

TRABALHO DE CONCLUSÃO DE CURSO II

Agronomia

**HEALTH QUALITY AND GERMINATION OF BUTIA
CAPITATA SEEDS TREATED WITH BACTERICIDE AND
FUNGICIDE**

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BACTERICIDE AND FUNGICIDE**

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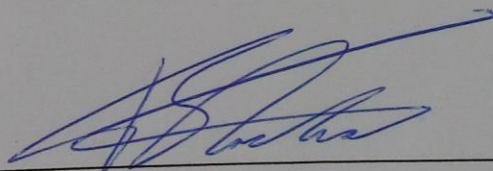
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Vander Rocha Lacerda. **HEALTH QUALITY AND GERMINATION OF BUTIA CAPITATA SEEDS TREATED WITH BACTERICIDE AND FUNGICIDE**

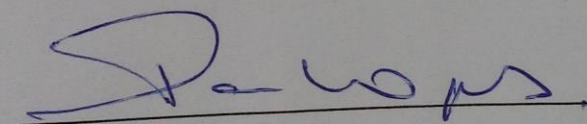
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Montes Claros, 11 de Maio de 2017.

Dedico a toda minha família Rocha e Lacerda
pelo incentivo, especialmente meus pais e
irmão.

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*“Aquele que leva a preciosa semente, andando
e chorando, voltará, sem dúvida, com alegria,
trazendo consigo os seus molhos. ”*

(Salmo 126.6)

Health quality and germination of *Butia capitata* seeds treated with bactericide and fungicide

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ABSTRACT

Lacerda, V. R.; Mendes, D.S.T.; Rocha, F.S.; Fernandes, M.F.G.; Lopes, P.S.N.; Civil, N.; Catão, H.C.R.M. Health quality and germination of *Butia capitata* seeds treated with bactericide and fungicide. *Summa Phytopathologica*, v.42, n.4, p.303-307, 2016.

Propagation of “coquinho-azedo” (*Butia capitata*) is limited due to dormancy and high incidence of fungi in its seeds. The aim of this study was to evaluate the effect of treating “coquinho-azedo” seeds with different concentrations of bactericide and/or fungicide on the incidence of fungi and on the germination of seeds. Thus, *B. capitata* seeds were immersed in four doses of the bactericide kasugamycin (0, 1.5, 3.0 and 4.5 mL/L) and/or in four concentrations of the fungicide carbendazim+ thiram (0, 2.92, 5.84 e 8.75 mL/Kg), totaling 16 treatments. After the treatments, the operculum was removed from the seeds, which were placed on trays containing autoclaved sand, covered with transparent plastic film and kept in the nursery. In the germination test, treatments were distributed in randomized block design, factorial arrangement

of 4x4 (bactericidal doses x fungicide doses), with four replicates of 50 seeds per plot. For the test of seed health quality, the *Blotter test* was employed in completely randomized design, using 20 replicates and 10 seeds. Health quality and germination were evaluated at 15 and 40 days after the treatment of seeds, respectively. There was no interaction between the bactericide and the fungicide on germination and control of fungi associated with seeds. However, there was an effect of fungicide concentrations on the evaluated parameters. We observed a negative correlation between the concentrations of the fungicide carbendazim + thiram and fungal incidence, and a positive correlation between the fungicide concentrations and seed germination. Therefore, fungicide treatment reduces the fungal incidence and provides better germination of *B. capitata* seeds.

Keywords: Carbendazim, thiram, “coquinho-azedo”, fungus, incidence.

RESUMO

Lacerda, V. R.; Mendes, D.S.T.; Rocha, F.S.; Fernandes, M.F.G.; Lopes, P.S.N.; Civil, N.; Catão, H.C.R.M. Qualidade sanitária e germinação de sementes de *Butia capitata* tratadas com bactericida e fungicida. *Summa Phytopathologica*, v.42, n.4, p.303-307, 2016.

A propagação do coquinho-azedo (*Butia capitata*) é limitada devido a dormência e a alta incidência de fungos em suas sementes. O objetivo deste trabalho foi avaliar o efeito do tratamento de sementes de coquinho-azedo com diferentes concentrações de bactericida e/ou fungicida na incidência de fungos e na germinação das sementes. Para isto, sementes de coquinho-azedo foram imersas em quatro doses do bactericida kasugamicina (0, 1.5, 3.0 e 4.5 mL/L) e/ou em quatro concentrações do fungicida carbendazim + thiram (0, 2.92, 5.84 e 8.75 mL/Kg), totalizando 16 tratamentos. Após o tratamento, retirou-se o opérculo das sementes, as quais foram colocadas em bandejas contendo areia autoclavada, revestidas com filme plástico transparente, e mantidas em viveiro. No teste de germinação, os tratamentos foram distribuídos em delineamento de blocos casualizados em esquema fatorial 4 x 4 (doses

do bactericida e doses do fungicida), com 4 repetições de 50 sementes por parcela. Para o teste de sanidade foi empregado o método do “*Blotter test*” em delineamento inteiramente casualizado utilizando 20 repetições com 10 sementes. A qualidade sanitária e a germinação foram avaliadas 15 e 40 dias após o tratamento das sementes, respectivamente. Não houve interação entre o bactericida e o fungicida sobre a germinação e o controle dos fungos associados às sementes. Porém, houve efeito das concentrações de fungicida sobre os parâmetros avaliados. Observou-se correlação negativa entre as concentrações do fungicida carbendazim + thiram e a incidência fúngica e correlação positiva entre as concentrações do fungicida e a germinação das sementes. Portanto, o tratamento fungicida reduz a incidência fúngica e propicia melhor germinação das sementes do coquinho-azedo.

Palavras-chave: Carbendazim, thiram, coquinho-azedo, fungo, incidência.

“Coquinho-azedo” (*Butia capitata* Mart. Becc.) is a palm tree native to the Cerrado biome and is especially found in the states of Bahia, Goiás and Minas Gerais in Brazil (1). Its fruits are commonly used in regional cuisine for fresh consumption and in the preparation of juices, ice cream, sweets and popsicles (17). The fruits are only exploited through intense extraction and in a predatory manner due to their high demand on the market (16) and play an important socio-economic role in regional communities where they naturally occur.

The dormancy phenomenon in *B. capitata* makes its germination

low, slow and uneven (14). This phenomenon limits the production of seedlings for commercial crops, causing an unsustainable exploitation of this resource. The operculum of the endosperm is the main structure that limits the embryo emergence (22); thus, it is classified as physical dormancy, according to the classification system proposed by Baskin and Baskin (2). Therefore, an efficient technique to overcome the dormancy of *B. capitata* is to remove this structure (7). However, the operculum removal procedure greatly increases the risk of seed contamination by several fungi since, in addition to exposing the

embryo, it causes injury to the operculum insertion point in the seed.

Some pathogenic fungi require the activities of the enzymes polygalacturonase and pectate lyase for cell wall degradation in the process of penetration and colonization of plant tissues. Studies about the health quality of *B. capitata* seeds have identified several fungal species such as *Aspergillus flavus*, *A. niger*, *Penicillium* spp. and *Fusarium* spp. (13). These fungi are producers of enzymes that cause seed deterioration (11, 28) and may directly reduce the germination potential and the vigor of seeds, consequently limiting the production of seedlings. Other enzymes produced by *Aspergillus* and *Penicillium* involved in the degradation of plant cell wall polysaccharides and the regulation of gene expression of these enzymes were revised (28). The procedure to minimize this contamination has been carried out under laminar flow chamber conditions and by using completely sterilized equipment (7). However, this method is difficult to be used in a rural environment and in poorly technified nurseries of seedling production.

Seed treatment with fungicide aiming to reduce contamination by fungi is an alternative to ensure the initial stand of plants in the cultivated area (1). Some systemic fungicides used for seed treatment would propitiate good results due to their protective and curative action on the plant tissue. Moreover, some products used in the treatment of embryos and seed disinfection usually affect the germination and the development of seedlings, since various fungicides produce phytotoxic effects at some dosages. Thus, determination of the concentrations of substances used in the control of microbial contamination is important for the establishment of protocols (26).

Considering the lack of studies involving chemical control in *B. capitata* seeds, the aim of this study was to evaluate the incidence of fungi in *B. capitata* seeds treated with different bactericidal doses (kasugamycin) and fungicide mixture (carbendazim + thiram), as well as their effect on seed germination.

MATERIAL AND METHODS

Fruits of *B. capitata* were collected in the municipality of Mirabela, state of Minas Gerais, in Brazil, from a natural population of plants. They were then taken to the Plant Propagation Laboratory at Federal University of Minas Gerais and stored during 4 months at room temperature. To remove the mesocarp (pulp) of these fruits, a low speed industrial blender was used. After pulping, the pyrenes (seed and endocarp) were dried in the shade for five days and stored in paper bags at room temperature for 30 days. Thereafter, with the aid of a lathe manual bench, the endocarp was removed to obtain the seeds. The humidity test of seeds was done by adopting the dry incubator method at 105 °C for 24 hours (4), a sample of 5 replicates with 10 seeds.

Treatments with the fungicide and the bactericide were performed by immersing the seeds for 1 and 10 minutes, respectively. The experiment was conducted in 4 x 4 factorial arrangements, with four doses of the bactericide kasugamycin of 0, 1.5, 3.0 and 4.5 mL/L and/or four doses of the fungicide carbendazim + thiram of 0, 2.92, 5.84 and 8.75 mL/kg of seeds, totaling 16 treatments. First, the seeds were immersed in the bactericide, and then in the fungicide. After treatment, the seeds were subjected to the operculum removal process according to the methodology proposed by Fior et al. (7). Then, for the germination test, the seeds were placed on trays containing sterilized and autoclaved sand and added of distilled water at 70% of its holding capacity. These trays were covered with transparent plastic film (PVC), forming thus a moist chamber around the seeds. The trays were kept in a greenhouse with 50% shade cloth. The temperature was daily monitored during

the experiment. Each treatment consisted of four replicates of 50 seeds per plot in a randomized block design. After 40 days, the effects of treatment with bactericide and/or fungicide on the percentage of germination (emergence of the embryo) and the elongation of embryos were evaluated according the method proposed by Dias et al. (5), which considered germinated embryos ≥ 2 mm long.

Verification of the health quality of *B. capitata* seeds was performed based on the *Blotter test*, according to Machado (12), with modifications. The seeds were treated with the fungicide and the bactericide as described above and incubated for 15 days under a photoperiod of 12 hours light and temperature of 25 ± 2 °C. The experimental design was randomized, using 20 replicates with 10 seeds. Quantitative and qualitative assessments of the fungi were obtained by individually examining the seeds under a stereoscopic microscope. The fungi present in the seeds were identified by using the identification key described by Klich and Pitt (10). In some cases, identification was confirmed by visualization of morphological structures of fungi under an optical microscope.

The normality of the data was analyzed according to Saphiro-Wilk test. As the data were not normally distributed, generalized linear models (GLM) were created. The quasibinomial error distribution test was used to analyze the relationship between the incidence of the three most abundant fungi (*Curvularia* sp., *Aspergillus* sp. and *Penicillium* sp.) and the different doses of bactericide and fungicide, as well as their interactions with the explanatory variables. The same error distribution was used for analysis of the final percentage of germinated seeds, non-germinated and elongated embryos according to the different doses of kasugamycin and carbendazim + thiram, and their interactions. Then, the analysis contrast with the CAR package was performed to compare means. We also tested the correlations of the effects of fungi, the percentage of germinated seeds, non-germinated and elongated embryos at different doses of bactericide and fungicide, using the Spearman correlation. All analyses were performed by using the statistical software R. The p-value lower than 0.05 was considered significant.

RESULTS AND DISCUSSION

There was no significant interaction between the bactericide kasugamycin and the fungicide carbendazim + thiram on seed germination and fungal control. There was not any effect of the bactericide application on fungal incidence or seed germination. By analyzing the GLM, we observed a significant effect ($P < 0.05$) of fungicide doses on all evaluated parameters. By analyzing the humidity test, the water content of the seeds used in the experiment was 8%. Seed storage with high levels of water remains the major preservation method for recalcitrant seeds; however, it provides favorable conditions for the development of microorganisms (18).

The fungi showing the highest incidence on *B. capitata* seeds were *Curvularia* sp. (70.63%), *Penicillium* spp. (13.76%) and *Aspergillus* spp. (6.89%) in the treatments without thiram + carbendazim (Table 1). Other fungi detected in the seeds were *Bipolaris* spp., *Phomopsis* sp. and *Drechslera* sp. with incidence of less than 2.5%. In *B. capitata* seeds with and without endocarp, occurrence of *Aspergillus flavus*, *A. niger*, *Cladosporium* sp., *Epicoccum* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp. and *Trichoderma* sp. has been reported (13). Among the fungi reported in the literature to be detected in *B. capitata* seeds, some species of *Fusarium* and *Aspergillus* and *Penicillium* genera are associated with damping off of seedlings and deterioration of seeds.

Table 1. Incidence of fungi detected on *Butia capitata* seeds treated with different doses of the fungicide carbendazim + thiram.

Doses (mL/Kg)	Incidence (%)		
	<i>Curvularia</i> sp.	<i>Penicillium</i> spp.	<i>Aspergillus</i> spp.
0	70.63±4.13 a	13.76±3.63 a	6.89±2.53 a
2.92	15.63±3.15 b	16.26±5.15 a	2.52±1.70 b
5.84	1.27±0.85 c	1.27±0.85 b	0.25±0.00 c
8.75	1.89±1.00 c	0.25±0.00 b	0.25±0.00 c

Means followed by the same letter in the column do not differ according to contrast analysis at 5% probability. Data represent the average percentage values of 20 replicates. Values represent Mean ± standard error.

Table 2. Average percentage values of germinated seeds, non-germinated seeds and elongated embryos of *Butia capitata* after seed treatment with the fungicide carbendazim + thiram at different doses.

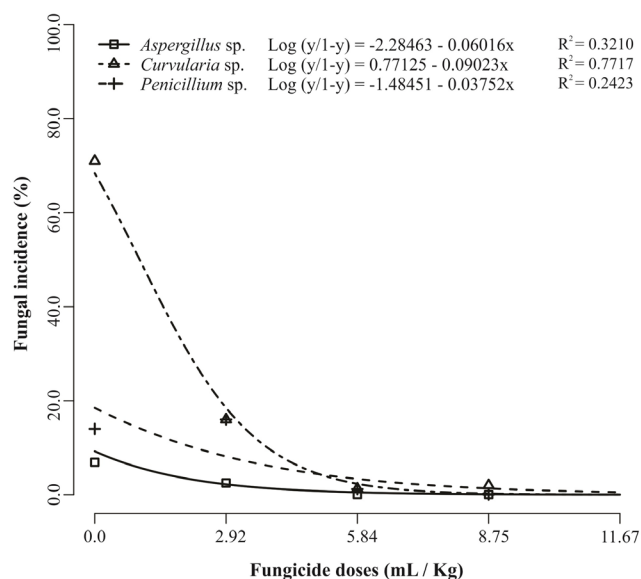
Doses (mL/Kg)	Seed germination (%)	Non-germinated seeds (%)	Elongated embryos (%)
0	10.00±2.58 c	83.13±3.33 a	6.26±2.56 b
2.92	39.38±3.59 b	40.63±4.32 b	20.62±2.49 a
5.84	56.88±1.98 a	27.50±3.22 c	15.63±2.03 a
8.75	62.50±1.44 a	23.75±2.87 c	18.75±3.01 a

Means followed by the same letter in the column do not differ according to contrast analysis at 5% probability. Data represent the average percentage values of 4 replicates. Values represent Mean ± standard error.

Contamination of *B. capitata* seeds occurs in the field, especially when the fruit falls to the ground, and during the fruit storage period. The fungi need enzymes such as cellulases, pectinases, amylases, lipases, proteases and nucleases to break the cell wall and promote the deterioration of seeds. In this study, a higher incidence of *Curvularia* sp. in *B. capitata* seeds was observed, as well as lower incidence of *Penicillium* spp. and *Aspergillus* spp. (Table 1). The genus *Curvularia* is considered of weak pathogens but is common in several seeds of plants of economic importance (24). However, considerable fungal infestations can compromise seed germination and vigor (23). Furthermore, *Curvularia pallescens*, detected in the seeds of various cereals, produces a large quantity of amylase and its virulence is associated with greater production of this enzyme (9). Masumi et al. (15) demonstrated the production of the enzyme amylase by thirty-three fungi endophytic from *Thymus* sp. but the greatest enzyme activity was produced by *Curvularia akaii*.

The fungi of the genera *Penicillium* and *Aspergillus* found at low incidence on *B. capitata* seeds are known to produce toxins and enzymes associated with the deterioration process. Fawole et al. (6) reported the production of pectinases and cellulases by *Aspergillus flavus* and *Penicillium* sp. and attributed the reduced germination of cowpea seeds and the virulence of these fungi to enzymatic activity. Rocha et al. (23) studied the physiological quality of soybean seeds at three inoculum levels and found 80% reduction in germination and seedling vigor and total tissue deterioration in seeds with incidence of up to or above 50% for *A. ochraceus*. In this study, the percentage of germinated seeds and elongated embryos treated with fungicide, compared to control, showed that the incidence of *Curvularia* sp., *Penicillium* spp. and *Aspergillus* spp. reduces the physiological quality of *B. capitata* seed, which may be due to their enzyme potential to deteriorate the cell wall (Tables 1 and 2).

Seed treatment with the studied fungicide doses (2.92, 5.84 and 8.75 mL/kg) reduced the occurrence of fungi on *B. capitata* seeds (Table 1 and Figure 1). At higher doses, germination percentage with fungicide was also higher, compared to control (Table 2 and Figure 2). Seed treatment with fungicide addition to reduce the incidence of fungi also promotes better germination rate and emergence of plants (8, 19). Several studies have demonstrated fungal control on the seed

**Figure 1.** Incidence of *Curvularia* sp., *Penicillium* sp. and *Aspergillus* sp. associated with *Butia capitata* seeds treated with different doses of the fungicide carbendazim + thiram at 15 days after treatment.

by the fungicide carbendazim + thiram in several plant species (8). Tavares (27) observed that processing soybean seeds with the fungicide carbendazim + thiram at a dose of 2 mL/kg of seed reduced the incidence of *Penicillium* spp., *Rhizopus* spp., *Aspergillus* spp. and *Fusarium* by more than 90% and had better performance than the fungicides fipronil and fludioxinil + metalaxyl-M. In another study, Silva et al. (25) found lower incidence of *Curvularia* sp., *Fusarium* spp., *Alternaria* spp. and *Bipolaris* in rice seeds treated with carbendazim + thiram at the dose of 3 mL/kg of seed, as well as higher germination percentage.

Low doses (0.1 to 1%) of the fungicide carboxin + thiram are not effective in controlling fungi and do not have negative effect on germination and development of seedlings of *B. capitata* (26). But a high dose of carboxin + thiram (8.75 mL/kg) promoted a reduction of

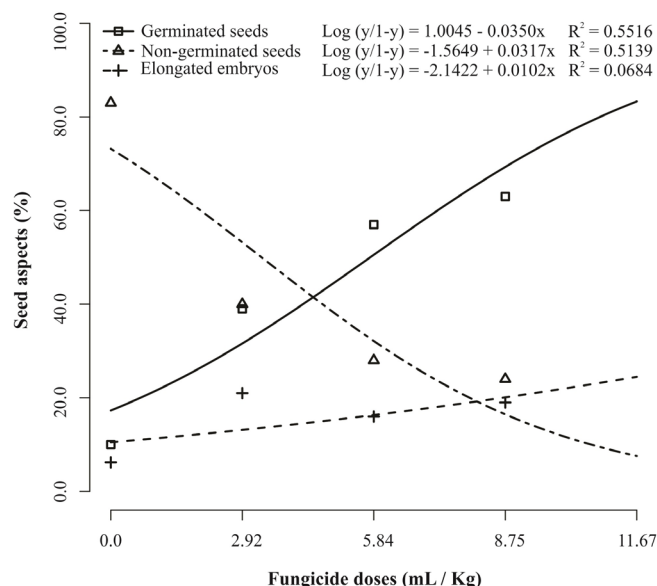


Figure 2. Percentage of germinated seeds, non-germinated seeds and elongated embryos according to the doses of the fungicide carbendazim + thiram at 40 days after treatment.

96, 97 and 98% in the incidence of *Aspergillus* spp., *Curvularia* sp. and *Penicillium* spp., respectively, as well as a 52.5% increase in seed germination (Tables 1 and 2). There was a negative correlation between seed treatment with fungicide and incidence of *Curvularia* sp. ($r = -0.816$), *Penicillium* spp. ($r = -0.537$), *Aspergillus* spp. ($r = -0.420$) and a positive correlation with seed germination ($r = 0.866$) and embryo elongation ($r = 0.321$). However, despite the reduced fungal effect and increased germination rate of seeds after treatment with fungicide, there was a little increase in the germination percentage, compared with the studies of Fior et al. (7), who managed 90% germination under aseptic conditions. The temperature of 30 °C is defined as most favorable for *B. capitata* germination (7). In this study, the average daily temperature in the experimental plots was 30.9 °C, which is consistent with the temperature required for germination. On the other hand, the *B. capitata* fruits used in this experiment were stored during 4 months under uncontrolled environmental conditions. Apparently, this factor, combined with fungal occurrence, may have contributed to reduce the germination potential of seeds, decreasing their physiological quality due to intensification of the deterioration phenomenon.

There were few seeds with symptoms of bacterial exudation during the operculum removal process and the experiment. This suggests that the low bacterial infestation on the seed did not allow the identification of significant interaction between the bactericide kasugamycin and the fungicide carbendazim + thiram, or its effect on control and germination.

In summary, this study demonstrated the potential of the chemical treatment of *B. capitata* seeds with the fungicide carbendazim + thiram to control *Curvularia* sp., *Penicillium* spp. and *Aspergillus* spp., resulting in increased germination percentage, which can be an effective measure to produce good quality seedlings.

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