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Articles

Quality of *Bixa orellana* L. seeds subjected to hot water treatment

Qualidade de sementes de *Bixa orellana* L. submetidas à termoterapia via úmida

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ABSTRACT

The species *Bixa orellana* is propagated through sexual reproduction but this method may present problems due to the low germination rate caused by pathogens associated with the seeds. This study aimed to verify the efficiency of hot water treatment in controlling fungi associated with urucum seeds and its effect on their physiological quality. The experiment was conducted at the Laboratory of Phytopathology of the Federal University of Paraíba, using urucum seeds collected from a mother plant in the municipality of São José de Espinharas, PB, Brazil. The treatments consisted of hot water treatment at constant temperatures of 40, 50, 60, and 70°C for five minutes and a control treatment with only disinfested seeds. The treated seeds underwent seed health, seed germination, and seedling emergence tests. The experiment was conducted in a completely randomized design and the data were subjected to analysis of variance and linear and quadratic regressions, with the means compared by the Dunnett test ($p<0.05$). The fungi associated with the seeds were *Colletotrichum* sp., *Cladosporium* sp., *Curvularia* sp., *Aspergillus* sp., *Aspergillus niger*, *Rhizopus* sp., *Alternaria* sp., and *Fusarium* sp. The hot water treatment at 60°C was promising in treating urucum seeds for producing high-quality seedlings, as it promoted the development of vigorous seedlings and controlled 100% of the incidence of *Colletotrichum* sp.

Keywords: Seedling emergence; Seed germination and health; Urucum

RESUMO

A propagação da espécie *Bixa orellana* é realizada por reprodução sexuada, no entanto, este método pode apresentar problemas devido à baixa taxa de germinação causada pelos patógenos associados às sementes. O objetivo deste trabalho foi verificar a eficiência da termoterapia, via calor úmido, no controle de fungos associados às sementes de urucum e seu efeito na qualidade fisiológica das mesmas. O experimento foi conduzido no Laboratório de Fitopatologia, da Universidade Federal da Paraíba, utilizando-se sementes de urucum coletadas em matriz localizada no município de São José de Espinharas/PB. Os tratamentos foram termoterapia em água aquecida nas temperaturas constantes de 40°C, 50°C, 60°C e 70°C por cinco minutos e um tratamento controle com sementes apenas desinfestadas. Após tratadas, foram realizados os testes de sanidade de sementes, teste de germinação de sementes e teste de emergência de plântulas. O experimento foi conduzido em delineamento inteiramente casualizado (DIC) e os dados submetidos a análise de variância (ANOVA) e regressão linear e quadrática, sendo as médias comparadas pelo teste de Dunnett (p <0,05). Os fungos associados às sementes foram *Colletotrichum* sp., *Cladosporium* sp., *Curvularia* sp., *Aspergillus* sp., *Aspergillus niger*, *Rhizopus* sp., *Alternaria* sp. e *Fusarium* sp. O tratamento hidrotérmico a 60ºC mostrou-se promissor no tratamento de sementes de urucum para produção de mudas de alta qualidade, pois promoveu o desenvolvimento de plântulas vigorosas e controlou 100% da incidência de *Colletotrichum* sp.

Palavras-chave: Emergência de plântulas; Germinação e sanidade de sementes; Urucum

1 INTRODUCTION

The search for a healthier diet has become a global trend, leading to an increase in the consumption of natural products, including dyes and condiments used during food preparation (Corradini, 2018). The species *Bixa orellana* L., popularly known as urucum or annatto, is a shrub species belonging to the family Bixaceae, widely used as raw material to obtain natural dye (Dequigiovanni *et al*., 2018) from the mature pericarp of its seeds due to the presence of the carotenoids bixin, norbixin, and nobixate (Oliveiras *et al*., 2021).

Brazil is considered the world's largest producer of urucum, with cultivation present in all regions of the country (Santos *et al*., 2019). In 2020, Brazil produced over 13 thousand tons of urucum, with an average yield of 993 kg/ha (IBGE, 2022). The sanitary quality of seeds is one of the most important requirements for maintaining their vigor, as the presence of pathogenic microorganisms causes damage before and during storage (Rosário *et al*., 2022).

Chemical control is the conventional method for disease management, but its excessive use causes adverse effects on the environment, such as increased resistance of phytopathogenic microorganisms and contamination of drinking water sources (Parisi *et al*., 2019). The search for alternative methods such as the use of essential oils, plant extracts, biological control, and thermotherapy has attracted worldwide attention, as they have a lower environmental impact and are more sustainable (Carvalho *et al*., 2019; Mendes *et al*., 2019).

Hot water treatment is considered effective in eradicating microorganisms due to its mode of action, that is, the denaturation of important proteins for maintaining the cellular metabolism of pathogens (Mascarenhas; Brito; Oliveira, 2019).

This study aimed to verify the efficiency of hot water treatment in controlling fungi associated with urucum seeds and its effect during the process of seed germination and seedling emergence.

2 MATERIALS AND METHODS

Urucum fruits were collected directly from a mother plant in August 2021 in the municipality of São José de Espinharas, PB, Brazil (6°50'29" S and 37°19'10" W). The experiment was conducted between February and March 2022 at the Laboratory of Phytopathology (LAFIT) in the Center for Agricultural Sciences (CCA) of the Federal University of Paraíba (UFPB), located in the municipality of Areia, PB, Brazil (6°57'42" S and 35°41'43" W).

The seeds were manually extracted from the fruits, with subsequent screening to eliminate those with physical and biological damage or poor formation, aiming to obtain a homogeneous sample. The seeds were stored in PET bottles for six months at room temperature (25 ± 2°C) until use.

The seeds were disinfected by immersion in a 1% sodium hypochlorite solution for three minutes, followed by double washing in sterilized distilled water (SDW). The seeds of the hot water treatment were placed in mesh bags and immersed in water heated in a water bath (KACIL BM-02) at constant temperatures of 40, 50, 60, and 70°C for five minutes. In addition, a control treatment consisted of only disinfested seeds.

The treated seeds were subjected to health testing, seed germination testing, and seedling emergence testing. The health testing was performed using 100 seeds per treatment, divided into 10 replications of 10 seeds each. The seeds were incubated in Petri dishes measuring nine centimeters in diameter on a double layer of sterilized filter paper moistened with SDW (BRASIL, 2009). The dishes remained incubated for seven days at a temperature of 25 ± 2°C and a 12-hour photoperiod. The fungi were detected and identified using an optical stereoscopic microscope and compared with descriptions in the literature (Seifert *et al*., 2011).

The germination testing was performed using 100 seeds, divided into four replications of 25 seeds each. The seeds were distributed on previously sterilized Germitest® paper moistened with SDW in a proportion of 2.5 times its dry weight. The paper rolls were placed in a regulated Biochemical Oxygen Demand (B.O.D.) chamber, alternating for 12 hours at a temperature of 25°C.

The variables analyzed in the germination testing were percentage of germination (GE), germination speed index (GSI), shoot length (SL), root length (RL), seedling length (SLL), shoot dry matter (SDM), root dry matter (RDM), and seedling dry matter (SLDM). The number of germinated seeds was counted daily until the 20th day after installing the test, following the criteria indicated for the evaluation of the germination testing (BRASIL, 2013). The germination speed index (GSI) was determined using the Maguire (1962) equation.

SL, RL, and SLL were assessed using a ruler graduated in cm. The seedlings were packed in Kraft paper bags and kept in a forced-air circulation oven at 65°C until constant weight to determine SDM, RDM, and SLDM. Subsequently, weight was determined on a semi-analytical scale with a 0.001-g precision.

The emergence testing was conducted in a greenhouse and consisted of the sowing of treated seeds in polypropylene plastic trays (43 x 335 x 664 mm) filled with sterilized washed sand as substrate and watered twice a day. One hundred seeds were used per treatment, being distributed in four replications of 25 seeds each. The percentage of emergence (EM), emergence speed index (ESI), shoot length (SL), root length (RL), seedling length (SLL), shoot dry matter (SDM), root dry matter (RDM), and seedling dry matter (SLDM) were evaluated. The number of normal emerged seedlings was counted up to the 20th day after sowing to evaluate EM (BRASIL, 2009). The other variables were analyzed according to the description of the germination testing.

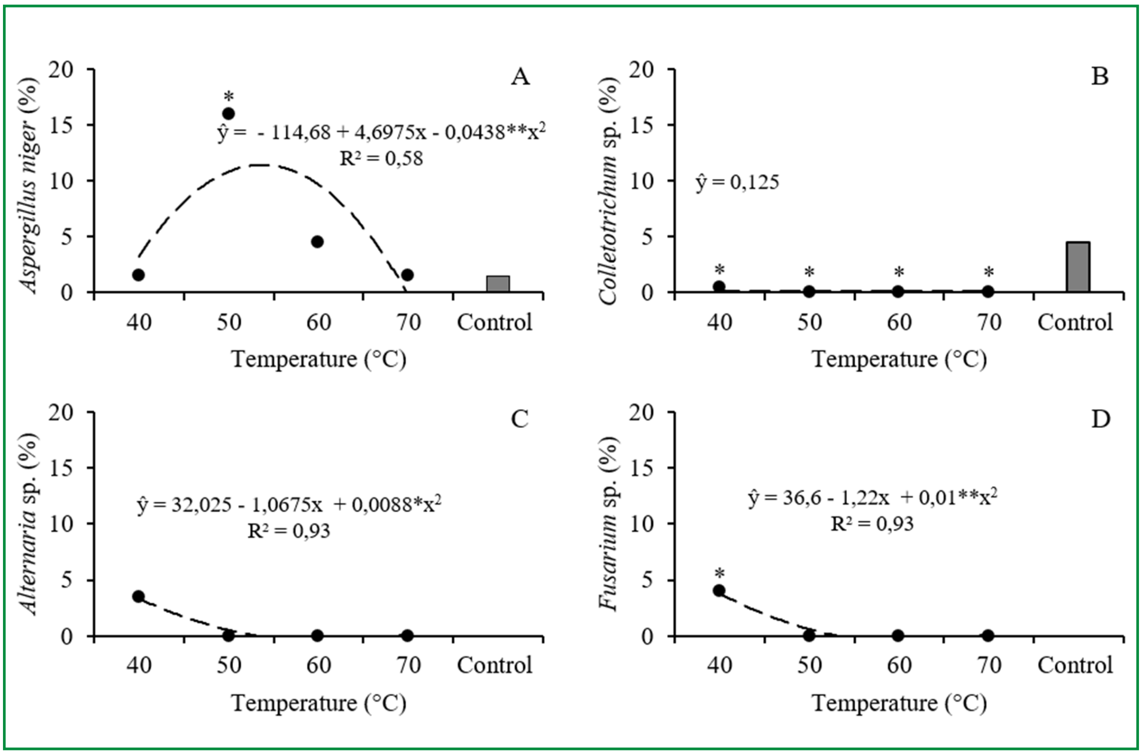
The experiment was conducted in a completely randomized design. The data were subjected to analysis of variance, linear and quadratic regressions, and the means were compared using the Dunnett test at the 5% significance level, using the statistical software Sisvar (Ferreira, 2020).

3 RESULTS AND DISCUSSION

Figure 1 shows the mean values referring to the sanitary quality of urucum seeds. The incidence of the genera *Colletotrichum* sp. (5%), *Cladosporium* sp. (4%), *Curvularia* sp. (1%), *Aspergillus* sp. (4%), and *Rhizopus* sp. (1%) did not fit tested regression models and except for *Colletotrichum* sp., no temperature in this study influenced the percentage of fungal incidence.

The incidence of *Aspergillus niger*, *Alternaria* sp., and *Fusarium* sp. adjusted to the quadratic regression model (p<0.05). The temperature of 50°C stimulated the incidence of *A. niger* and the temperature of 40°C stimulated the incidence of *Fusarium* sp. This behavior may be related to the fact that these temperatures provide an optimal development condition for these species. Determining the temperature at which mycotoxin-producing fungi, such as *Fusarium* spp., best develop is essential because it is the most important environmental factor in the production of food contaminants (Maia *et al*., 2021).

Figure 1 – Fungal incidence associated with hot water treated urucum (*Bixa orellana* L.) seeds



Source: Authors (2022)

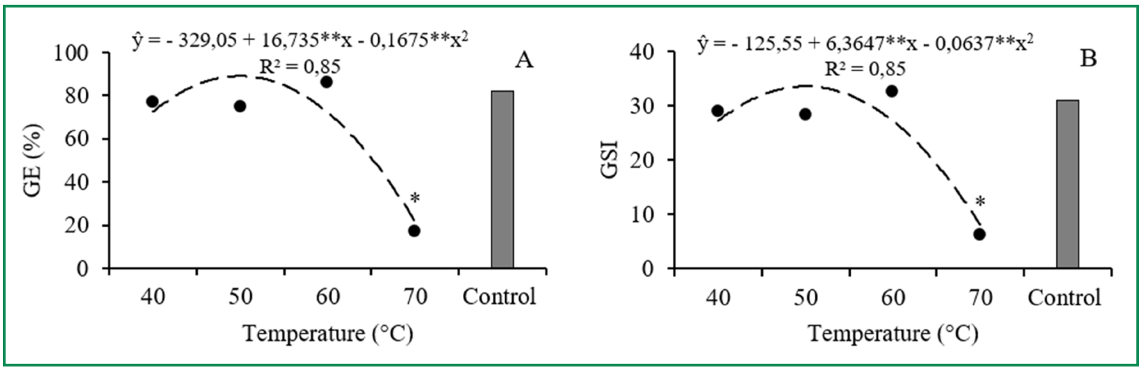
In where: Means followed by * differ statistically from the control treatment (only disinfested seeds) by the Dunnett test at the 5% probability level.

All tested temperatures were efficient in controlling *Colletotrichum* sp., showing a significant difference compared to the control treatment. No influence of treatments was observed on *Alternaria* sp. The efficiency of sanitary seed treatment depends on the vigor, availability of substances, and the type and location of the pathogen present in the seed lot, which may be mainly adhered to the seed coat or inside the seed (Medeiros *et al*., 2019).

Heat treatment at a temperature of 70°C for 10 to 15 minutes reduced the percentage of fungi associated with *Acacia mangium* seeds (Araújo *et al*., 2018). According to Raad *et al.* (2022), thermotherapy proved to be efficient in reducing the incidence of fungi in corn (*Zea mays*) seeds, and temperatures of 60 and 80°C managed to eradicate *Fusarium moniliforme*.

Figure 2AB shows that the percentage of germination (R^2 = 0.85) and GSI (R^2 = 0.85) adjusted to the quadratic regression model ($p<0.05$). The temperature of 70°C caused physiological damage to urucum seeds, as it reduced 65% of the germination rate and 79.8% of GSI, differing from the control treatment, which presented GE and GSI of 82% and 30.90, respectively. The other temperatures did not influence these variables. Therefore, hot water treatment at 60°C can be used on urucum seeds without reducing vigor.

Figure 2 – Seed germination (GE) and germination speed index (GSI) of urucum (*Bixa orellana* L.) subjected to hot water treatment



Source: Authors (2022)

In where: Means followed by * differ statistically from the control treatment (only disinfested seeds) by the Dunnett test at the 5% probability level.

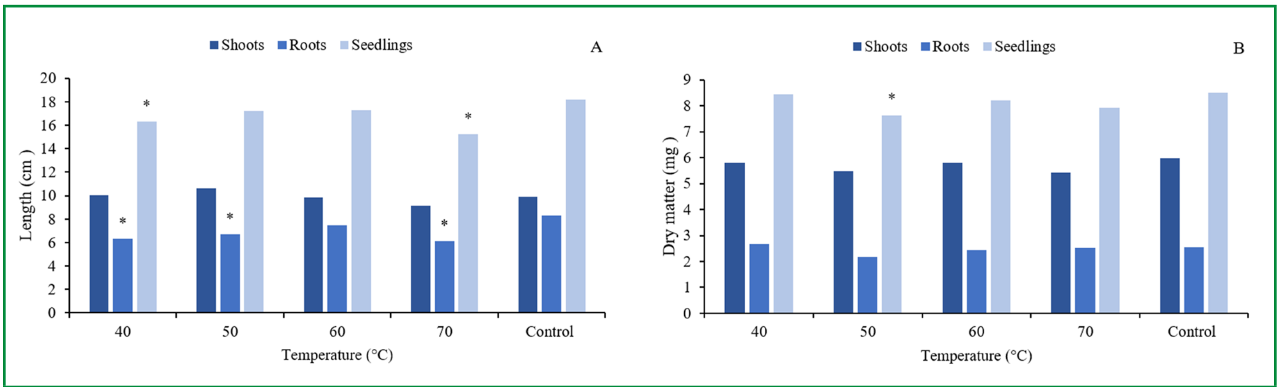
High-vigor seeds provide a more uniform stand and enable increased crop yield due to their higher speed of germination and emergence and better initial development of seedlings in the field (Cardoso *et al*., 2021). Seedlings from high-vigor seeds begin the photosynthetic process earlier, favoring shoot and root system growth and providing advantages in the use of water, light, and nutrients (Bagateli *et al*., 2020).

Treatments did not affect shoot length (Figure 3A) compared to the control. Root and seedling lengths were reduced at temperatures of 40 and 70°C. However, the temperature of 50°C did not compromise the final seedling length. Heat stress likely altered the balance between reactive oxygen species (ROS) production and ROS scavenging enzymes, partially reducing the metabolic activity necessary to continue and stimulate normal root growth (Rashid *et al*., 2017).

The temperature of 60°C caused no physiological damage to urucum seeds, as it allowed the development of normal seedlings, with a mean length similar to that of seedlings from the control treatment (18.2 cm). Seedling height is an agronomic parameter of great importance, as it guarantees less susceptibility to stress in the field due to the higher rate of interception of solar radiation, thus providing the seedling with favorable development conditions (Goes *et al*., 2019).

The mean shoot and root dry matter (Figure 3B) did not change with the use of hydrotherapy, that is, the treatments did not affect the mechanism of nutrient accumulation in the seedling, not statistically differing from the mean of the control treatment. Seedling dry matter was negatively affected only at a temperature of 50 °C, with a 10.2% reduction in total accumulated dry matter.

Figure 3 – Shoot, root, and seedling lengths, in cm (A), and shoot, root, and seedling dry matter, in mg (B), of urucum (*Bixa orellana* L.) from seeds submitted to hot water treatment



Source: Authors (2022)

In where: Means followed by * differ statistically from the control treatment (only disinfested seeds) by the Dunnett test at the 5% probability level.

Figure 4 shows that the percentage of emergence, emergence speed index, root length, and seedling length were adjusted to the quadratic regression model

($p<0.05$). The shoot length did not fit the tested regression models. The percentage of emergence (Fig. 4A) indicated that the hot water temperatures of 40, 50, and 60°C for 5 minutes did not cause physiological disturbances in urucum seeds, as they did not differ statistically from the control treatment.

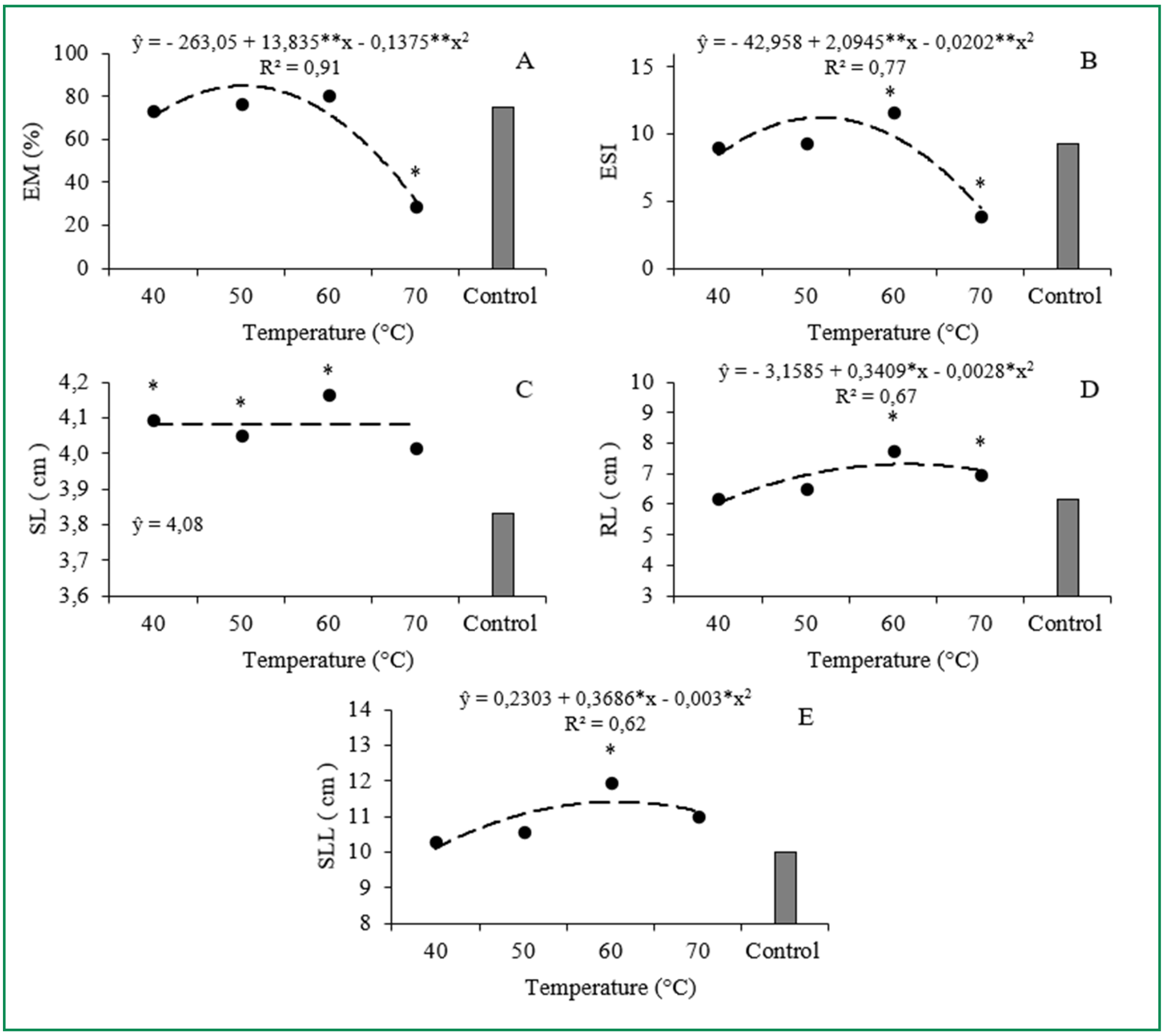
The temperature of 70°C was harmful to urucum seeds because it caused a 46% reduction in the percentage of emergence. The highest emergence speed index (Fig. 4B) was observed in seeds treated at 60°C (11.66) and the lowest value was observed at 70°C (3.87). The other treatments were not influenced, as they did not present significant differences relative to the control treatment.

Figure 4C shows that temperatures of 40, 50, and 60°C had higher seedling shoot lengths, with a significant difference of 0.26, 0.22, and 0.33 cm, respectively, compared to the control treatment.

The temperature of 70°C was harmful to urucum seeds, as it caused damage to the germination and emergence processes, leading to the development of abnormal seedlings, with necrotic and poorly developed roots. Knowledge about seed germination is useful for more efficient propagation of species, and adequate temperature is an essential factor in this physiological process, as it influences the biochemical reactions responsible for regulating the metabolism necessary to initiate the germination process, the speed of this process, and the water absorption rate (Silva *et al*., 2021; Arantes *et al*., 2022).

Temperatures of 60 and 70°C in urucum seeds promoted the emission of longer roots (Fig. 4D), with mean values of 7.76 and 6.96 cm, respectively. The emergence of more vigorous seedlings, with higher total length (Fig. 4E), was determined in seedlings from seeds treated at 60°C (11.92 cm). The other treatments showed no significant differences from the control treatment (10.00 cm).

Figure 4 – Percentage of seed emergence (A), emergence speed index (ESI) (B), seedling shoot length (C), seedling root length (D), and seedling length (E) of urucum *(Bixa orellana* L.) from seeds subjected to hot water treatment



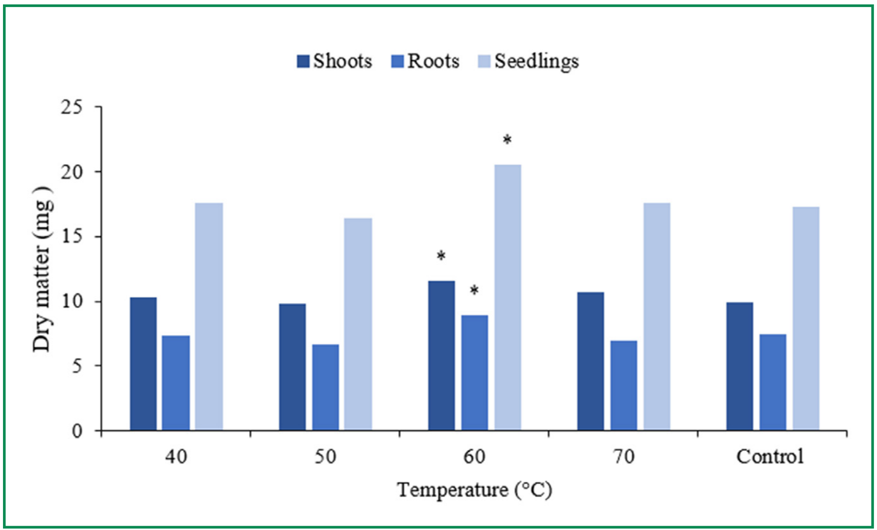
Source: Authors (2022).

In where: Means followed by * differ statistically from the control treatment (only disinfested seeds) by the Dunnett test at the 5% probability level.

The use of water heated to 60°C can be used on urucum seeds when higher emergence speed indices and longer emerged seedlings are desired. These agronomic parameters are extremely important, as they guarantee the production of high-quality seedlings, a homogeneous stand, and a high crop yield (Cavalcanti *et al*., 2019).

Figure 5 shows that temperatures of 40, 50, and 70°C did not influence dry matter accumulation in emerged seedlings.

Figure 5 – Dry matter (mg) of urucum (*Bixa orellana* L.) seedlings from the emergence testing using seeds subjected to hot water treatment



Source: Authors (2022).

In where: Means followed by * differ statistically from the control treatment (only disinfested seeds) by the Dunnett test at the 5% probability level.

The temperature of 60°C promoted the highest dry matter accumulation in the shoot, roots, and seedlings (11.57, 8.90, and 20.47 mg, respectively). In this sense, this temperature stimulated vegetative development when used to treat urucum seeds, as it generated seedlings with longer lengths (Figure 4) and higher accumulation of nutrients (Figure 5). This result is probably related to better use of solar radiation and, consequently, more efficient photosynthetic assimilation (Sousa, 2020).

Research has demonstrated the success of thermotherapy in controlling pathogens in seeds. Santo *et al*. (2018) used sunflower (*Helianthus annuus* L.) seeds and observed a difference in the vigor of seedlings whose seeds were subjected to thermotherapy, with the best results being obtained at 40°C for 20 minutes. Temperatures of 42, 46, and 50°C for 15 and 30 minutes did not interfere with the germination of castor bean (*Ricinus communis* L.) seeds (Marroni *et al*., 2021). According to Cardoso *et al*. (2020), thermotherapy at 55ºC for 15 minutes was the highest temperature and exposure time without affecting the physiological quality of kale seeds (*Brassica oleracea* L.).

Temperatures of 42ºC, 46ºC and 50ºC, via moist heat for 15 and 30 minutes, did not interfere with the germination of castor bean seeds (*Ricinus communis* L.) (MARRONI et al., 2021). According to Cardoso *et al*. (2020), thermotherapy at 55 ºC for 15 minutes was the highest temperature and exposure time without affecting the physiological quality of kale seeds (*Brassica oleracea* L.).

4 CONCLUSIONS

Thermotherapy, via moist heat (60ºC), showed promise for use as a disease management strategy in annatto seeds, being efficient in controlling *Colletotrichum* sp.

Thermotherapy, via moist heat at 60ºC for 5 minutes, does not compromise the physiological quality of annatto seeds and can be used as a sustainable alternative for producing high-vigor seedlings.

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