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Hydrothermal treatment in the management of anthracnose in 'Prata-Anã' banana produced in the semiarid region of Minas Gerais, Brazil

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Abstract - Anthracnose stands out among rot diseases that can occur in the post-harvest phase of banana. The aim of this study was to evaluate the thermotherapy in the control of anthracnose in 'Prata Anã' banana produced in northern state of Minas Gerais. Bananas 'Prata Anã' variety were divided into groups of three fruits and inoculated with *Colletotrichum musae*. Groups were submitted to thermotherapy using five temperatures (40°C, 44°C, 48°C, 52°C and 56°C) and four immersion times (4, 8, 12 and 16 minutes) and control. Treatments were repeated five times in a completely randomized design. Area under the incidence progress curve (AUIPC) and area under the severity progress curve (AUSPC) were calculated. To evaluate the thermotherapy efficiency compared to chemical control, fruits were submitted to the following treatments: thermotherapy with and without fungicide application and fruits treated with fungicide only, the control fruits without thermotherapy and without fungicide application. After fifteen days of storage, fruits were evaluated for anthracnose severity. The results of experiments were submitted to analysis of variance and regression and means were compared by the Scott-Knott test ($p<0.05$). Control was compared to treatments by the Dunnett test ($p<0,05$). The lowest anthracnose AUIPC values were observed when using temperature of 48°C for eight and 12 minutes. Thermotherapy at 52°C from eight minutes of immersion, in addition to reducing the anthracnose AUSPC in fruits up to 81.6%, delayed maturation of fruits. Fruits submitted to thermotherapy at 56°C presented higher severity of the disease and resulted in the maturation of fruits. Thermotherapy reduces anthracnose severity in fruits in a manner similar to fungicide use, but the association of both results in better control of the disease in 'Prata Anã' banana. Thermotherapy is an efficient technique to reduce the severity of the disease in 'Prata Anã' banana produced in northern state of Minas Gerais.

Index terms: *Colletotrichum musae*, thermotherapy, incidence, severity.

Tratamento hidrotérmico no manejo da antracnose em banana 'Prata-anã' produzida no semiárido mineiro

Resumo - A antracnose destaca-se dentre as podridões que podem ocorrer na fase pós-colheita da banana. O objetivo do trabalho foi avaliar a termoterapia no controle da antracnose em banana 'Prata-Anã' produzida no norte de Minas Gerais. Bananas da variedade Prata-Anã foram divididas em buquês de três frutos e inoculadas com *Colletotrichum musae*. Os buquês foram submetidos à termoterapia com cinco temperaturas (40°C, 44°C, 48°C, 52°C e 56°C) por quatro tempos de imersão (4; 8; 12 e 16 minutos) e testemunha. Os tratamentos foram repetidos cinco vezes em delineamento inteiramente casualizado. Calculou-se a área abaixo da curva de progresso da incidência (AACPI) e a área abaixo da curva de progresso da severidade (AACPS). Para a avaliação da eficiência da termoterapia comparada ao controle químico, os frutos foram submetidos aos seguintes tratamentos: termoterapia com e sem aplicação de fungicida e frutos tratados somente com fungicida, sendo a testemunha os frutos somente inoculados com o fungo. Após quinze dias de armazenamento (25°C e 80% UR) os frutos foram avaliados quanto à severidade de antracnose. Os resultados dos experimentos foram submetidos à análise de variância e regressão, e as médias, comparadas pelo teste de Scott-Knott ($p<0,05$). A testemunha foi comparada aos tratamentos pelo teste de Dunnett ($p<0,05$). Os menores valores da AACPI da antracnose foram observados ao utilizar a temperatura de 48°C por oito e 12 minutos. A termoterapia a 52°C a partir de oito minutos de imersão, além de reduzir a AACPS da antracnose nos frutos em até 81,6%, retardou sua maturação. Os frutos submetidos à termoterapia a 56°C apresentaram maior severidade da doença e resultou no avanço da maturação dos mesmos. A termoterapia reduz a severidade de antracnose nos frutos de maneira semelhante ao uso de fungicida, porém a associação de ambos resulta em melhor controle da doença em banana 'Prata-Anã'. A termoterapia é uma técnica eficiente em reduzir a severidade da doença em banana 'Prata-Anã' produzida no norte de Minas.

Termos para indexação: *Colletotrichum musae*, termoterapia, incidência, severidade.

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Introduction

Several rot diseases may occur in the post-harvest phase of banana cultivation, most notably anthracnose caused by fungus *Colletotrichum musae* (Berk & Curt.), which is one of the main post-harvest diseases and leads to significant production losses of up to 40% (COELHO et al., 2010).

The control of banana anthracnose is carried out mainly by chemical and physical methods. Among physical methods, hydrothermal treatment has been tested by several researchers for the post-harvest management of the disease (SPONHOLZ et al., 2004; MORAES et al., 2005; MORAES et al., 2006; NOLASCO et al., 2008; SILVA et al., 2008; RAMIREZ et al., 2011).

Heat can directly control pathogens by protein denaturation, lipid release, destruction of hormones, reduction of metabolic reserves or injuries, inhibition or retardation of germinative tube elongation, or inactivation of spores. The use of high temperatures also targets the control of fungal diseases by the expression of plant defense genes (BARKAI-GOLAN and PHILLIPS, 1991; FERGUSON et al., 2000). However, the ideal temperature for the control of anthracnose and the time of fruit exposure varies among the different works carried out by researchers.

The climatic conditions of the region where fruits were produced can also influence the response to hydrothermal treatment. Field studies have shown that environmental factors directly influence the banana physiology (CABRERA and GÁLAN SAÚCO, 2005; TAULYA et al., 2014; LIMA et al., 2015).

According to Golan and Phillips (1991), the efficiency of hydrothermal treatment in fruits is related to climatic conditions close to the harvest of bunches. Differences in thermotherapy efficiency were also observed by Shiffmann-Nadel and Cohen (1966) in fruits produced at different growth temperatures and cultural practices of cultivars and harvested at several maturity stages.

Environmental factors are directly involved in the incidence and severity of the disease, influencing the various phases of the pathogen's life cycle and also the host development, being important for the pathogen infection (ADASKAVEG et al., 2002; MAFIA et al. 2011; OLIVEIRA et al., 2011).

Thus, the aim of this work was to evaluate thermotherapy in the management of anthracnose in 'Prata Anã' banana produced in northern state of Minas Gerais.

Material and Methods

'Prata Anã' banana bunches came from an commercial planting area in the municipality of Nova Porteirinha - MG, located at 15 ° 48 '09 "S and 43 ° 18' 32" W, with average elevation of 533 m a.s.l. and 27.5 ° C and climate, according to Köppen classification of type Aw, characterized by rainfall concentrated in summer and dry in winter.

Bunches were harvested in November and December in pre-climacteric stage or maturation stage 2 (green fruits with yellow spots), according to the Von Loesecke's scale (PBMH and PIF, 2006), selecting central hands aiming at uniformity of fruits during maturation. Hands were transported to the Laboratory of Post-Harvest Pathology in plastic boxes protected with paper to avoid injuries.

In the laboratory, hands were subdivided into groups of three fruits. Groups were washed with neutral detergent and dried to ambient. They were then atomized with 2.5×10^{6} spores mL^{-1} of *C. musae* obtained from colonies grown in BDA for seven days in order to standardize infection. After inoculation, groups were incubated under moist chamber at 25 ° C for 24 hours. After this period, groups were immersed in a bath-thermostat with water heated at different temperatures: 40 ° C, 44 ° C, 48 ° C, 52 ° C and 56 ° C, for different immersion times: 4, 8, 12 and 16 minutes. After treatment, groups were cooled in water to room temperature. The additional treatment (control) consisted of fruits without thermotherapy.

The experimental design was completely randomized, in a 5 x 4 factorial scheme (5 temperatures x 4 immersion times) and control. Each treatment consisted of five replicates each containing one group with three fruits.

Groups were stored in expanded polystyrene trays and kept in refrigeration chamber (25 ± 1 ° C and 80 ± 5% RH) for 3, 6, 9, 12 and 15 days when disease intensity was evaluated.

The anthracnose intensity in fruits was evaluated by incidence and severity. Incidence was obtained by the number of fruits affected by repetition, being these values expressed in percentage by treatment. For the severity variable, the diagrammatic scale developed by Moraes et al. (2008) with disease severity ranging from 0.5 to 64% was used. The results were used to calculate the areas under the incidence progress curve (AUIPC) and area under the severity progress curve (AUSPC), and disease progress curves were constructed according to the Shaney and Finney formula (1977).

To evaluate the thermotherapy efficiency compared to the chemical control in the control of anthracnose, groups containing three fruits were atomized with suspension of 2.5×10^{6} spores mL^{-1} of *C. musae*, obtained from colonies grown in BDA for seven days. After inoculation, groups were incubated under moist chamber at 25 ° C for 24 hours. After that period, groups were submitted to the following treatments: hydrothermal treatment (52 ° C for eight minutes) with subsequent fungicide immersion (imazalil at 2 mL L^{-1} concentration for two minutes), fruits submitted to hydrothermal treatment without fungicide application and fruits treated only with fungicide. Control consisted of fruits without hydrothermal treatment and without fungicide application.

Fruits were stored in expanded polystyrene trays and kept in refrigerated chamber (25 ± 1 ° C and 80 ± 5% RH). The statistical design was completely randomized.

The anthracnose intensity in fruits was evaluated by means of the severity evaluation after 15 days of storage using the of Moraes et al. (2008) scale.

Data obtained in experiments were submitted to analysis of variance and regression and averages were compared by the Scott-Knott test at 5% probability using the Sisvar software (FERREIRA, 2011). Control was compared with the other treatments by the Dunnett test at 5% probability using the SAS software.

Results And Discussion

Significant interaction ($p < 0.05$) was observed between water temperature and immersion time in the assessment of anthracnose AUIPC. However, as there was no adjustment of regression models, the mean anthracnose AUIPC values in ‘Prata Anã’ banana were compared by the Scott-Knott test ($p < 0.05$).

By evaluating the binomial water temperature and fruit immersion time, the lowest anthracnose AUIPC values were observed when using the hydrothermal treatment at 48 ° C for eight and 12 minutes, not differing from each other. The reduction of AUIPC in treated fruits compared to control was observed in the treatments at 40 ° C for 8 and 12 minutes, 44 ° C for four, eight, 12 and 16 minutes, 52 ° C for 8, 12 and 16 minutes and 56° C for eight minutes, all with disease reduction of 11.11%, with the exception of hydrothermal treatments at 48 ° C for eight and 12 minutes, both reduced anthracnose AUIPC values by 40.74% (Table 1).

Table 1- Area under the incidence progress curve (AUIPC) for anthracnose in ‘Prata Anã’ banana submitted to thermotherapy at different temperatures and immersion times.

Immersion time	Water Temperature (°C)[1]				
(minutes)	40	44	48	52	56
4	468.75 aA	450.00 aA*	468.75 aB	468.75 aA	468.75 aA
8	450.00 bA*	450.00 bA*	300.00 aA*	450.00 bA*	450.00 bA*
12	468.75 bA	450.00 bA*	300.00 aA*	450.00 bA*	468.75 bA
16	450.00 aA*	450.00 aA*	468.75 aB	450.00 aA*	468.75 aA
VC (%)	5.36				
Control	506.25				

[1] Means followed by the same letter, lowercase in the row and upper case in the column, do not differ by the Scott-Knott’s test ($p < 0.05$). Means with asterisks (*) differ from control by the Dunnett test ($p < 0.05$).

Most phytopathogenic microorganisms present a lethal thermal point at temperatures ranging from 45 to 60 ° C (COCHRANE, 1958; DEVERALL, 1965; LIU et al., 1997), as observed by Tanaka et al. (2003) to control the incidence of species of the genus *Colletotrichum* spp. in the solar thermal treatment of water.

Several studies carried out in other pathosystems have demonstrated, similarly to this work, that thermotherapy was effective in reducing, but not suppressing the incidence of diseases. Sponholz et al. (2004) working with ‘Prata’ banana treated at 45 ° C verified that thermotherapy did not prevent the incidence of anthracnose and the percentage of the injured area reached its maximum at 12 days after treatment. In cajá, Brito et al. (2008) also observed that hydrothermal treatment at 50 ° C did not control the appearance of rot in fruits.

Regarding anthracnose AUSPC, significant interaction ($p < 0.05$) was observed between water temperature and immersion time.

There was a quadratic behavior in the AUSPC regression plots of the disease in fruits as a result of the increase in water temperature (Figure 1).

Thermotherapy with fruit immersion for 4, 8 and 12 minutes presented AUSPC values that decreased and obtained a subsequent increase from temperatures of 47.7 ° C, 45.8 ° C and 48.16 ° C, respectively. The increased anthracnose severity can be attributed to the fact that higher exposure temperatures resulted in physical damages to fruits, facilitating the infection of *C. musae*. The increased action of the fungus with the increase of the exposure period was also observed by Moraes et al. (2005), confirming an increase in the incidence of *C. musae* when the time of exposure of fruits to heat treatment was extended.

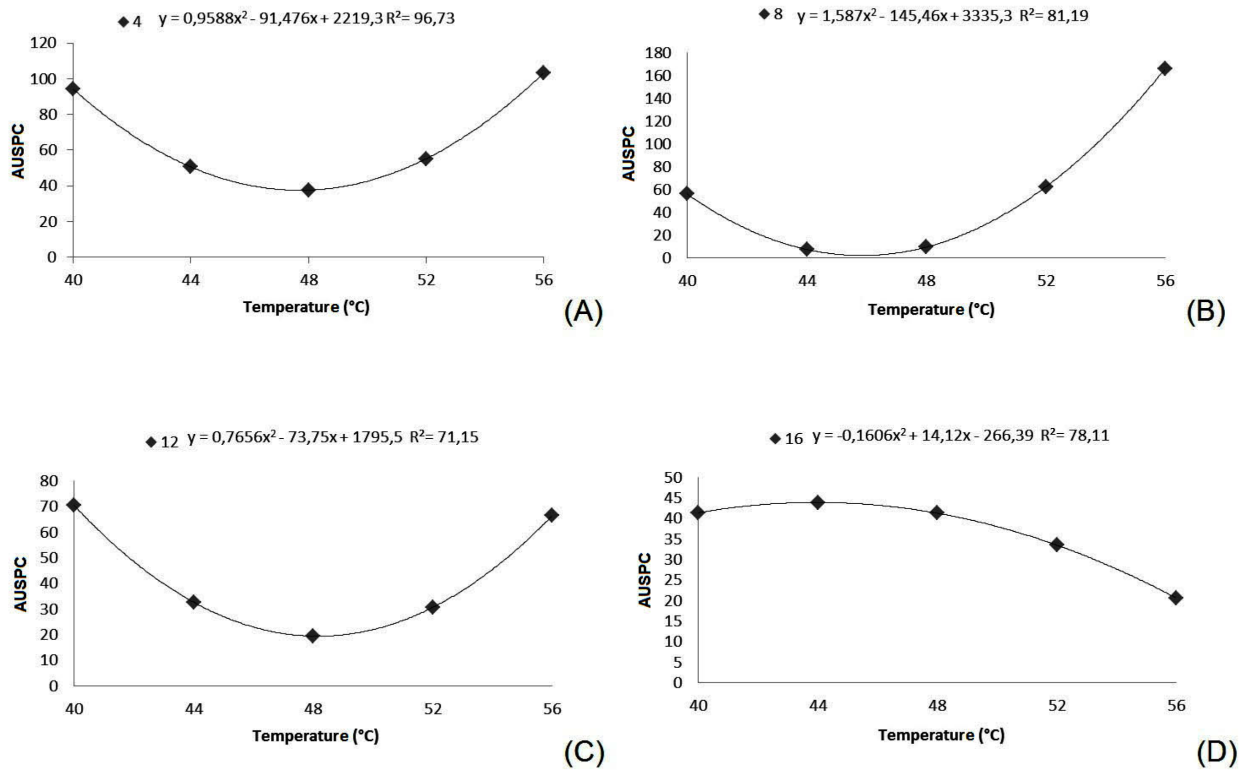

Figure 1- Area under the severity progress curve (AUSPC) for anthracnose in 'Prata Anã' banana, during 15 days, submitted to thermotherapy for 4 minutes of immersion (A), 8 minutes of immersion (B), 12 minutes of immersion (C) and 16 minutes of immersion (D).

In the immersion time of 16 minutes, an inverse behavior was observed, as AUSPC progressed until reaching the temperature of 43.6 ° C, followed by reduction with the elevation of temperature. The reduction of AUSPC in the immersion time of 16 minutes occurred due to the higher fruit exposure to elevated temperatures.

One of the mechanisms responsible for the death of phytopathogens is the denaturation of proteins and enzymes, important for cellular metabolism. Thus, immersion of fruits in water heated from 50 ° C to 55 ° C for 10 minutes has been considered a standard method for controlling various post-harvest fungal diseases (LIU et al., 1997).

Lower anthracnose AUSPC values by the regression plot were observed in treatments at 48 ° C when immersed at 4, 8 and 12 minutes, verifying that there is no need for temperatures so high to obtain satisfactory results in 'Prata Anã' bananas.

The control of pathogens by hydrothermal treatment occurs when spores in quiescent infections are present on the surface or the first cell layers of the fruit (SILVEIRA et al., 2005), a fact that occurs with fungus *C. musae*, which causes anthracnose in bananas. Some authors report that the effect of thermotherapy on post-harvest disease control is due to the reduction in fungal spores viability and resistance induction (CABRERA and DOMÍNGUEZ, 1998; PESSOA et al., 2007).

Analyzing the interaction between water temperature and fruit immersion time, it was observed that in the time of four minutes, temperatures of 44 ° C, 48 ° C and 52 ° C showed the lowest anthracnose AUSPC values, not differing from each other (Table 2).

In the immersion time of eight minutes, it was observed that thermotherapy at 56 ° C presented the highest AUSPC value, differing from the other temperatures used. When fruits were immersed for 12 minutes, it was verified that the lowest AUSPC values were found at temperatures of 48 ° C and 52 ° C, with reduction of 81.2% and 88.6%, respectively, with no difference from each other. At 16 minutes of immersion, there was no difference between temperatures in treated fruits.

Sponholz et al. (2004) reported that in the exposure of 'Prata' banana fruits at 50 ° C for 15 minutes of immersion, at 12 days after treatment, the percentage of injured area was 25%, higher than that found in this study. Nolasco et al. (2008) were able to control 'Prata' banana rot through the 12th day of evaluation with the temperature / immersion time of 50 ° C for 6 and 12 minutes.

By setting the water temperature at 40 ° C, the lowest AUSPC values were observed and after eight minutes of immersion and reduction of up to 63.8% was observed compared to control. At temperature of 44 ° C, the immersion time of eight minutes resulted in a 62.6% reduction in relation to control, but there was no difference among immersion times used. At 48°C, all immersion times reduced AUSPC, differing from control by up to 81.6%.

Table 2-Area under the severity progress curve (AUSPC) for anthracnose in 'Prata Anã' banana submitted to hydrothermal treatment at different temperatures and immersion times.

Immersion time (minutes)	Water Temperature (°C)[1]				
	40	44	48	52	56
4	91.25 bB	56.50 aA	38.75 aA*	47.75 aA	107.75 bC
8	38.50 aA*	39.25 aA*	19.25 aA*	17.75 aA*	187.00 bC*
12	61.25 bA	51.00 bA	19.75 aA*	12.00 aA*	75.75 bB
16	38.00 aA*	51.50 aA	39.00 aA*	19.00 aA*	23.25 aA*
VC (%)	23.84				
Control	104.89				

[1]Means followed by the same letter, lowercase in the row and upper case in the column, do not differ by the Scott-Knott's test ($p < 0.05$). Means with asterisks (*) differ from control by the Dunnett test ($p < 0.05$).

There was no difference among immersion times at temperature of 52°C. There was difference in immersion times compared to control from eight minutes, with reduction percentage of up to 88.6%. This temperature had the lowest anthracnose AUSPC values compared to control, proving to be the most efficient treatment. In addition, fruits treated at this temperature were maintained with green traits, presenting late maturation.

This confirms, as reported by Lobo et al. (2000) working with Cavendish bananas treated at 50 ° C for 15 minutes, that thermal immersion treatments at high temperatures produce changes in maturation. Similar results were found by Wall (2004) when applying thermotherapy (48 ° C, 49 ° C and 50 ° C) for times of 15 and 20 minutes in bananas, where changed in ethylene synthesis were observed. In this work, Chillet et al. (2006) observed that fruits at advanced maturity stage are more susceptible to *C. musae* infections, while green or early maturation fruit are more resistant to infection. Ramirez et al. (2011) also reported that treatment at 55 ° C and a five-minute immersion time delays the maturation of Cavendish bananas.

Generally, green fruits are more resistant to pathogens due to the presence of phytoalexins and other compounds (Pessoa and Oliveira 2006). Tannins found in these fruits are examples of high molecular weight phenolic compounds that precipitate proteins, playing a role in plant protection against pathogens, since they have the capacity to form complexes with proteins and polysaccharides, inactivating enzymatic reactions. These biochemical reactions de-characterize enzymes, preventing them from being used in normal fungal growth processes (HASLAM, 1996; EFRAIM et al., 2006).

Using water temperature at 56 ° C, it was observed that all exposure times were detrimental to fruits. Immersion for four and eight minutes caused a rapid maturation in fruits, which favored the development of the disease, increasing the AUSPC values by 78.3%. From 12 minutes of immersion, scalding in treated fruits was verified, which resulted in a reduction of AUSPC, but making the fruits unfeasible for marketing.

According to Moraes et al. (2005), the injuries caused by the hydrothermal treatment can cause an increase in weight loss, bark discoloration, increased susceptibility to fungi and reduced post-harvest life. Sponholz et al. (2004) and Moraes et al. (2005), working with *C. musae* on banana observed that the increase in temperature reduced the injured area. However, very high temperatures around 56 ° C, associated with longer exposure times of 9 and 12 minutes, led to bark lesions that are more detrimental to marketing than the pathogen action due to poor visual appearance of fruits. Ramirez et al. (2011) evaluated the effect of thermotherapy on the secretion of latex from freshly cut banana and found that bananas exposed to 55 ° C were darker and with burn symptoms due to high temperature.

Temperatures of 50 ° C, 55 ° C and 56 ° C are detrimental to fruits, causing scalding and pulp hardening, impairing commercialization due to poor visual appearance of fruits (ARMSTRONG, 1982; RAHMAN *et al*., 1994; REYES *et al*., 1998; PESSOA *et al*., 2009). The heat treatment can promote external and / or internal damages to fruit tissues. Control of temperature and exposure time is of extreme importance for its use alone or in combination with other control methods (CHITARRA and CHITARRA, 2005).

Figure 2 shows that there was a difference in the anthracnose severity between fruits submitted to hydrothermal treatment with fungicide imazalil and fruits submitted only to hydrothermal treatment and only with fungicide. Thermotherapy reduced the anthracnose severity in fruits in a manner similar to the use of fungicide, without statistical differences, but more efficient results were those in which bananas were treated with thermotherapy together with fungicide imazalil, that practically controlled the disease, presenting only 0.5% of anthracnose in fruits. Thus, the association of hydrothermal treatment / chemical treatment was efficient in the management of anthracnose in 'Prata Anã' banana.

The Dunnett test at 5% probability showed that there was a difference in disease severity between control and fruits submitted to hydrothermal treatment with fungicide and hydrothermal treatment without fungicide, which could be expressed as a disease reduction of 20.99% and 19.24%, respectively. Even with the appearance of the disease, the lesion caused in fruits treated with fungicide alone was significantly lower than those in the control, with reduction of 19.58% of the disease.

These results corroborate those found by Coelho et al. (2010) studying the post-harvest control of anthracnose of ‘Prata Anã’ banana treated with fungicide imazalil. The authors verified a lower effect of *C. musae* on control, not totally controlling the disease.

Despite the existence of *C. musae* resistant to fungicides, Chillet et al. (2006) suggest that the appearance of the disease should not only be explained by this reason, but also by the quality of fruits, much influenced by edaphoclimatic factors. According to research conducted by the authors, there may be a relationship between the mineral status of the plant and the susceptibility of fruits to the disease.

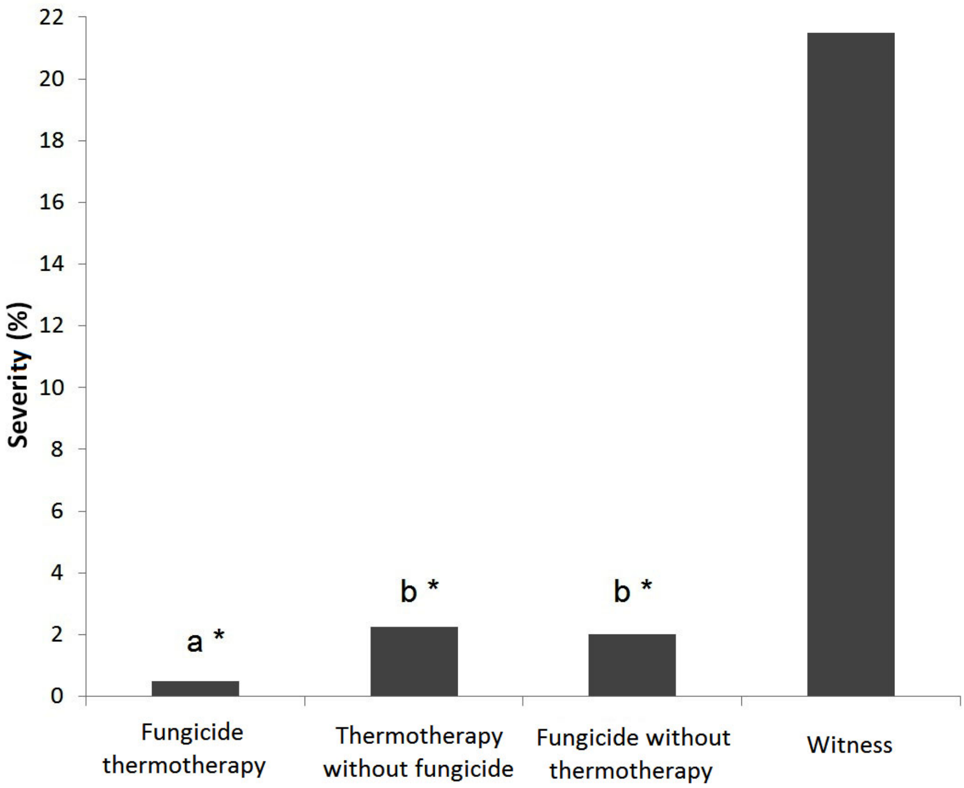

Figure 2. Anthracnose severity in ‘Prata Anã’ banana submitted to hydrothermal treatment (52°C/8’), chemical treatment (imazalil 2 mL L^{-1}) and association of both, after fifteen days of storage, expressed as percentage of injured area / fruit. Means followed by the same letter do not differ by the Scott-Knott test (p <0.05). Means with asterisks (*) differ from control by the Dunnett test (p <0.05).

Conclusions

Thermotherapy at 52 ° C from eight minutes of immersion reduces anthracnose AUSPC in ‘Prata Anã’ banana in up to 81.6% of the injured area and delays their maturation.

The association of hydrothermal treatment with fungicide imazalil results in better control of anthracnose in ‘Prata Anã’ banana.

Thermotherapy is an efficient technique to reduce disease severity in ‘Prata Anã’ banana produced in northern Minas Gerais.

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