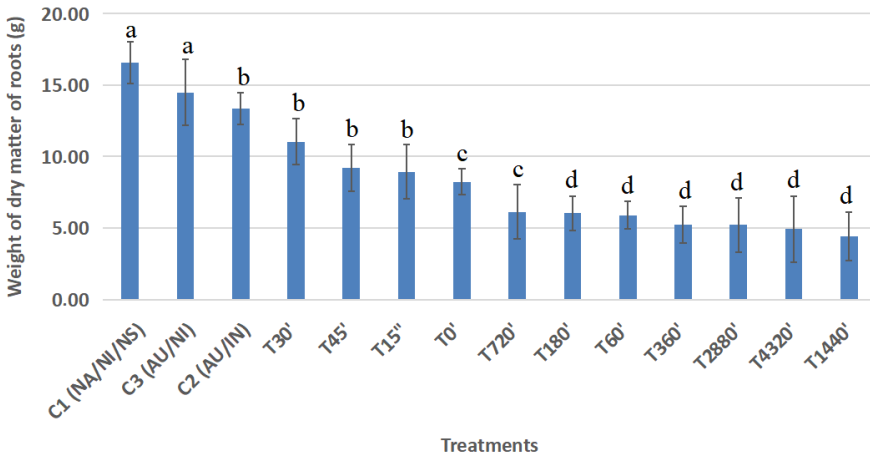

Figure 9: Variation in Root Dry Matter Weight by Treatment in Experiment 2 of the Clone 125 variety. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (NA= not autoclaved, NI= not inoculated, NS= not solarized, AU= autoclaved, IN= inoculated, T= time, ' = minutes).

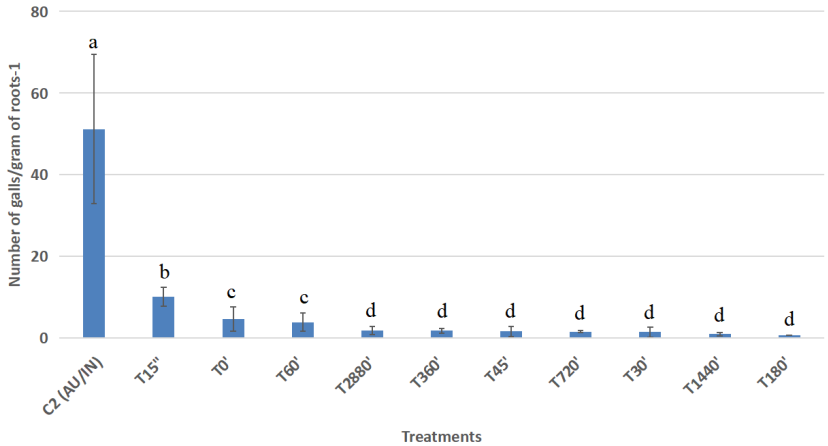

Figure 10: Variation in the number of root galls per treatment in experiment 1 of the variety BRS 2357. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (AU/IN= autoclaved/ inoculated, T= time, ' = minutes).

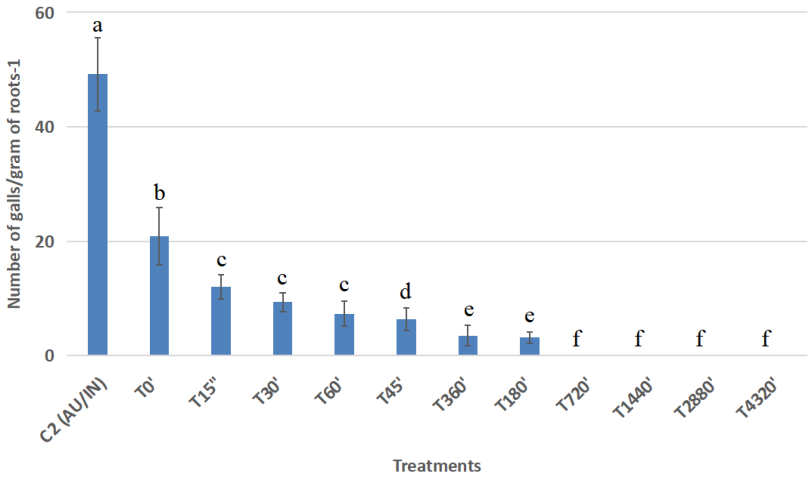

Figure 11: Variation in the number of root galls per treatment in experiment 2 of the Clone 125 variety. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (AU/IN= autoclaved/ inoculated, T= time, ‘ = minutes).

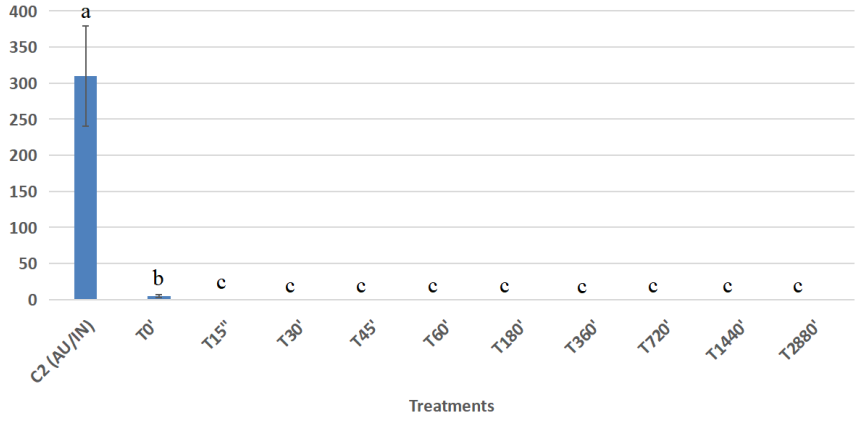

Figure 12: Variation in the number of eggs-gram of roots per treatment in experiment 1 of the variety BRS 2357. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (AU/IN= autoclaved/ inoculated, T= time, ‘ = minutes).

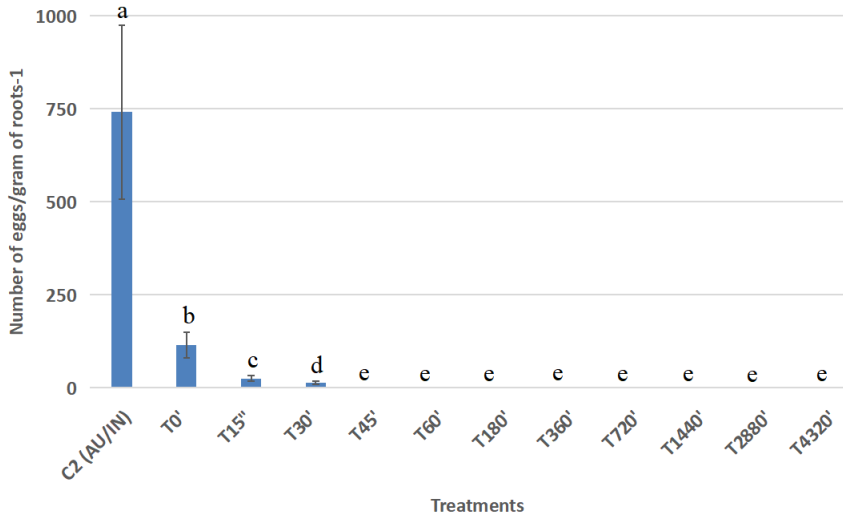

Figure 13: Variation in the number of eggs-gram of roots per treatment in experiment 2 of the Clone 125 variety. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability. Bars indicate the standard deviation of the mean. Legend: (AU/IN= autoclaved/ inoculated, T= time, ‘ = minutes).

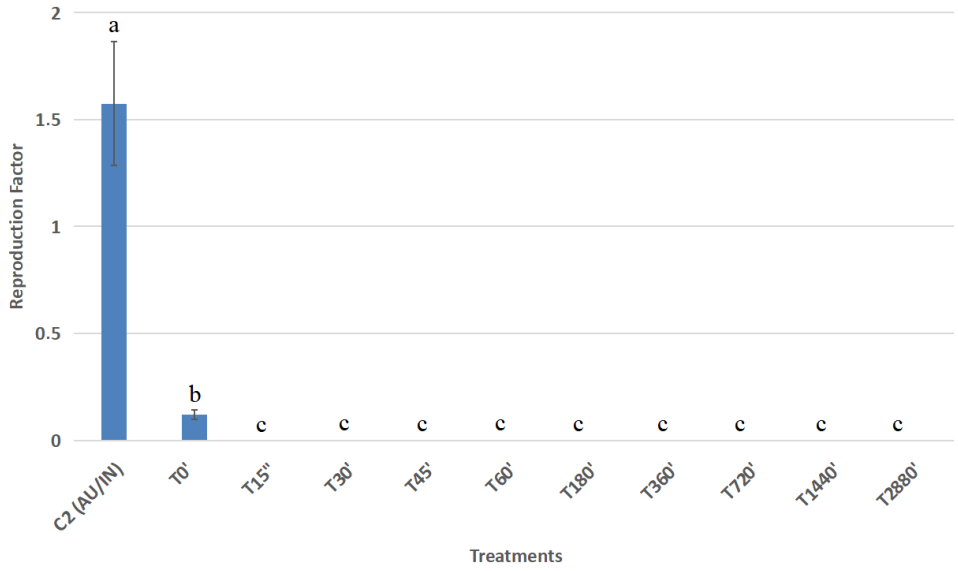

Figure 14: Variation of the Reproduction Factor by treatment in experiment 1 of the variety BRS 2357. Means followed by the same letters, constitute a statistically homogeneous group by the Scott-Knott test at 5%. Legend: (AU/IN= autoclaved/ inoculated, T= time, ‘ = minutes).

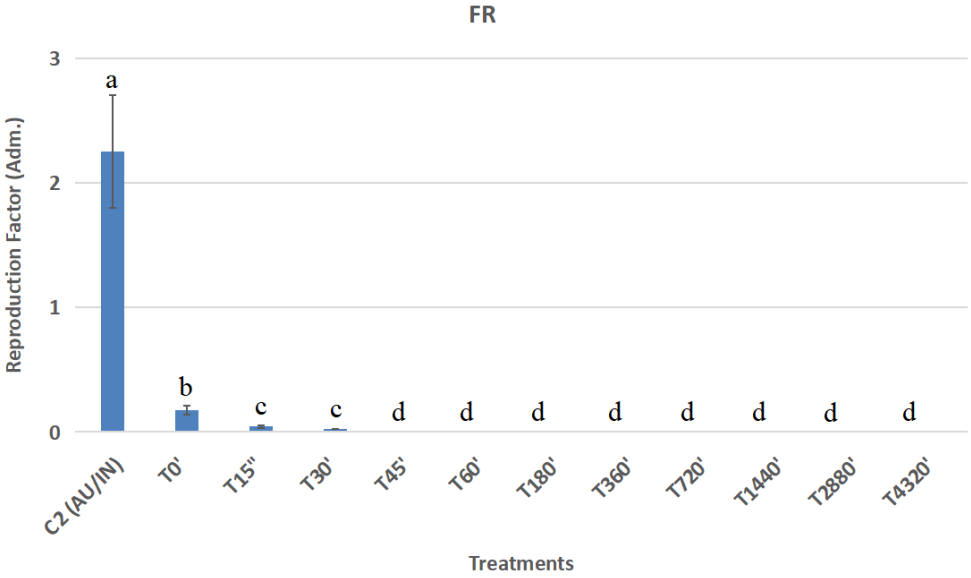

Figure 15: Variation of the Reproduction Factor by treatment in experiment 2 of the Clone 125 variety. Means followed by the same letters, constitute a statistically homogeneous group by the Scott-Knott test at 5%. Legend: Legend: (AU/IN= autoclaved/ inoculated, T= time, ‘ = minutes).

4 DISCUSSION

It is important to emphasize that the STS system reduced the time for heating the soil, allowing the treatment of a greater volume of substrate in one day, as the high-water temperatures observed (Figure 2 and 3) heat the soil at the bottom of the box. This is different from what was observed in the solarization method with a plastic film cover, which takes a long time to heat up to lethal temperatures for the nematode and reaches only surface layers of the soil (Mormile et al., 2016).

The uniformity of water and soil temperature (Figure 4 and 5) in the STS type solarizer system for an extended period ensures the production of substrate in a measurable volume, enabling the organization, control and planning of the substrate and seedling production unit. These results were also obtained by Dos Santos et al. (2021), who evaluated the temperature variation in a solar collector and found that the equipment caused heat peaks between 70 and 80 ºC in the soil inside the equipment.

These results were also verified by Miranda et al. (2006) who reported that coffee seedlings cultivar Paraíso H

419-1 showed growth retardation in the 15-day solarization treatment. In the work developed by Cruz (2020), a different behavior is observed, as there is no significant change in the weight of plant matter in an experiment evaluated in cabbage plants, planted in treated substrate and untreated in a solarizer.

The behavior observed in the dry matter weight of the aerial part of Experiments 1 (Figure 6) and Experiment 2 (Figure 7), were also verified by Miranda et al. (2006) who reported that coffee seedlings cultivar Paraíso H 419-1 showed growth retardation in the 15-day solarization treatment. In the work developed by Cruz (2020), a different behavior is observed, as there is no significant change in the weight of plant matter in an experiment evaluated in cabbage plants, planted in treated substrate and untreated in a solarizer.

The root dry matter weight of Experiments 1 (Figure 8) and Experiment 2 (Figure 9) shows behavior that may be the result of changes in the chemical and physical properties of the soil; according to Ghini (2003) this effect is due to the different processes developed in solarization, which cause biotic and abiotic changes in the soil; such as alteration of the soil microbial community, and interferences in macro and micronutrients present, release of volatile substances and soil structure.

In the number of root gram-knots that decreases with the increase of TLM, in Experiment 1 (Figure 10), and in Experiment 2 (Figure 11), which had a more uniform drop in the number of galls than in Experiment 1, has its results corroborated by Santos et al. (2006) who verified the efficiency of a solar collector on the number of galls of *Meloidogyne ssp.* on coffee seedlings, the authors report that all treatments showed a significant difference in terms of the decrease in the number of galls when compared to the contaminated controls, and that treatments with longer exposure to high temperatures did not present galls when compared to previous treatments.

In the work by Lambert et al. (2020) showed that heat treatment reduced the population of nematodes of the genus *Meloidogyne ssp.* (eggs + J2) in the roots of arabica coffee cv. Catuaí Vermelho IAC 144, in relation to seedlings that did not receive the treatment. These results coincide with those obtained in this work, as observed in the number of eggs per gram of root in experiment 1 (Figure 12) and experiment 2 (Figure 13), which had a decrease in the number of eggs when exposed to TLM; and consequently in the reproduction factor of experiment 1 (Figure 14) and 2 (Figure 15).

5 CONCLUSION

The Model Sandwich Solarizer – STS was efficient in treating soil for substrate against *M. incognita* to produce coffee seedlings, eliminating the presence of the pathogen after 45 minutes of exposure to TLM at 60ºc. A 60- minute exposure to TLM is recommended, as a safety margin for the treatment of substrates, to produce root-knot nematode-free seedlings.

6 AUTHORS' CONTRIBUTION

FPU, VASR, DMS, TCF, SCS and SARM wrote the manuscript and performed the experiment, JRVJ, CFF, MCE, RBR and FPU supervised the experiment and co-worked the manuscript, and JRVJ, CFF and FPU reviewed and approved the final version of the work, JRVJ and RBR conducted all statistical analyses.

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