academicJournals

Vol. 15(38), pp. 2091-2097, 21 September, 2016
DOI: 10.5897/AJB2016.15559
Article Number: A6382A360534
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Coating with fungicide and different doses of fertilizer in vinhatico seeds

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Received 7 July, 2016; Accepted 31 August, 2016

Plathymenia reticulata **Benth occurs in open formations of the Brazilian Cerrado and Atlantic Forest. The seeds are affected by pathogens that reduce germination. The use of fungicides is defined for many species, but, for forest species, further studies are necessary. Thus, the aim of this study was to evaluate and identify the physical and physiological quality of mahogany seeds that are coated with fertilizer and fungicide. The treatments were: seed coating with sand + lime + fungicide with different doses of fertilizers. The seeds were evaluated in the laboratory and in a greenhouse. The experiment was conducted in a completely randomized design with four replications of 50 seeds. The treatment with 50 g of fertilizer had the highest weight out of 1000 seeds. There were no infested seedlings among the coated seeds. The coating maintained the quality of the seeds and the coating with high doses of fertilizer inhibited the germination, germination speed index (GSI), emergence and emergence speed index (ESI) of the mahogany seed.**

Key words: *Plathymenia reticulata*, germination, vigor, fungicide.

INTRODUCTION

Plathymenia reticulata Benth is popularly known as *vinhático-do-campo* or *vinhático-do-cerrado* (Brazilian mahogany). It is a forest species that has hardwood used in construction, aside from being used in the recovery of degraded areas (Lorenzi, 2008).

Studies related to the cultivation of native species have increased significantly due to the increased demand for forest deployment for various purposes, but there is still a lot to advance regarding the specificities of each botanical group in the field of seed technology or in the field of ideal nutrition for planting. According to Gonçalves et al. (2012), there are few studies on nutritional requirements of forest species, but knowledge of proper nutrition of each species plays an important role in plant development. As the understanding of the seed value and the need to protect or enhance their performance increases, it also increases the quantity of products available for the treatment of seeds, for various purposes, either for protection, with the use of fungicides and insecticides, or nutrition, with the use of nutrients, when

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the main objective is to enhance seed performance in the physiological and economic aspect.

According to Netto and Faiad (1995), one of the important aspects of forest seeds is the phytosanitary quality, because as well as causing deterioration of seeds, diseases reduce the population of seedlings, causing debilitation and epidemics. The use of fungicides for the treatment of seeds is well established for large commercial crops, but for forestry species further studies are still needed. The supply of nutrients through seeds is effective due to the fact that the required amount of nutrients and their distribution become critical in soils in which occur adverse conditions for their solubilisation, thereby affecting the growth of roots (Kirkby and Römheld, 2007).

In this context, experiments using nutrients and fungicides in seed coating are important because they take part in the development of seedlings. The objective of this study was to evaluate and identify the effect of the coating with fungicide and with fertilizer doses on the quality of Brazilian mahogany (vinhatico) seeds.

MATERIALS AND METHODS

The *Vinhático* seeds were purchased from the *Empresa Caiçara Comércio de Sementes (Rascal* Seed Trade Company). Initially, the wings, empty and malformed seeds, were removed. After this step, the selected seeds were mechanically scarified by means of 36 grit sandpaper to ensure complete soaking. For coating, the proportions between the filling materials and seeds were 3:1 (w/w). For each 50 g of seed, 150 g of filler material were used, and this material was divided into twelve portions of 12.5 g each. Every two filler portions formed a layer, totaling six layers. As adhesive solution, Polyvinyl acetate (PVA) glue diluted in water heated at the temperature of 70°C was used at a ratio of 1: 1 (v/v), according to Mendonça et al. (2007). Captan fungicide was used in the last layer. 0.25 g of fungicide + 12.25 g of limestone was added in the last two portions of 12.5 g of filler.

As filler material, sand, limestone, and increased doses of NPK 4-14-8 fertilizer + boron and zinc was used; and fungicide mixed with 10 g of boric acid + 10 g of zinc sulfate and 200 g of NPK 4-14-8 fertilizer was also used. The fertilizer was applied in the intermediate layers of the coating so as to prevent it to come into direct contact with the surface of the seed, while it was also protected by the outer layers. The arrangement of portions of fillers in the coating of the seeds was as follows:

T1: 6 portions of sand + 4 portions of limestone + 2 portions of limestone and fungicide; T2: 5 portions of sand + 2 portions of sand and fertilizer (3.125g of fertilizer + 9.375g of sand per layer) + 3 portions of limestone + 2 portions of limestone and fungicide (0.25g of fungicide + 12.25g of limestone per layer); T3: 5 portions of sand + 2 portions of sand and fertilizer (6.25g of fertilizer + 6.25g of sand per layer) 3 portions of limestone + 2 portions of limestone and fungicide; T4: 5 portions of sand + 2 portions of fertilizer + 3 portions of limestone + 2 portions of limestone and fungicide; T5: 4 portions of sand + 4 portions of fertilizer + 2 portions of limestone + 2 portions of limestone and fungicide; T6: uncoated seeds.

The coating process was carried out in a coater, model N-10, Newpack. The equipment has stainless steel chamber spinning at a speed of 40 rpm, spray at pressure of 4 bar and hot air at 40°C to dry the seeds. The seeds were placed inside the coater along with a portion of filler material. Then, the spray of adhesive solution was applied 3 times, with one-minute intervals between each application, and then another portion of filler material was put on the seeds, followed by another application of the adhesive solution. Next, the hot air blower was activated for 1 min. This process corresponded to a coating layer. After coating, the seeds were evaluated for physical and physiological characteristics: The procedures used for evaluation were as follows:

Water content (WC) and weight of thousand seeds (WTS)

Germination test (GT)

This test was performed with four repetitions of 50 seeds, sown in rolls of paper for germination, previously moistened with water equivalent to 2.5 times the mass of the paper. Then, the rolls were taken to the germinator (BOD) at a constant temperature of 25°C, light/dark for 8/16 h. The evaluations were performed on days 10 and 16, computing the number of normal, abnormal seedlings, infested and non-germinated seeds, according to the criteria set in the Rule for Seed Analysis (Brasil, 2013).

Germination speed index (GSI)

This was conducted with the germination test, and evaluations were carried out every two days from sowing to the end of test. The index was calculated based on the formula of Maguire (1962).

Emergence at house of vegetation (%E)

Four repetitions of 50 seeds were sown in perforated plastic trays containing washed sand, corresponding to each of the treatments. The trays were kept in a greenhouse and the counting of normal seedlings emerged was held on the 16th day after sowing.

Emergency speed index (ESI)

This was conducted along with the evaluation of seedling emergence in greenhouse, and the evaluations carried out every two days from sowing until the 16th day after sowing using the formula of Maguire (1962).

Shoot (SL) and root (RL) length

The length of shoot and root was measured after 60 days in the greenhouse, in four repetitions of ten washed and cut plants, separating shoot and root from the neck of the plant and measured by a millimetre ruler.

Fresh and dry mass of shoot and root

After the measurements of shoot and root length, they were weighed on an analytical scale to obtain the values of fresh mass and both parts were packed separately using paper bags and put to dry in forced-air oven at 60°C for 72 h and were then weighed on an analytical scale.

Statistical analysis

The experiments in the laboratory and greenhouse were conducted in a completely randomized design with six treatments and four repetitions of 50 seeds. The data were submitted to analysis of

Table 1. Weight of a thousand seeds (g) and Coating Increase (g) on uncoated vinhático seeds and seeds coated with fungicide and increasing fertilizer doses

Treatments	Thousand seed weight	Coating Increase
Control	5.34[e]	0
Without fertilizer	7.18[d]	1.84
6.25 g fertilizer	9.13[b]	3.79
12.5 g fertilizer	8.03[c]	2.69
25 g fertilizer	7.11[d]	1.77
50 g fertilizer	10.66[a]	5.32

Averages followed by the same letter do not differ among themselves by Duncan's test, at the 5% level probability.

variance and comparison of means was carried out by Duncan test at 5% probability, with the help of the ASSISTAT program (Silva and Azevedo, 2002).

RESULTS AND DISCUSSION

Laboratory

For the thousand seed weight (TSW), a significant increase was observed after the coating and after the increase in the TSW, in the order of 1.77 to 5.32 g in coated seeds, when compared with uncoated seeds (Table 1). The coating with 50 g of fertilizer had the highest TSW. The difference in seed weight is possibly related to the difference of granulometry of the materials used, as the fertilizer has a higher particle size in relation to sand and limestone. Some authors are unanimous in saying that one of the advantages of the coating process is the increase of the size of the seeds, in order to facilitate manual and mechanical seeding (Nascimento et al., 2009). The coated seeds showed lower water content than the non-coated seeds (Figure 1A). The values of water content in the seeds that received coating fluctuated between 7.09 and 8.69%, below the uncoated seeds, which had 10.72% of water. These results indicate that the drying process of the coater, at 40°C, was efficient and that the materials have not retained moisture from the adhesive solution.

Conceição et al. (2009) also found lower water values in coated corn seeds, and claim that the coated seeds have less water content because the water contained in the coating is not absorbed by the seed and it is quickly lost in the process. Therefore, the coated seeds tend to have lower water content, when compared to uncoated seeds. The results of the first count showed decreasing linear behavior depending on the dose of fertilizer in the coating. As the doses of fertilizer in the coating of vinhatico seeds were increased, there was a reduction of the first count germination (Figure 1B). The first count germination values were greater for coated seeds without fertilizer, surpassing even the uncoated seeds, reaching 85% of emission of radicle compared to uncoated seeds that showed 60% of issued radicles. It is also important to note that among the treatments that were coated with doses of fertilizer, the only treatment that achieved less than the uncoated seeds was treated with 50 g of fertilizer.

The reduction of first count germination with the raise of fertilizer doses could be caused by the effect of fertilizer toxicity, and reduction was observed on the first germination count. Different results were found by Tavares et al. (2013). Studying the potassium action through soybean seed coating and the effects upon physiological quality and performance, it was possible to notice that the first count germination and germination variables did not significantly differ, regardless of the source and concentration used. However, Bays et al. (2007), working with the coating of soybean seeds with micronutrient, fungicide and polymer, observed that there was damage on the plant standard development, because the final volume from the mixture surpassed the recommendation of 300 ml/50 kg seeds, reaching a final volume of 400 ml/50 kg.

Fungueto et al. (2010) analyzing the rice seed coating with source zinc, fungicide and polymer did not observe differences on germination. Derré et al. (2013) worked with *Urochloa brizantha* cv. Xaraes and *Urochloa ruziziensis* cv Kennedy seeds and confirmed that coated seeds soak more slowly. For the germination, the data also adjusted better to the decreasing linear model. As the doses of fertilizer on vinhatico seeds were increased, there was reduction of germination (Figure 1C). According to the germination test, it can be observed that coated seeds without fertilizer present 82% of germination, while uncoated seeds presented 67% of germination (Figure 1C). As the fertilizer was added to the coating, reduction was observed in germination, reaching lower values when compared to uncoated seeds. This result resulted from the toxicity of high doses of fertilizer on the coating and/or due to exacerbated delay on seed soaking, caused by the presence of fertilizer on the coating.

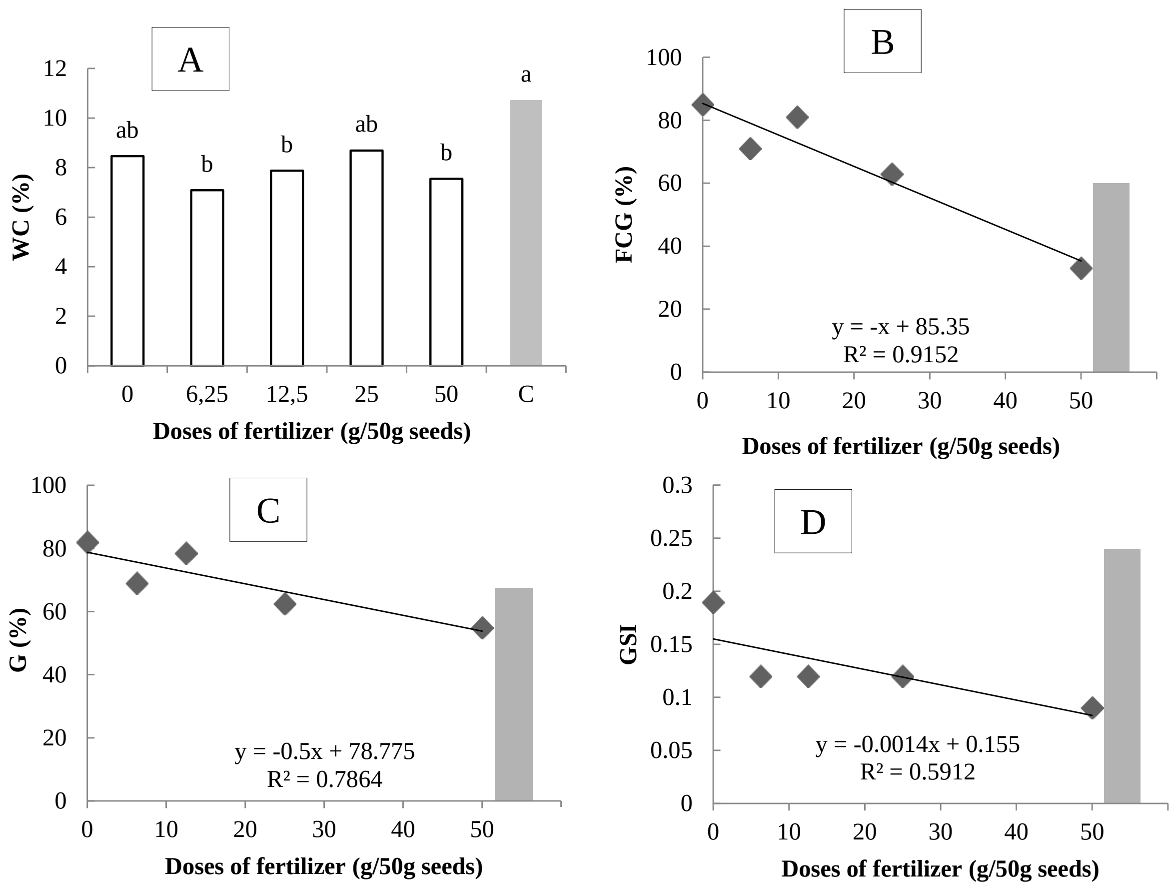

Figure 1. (A) Water content (WC %); B: first germination count (FCG %); C: germination (G %); D: germination speed index (GSI) on vinhatico seeds coated with fungicides and increasing fertilizer doses (0; 6.25; 12.5; 25 e 50 g/50 g of seeds) and uncoated seeds (gray column).

According to Xavier et al. (2015), the fertilizers interfere with the hydric potential of the coating around the seed, reducing the gradient between coating and seed surface. This limits the amount of water absorbed, reducing seed germination, which can be seen in this work, in which the nutrients added on the higher dose of fertilizer resulted in lower germination and first count germination (Figure 1). The results found on literature regarding germination of coated seeds varied. Some authors have observed reduction while others have noticed increase on germination. These results depend on the seed condition, on the studied species, on the type and amount of coating used during the process. Dutra et al. (2014), studying *maxixe* seeds observed germination and increase of strength on seeds treated with phytin, compared to the ones treated with dicalcium phosphate.

According to Soares et al. (2016), the use of soybean seeds coated with 0.6 to 0.8 g of monosodium phosphate per 100 g of seeds resulted in germination and strength increase, as well as emergence and emergence speed improvement. In relation to germination speed index, a linear behavior on the results was observed. The coated seeds presented reduction on the GSI as the doses of fertilizers were increased (Figure 1D). These results can be explained because coating, initially, reduces the speed of water soaking by the seed and can still hamper gas exchange between seed and environment. As a consequence, there is a delay of the seed respiratory process and standard metabolism, promoting reduction of germination speed index. This causes a positive effect on the germination of coated seeds without fertilizer and coated seeds with lower doses of fertilizer (Figures 1C and D).

The main responsible factor for the decrease in germination speed of coated seeds is the physical barrier imposed on the seed, which delays the root protrusion, aggravated by the increase in size and coating resistance. Pereira et al. (2011), working with pelleted *Brachiaria* treated with fungicide and insecticide, observed a higher germination speed index for seeds that were not pelleted. According to the authors, this happened because the coating layer acted as a physical barrier.

The percentage of non-germinated seeds (NGS)

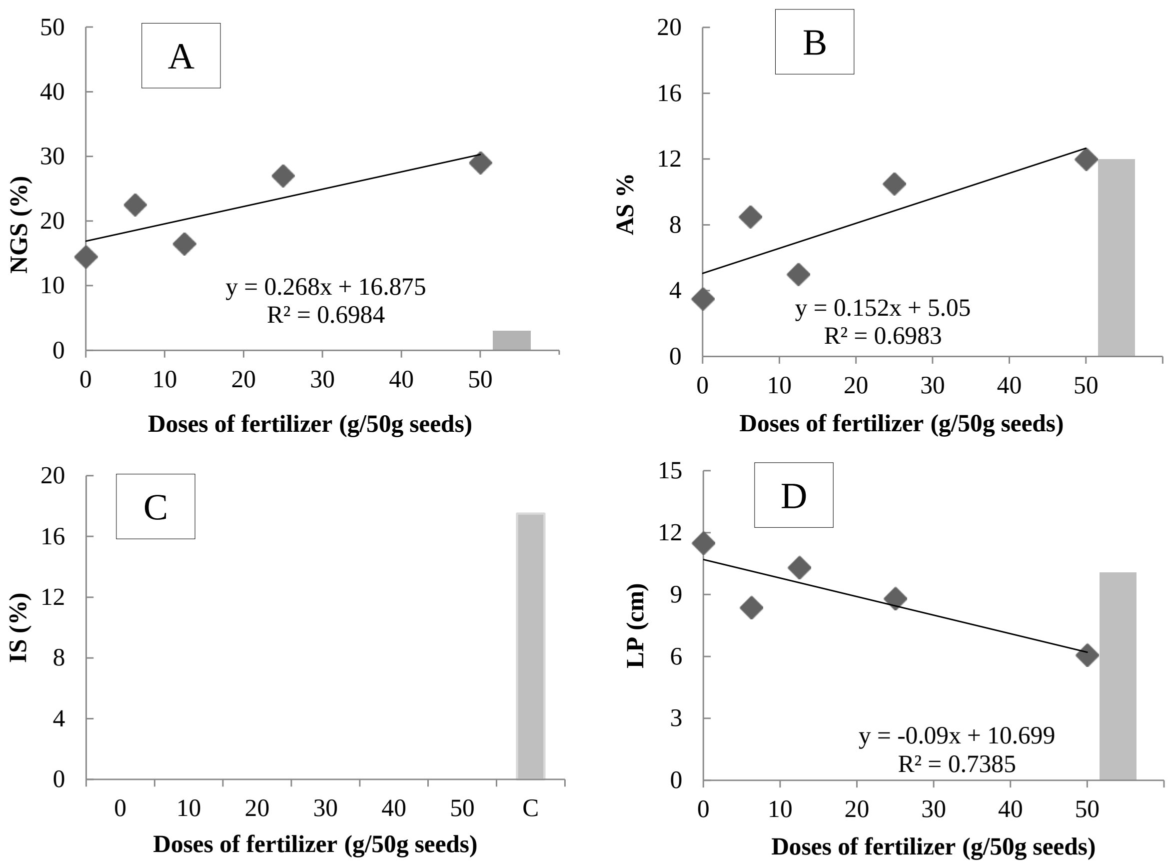

Figure 2. Non-germinated seeds (NGS %), abnormal seedlings (AS), infected seedlings (IS %) and seedling length (LP) on seedlings originated from vinhatico seeds coated with fungicides and increasing doses of fertilizer (0; 6.25; 12.5; 25 e 50 g/50 g of seeds) and uncoated seeds (gray column).

presented increasing linear behavior, that is, as the fertilizer dose was increased on the coating, there was a significant raise on the number of NSG (Figure 2A). This is another effect from higher doses of fertilizer used on seed coating, causing toxic effect added to the gas exchange restriction caused by coating. This can be proved when observing the increase of NSG as the seed is coated, reaching from 14 to 30% of NSG when the fertilizer (50 g) is added to the coating. Brites et al. (2011), working with scratched seeds and coated seeds with different tropical forage species, found a higher percentage of dead seeds after coating, comparing to the scarified seeds, reaching 73% in *Brachiaria humidicola*.

The unusual seedlings (US) results presented increasing linear behavior due to the increase of fertilizer doses on the coating (Figure 2B). For this variable, it was observed that uncoated seeds reached 12% of (US), against 3.5 and 5% of US on coated seeds with and without fertilizer, respectively, highlighting that uncoated seeds lost strength quickly while the coating protected the seeds, keeping their strength, reducing water absorption speed and reducing damages to the membranes during the soaking process. Only damages caused by high fertilizer doses on coating made the results of US stay closer to the control treatment, without coating. These results corroborate with the results found by Brites et al. (2011). Observing the germination of *Brachiaria* and *Panicum* seeds, scarified with sulfuric acid and coated, it was possible to conclude that coated seeds reduced the number of unusual seedlings compared to uncoated seeds, scarified and not scarified.

During the experiment, the presence of infested seedlings on coated seeds was observed, proving that the fungicide dose was efficient to combat fungi that were found on previous experiments. It is worth highlighting that uncoated seeds from the control treatment presented 17.5% of seedlings infested with fungi (Figure 2C). Ludwig et al. (2014), studying the strength and production of *crambe* seeds treated with fungicide, insecticide and polymer, concluded that the application of fungicide and/or insecticide associated or not to the polymer, affected the strength of the seed in laboratory tests, but in soil conditions, it did not affect germination and emergence of *crambe* seedlings.

Table 2. Percentage of emergence (%E), emergence speed index (ESI), length of aerial part length (LAP), length of root (LR), fresh and dry mass from the aerial part (FMAP/DMAP), fresh and dry mass from the root (FMR/DMR) of vinhatico seedlings in greenhouse 60 days after sowing.

Treatments	E (%)	IVE	CPA (cm)	CR (cm)	MFPA (g)	MSPA (g)	MFR (g)	MSR (g)
Control	74.5[ab]	0.11[a]	7.6[ab]	7.9[a]	1.94[b]	0.85[a]	1.07[c]	0.33[a]
Without fertilizer	79.5[a]	0.11[a]	7.9[a]	7.5[ab]	2.74[a]	0.85[a]	2.14[a]	0.44[a]
6,25g fertilizer	68.5[ab]	0.08[b]	7.2[b]	6.7[c]	2.16[b]	0.76[a]	1.18[c]	0.28[a]
12,5g fertilizer	67.5[ab]	0.09[b]	7.4[ab]	7.1[bc]	2.51[a]	0.77[a]	1.81[ab]	0.35[a]
25g fertilizer	70.5[ab]	0.09[b]	7.7[ab]	7.3[abc]	2.57[a]	0.85[a]	1.74[b]	0.39[a]
50g fertilizer	61.5[b]	0.07[c]	7.9[a]	7.4[abc]	2.53[a]	0.77[a]	1.60[b]	0.27[a]
CV (%)	11.96	6.69	4.78	4.7	7.68	7.19	15.97	32.31

Averages followed by the same letter do not differ among themselves by Duncan's test, at the 5% level probability.

Regarding the length of the seedling, the results indicated a linear behavior, with the length reduced due to the increase of fertilizer doses (Figure 2D). There is no loss to this variable when the seeds are coated, but the decrease is significant when the fertilizer is added to the coating.

Almeida et al. (2015), studying the soaking and physiological quality of white lupin seeds treated with micronutrients, observed no significant effects on the dry mass weight and on the length of the seedling between lots and pre-sowing treatments.

Greenhouse

With regard to the characteristics evaluated in the greenhouse, low correlation coefficient was verified in the regressions. After analysis of variance, the averages were compared by Duncan's test at the 5% level of probability. Analyzing the emergence in the greenhouse (Table 2), no significant reduction was observed after the increase of fertilizer doses on coating. However, it is important to highlight the benefit from the coating without fertilizer to the plant emergence (79.5%), even if not significant.

Santos et al. (2010) analyzed coated *Brachiaria brizantha* cv. Marandu seeds and concluded that the pelletization reduces both seedling and speed emergence. The presence of fertilizer on the coating reduced the emergence speed index (ESI), which did not happen to the coated seeds without fertilizer, demonstrating once more the negative effect of adding fertilizer to the coating of vinhatico seeds (Table 2).

Some authors affirm that the coating can improve seed quality when it is associated to the use of micronutrients, insecticides and fungicides as it was observed on rice (Tavares et al., 2012), carrot (Hölbig et al., 2010) and millet seeds (Peske and Novembre, 2011). However, the coating also delays the plant germination and emergence (Brites et al., 2011; Derré et al., 2013). The different responses to coating happen due to the species, to the specific material used, to the thickness of the coating layer placed on the seeds, to the way the material is applied and to the process used for the coating.

For the length of the aerial part, no treatment differed from the uncoated seeds. This indicates that until the evaluated phase, the coating treatment did not cause any damage regarding this characteristic (Table 2). The root length was reduced by intermediate doses of fertilizer on the coating, whereas higher doses was equal to the control treatment (Table 2). Soares et al. (2014) analyzed the soybean seeds coated with phosphorus and concluded that the coating caused soybean plant nodulation and growth raise.

For the fresh mass from the aerial part (FMAP) variable, significant increase was observed when the seeds were coated with higher doses of fertilizer, although it did not reflect on the dry mass from the aerial part (DMAP) (Table 2). These results corroborate with Yagi et al. (2006) who, studying the application of zinc on sorghum seeds, concluded that it does not affect the accumulation of shoot dry mass. On the other hand, Soares et al*.* (2014) observed that soybean seeds coated with phosphorus showed an increase in plant growth. For the fresh mass from the root (FMR) variable, the higher value (2.14 g) is noticed in coated seeds without fertilizer, which is 100% higher than the observed on uncoated seeds, demonstrating positive and significant effect of coating on FMR. Although this treatment reached a great value for dry mass from the root (DMR, 0.44 g), it was not significant compared to the other treatments, probably due to the high coefficient of variation, since the DMR value of this treatment was 33% higher than on uncoated seeds (Table 2).

These results show that the application of coating on vinhatico seeds promotes higher development of this plant until that evaluated phase, although it also delays the germination and emergence. Under greenhouse conditions, the negative effect of fertilizer on the coating is significantly reduced, probably due to the dilution of the coating compounds by the irrigation during the period of this experiment and also because of the adsorption and leaching of part of the compounds. An option to minimize the problems could be the reduction of fertilizer doses as well as the amount of inert material that is used on the coating (Ludwig et al., 2014).

Conclusions

1. The coated seeds without fertilizer reached better results of the count of first and final germination. As the doses of fertilizer on coated seeds were increased, reduction of the first germination count, of the germination and of the germination speed index was observed.
2. The coating with fertilizer provided higher values of seeds that were not germinated and abnormal seedlings. The dosage of fungicide used on the coating was efficient to combat fungi.
3. The plants originated from seeds coated with fertilizer reached higher FMAP and FMR. The length of aerial part length (LAP), length of root (LR), DMAP, DMR and the emergence were not affected, only the ESI was reduced on coated seeds with fertilizer.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Coordination for the Improvement of Higher Education Personnel) for the research grant concession.

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