What is the cause of low seed germination of *Zanthoxylum rhoifolium* Lam.? [1]

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ABSTRACT

This study aimed to determine the main causes of low germination of *Zanthoxylum rhoifolium* Lam. (Rutaceae) seeds. Initially, the physical and physiological qualities were evaluated in seeds from four areas (lots) (Step I). Then, tests were performed (Step II) to determine physical dormancy (imbibition in water and methylene blue), physiological (germination test with scarification) and morphological (analysis of the embryo), in addition to histochemical analysis and (Step III) bioassay in aqueous and hydroalcoholic extracts. In Step I, a low germination was observed in all evaluated lots (d” 1.0%); however, in the tetrazolium test, a high viability was verified for the Dois Vizinhos lot (88%). The lots showed differences in their physical aspects, with the predominance of seeds with damage. In Stage II, developed embryos were verified and there has no germination without the tegument. The methylene blue and histochemical test indicated the presence of an impermeable and thick tegmen. The bioessays with hydroalcoholic extracts indicated the presence of germination inhibitor(s). The main causes associated with low germination of *Z. rhoifolium* seeds are attributed to the combined dormancy due to an impermeable tegmen and some non-specific physiological dormancy, as well as the presence of damaged seeds. The seeds also contain germination inhibitors.

Keywords: histochemical analysis; physical dormancy; physiological dormancy; forestry seeds; Rutaceae

INTRODUCTION

The tree species *Zanthoxylum rhoifolium* Lam. (Rutaceae) occurs from Mexico to various parts of South America, including Brazil, where it is present in all vegetation formations. This species inhabits rainy to semideciduous forests, up to 1300 m in altitude, more frequently in clearings or edges of disturbed forests or with secondary development (Pirani & Groppo, 2020). It has timber importance in the manufacture of tool handles for carpentry and in internal works of civil and naval construction (Carvalho, 2006), has landscape importance and may be used for projects of recovering of degraded areas (Costa *et al*., 2014); and mainly, medicinal potential, with a range of studies that demonstrate the effects of its essential oil, bark and leaves (Krause *et al.*, 2013; Bessa *et al*., 2019).

Although the potential for multiple uses of *Z. rhoifolium* is evident, there are studies on the species report the difficulty in using seeds to produce seedlings due to low germination (Silva & Paoli, 2000; Carvalho, 2006; Souza Junior & Bracalion, 2016). Due this, different causes are suggested, such as the presence of damaged, empty seeds and physical and morphological dormancy (Carvalho, 2006). Beside this, there is no methodology for seeds analysis of this species (Brasil, 2013) and no information about the presence of inhibitory substances in this seed.

The presence of damaged seeds is usually caused by insect predation, which can lead to a reduction in the physiological quality. The damage can lead to the entry of pathogens, impairing the functioning of its physiological processes during storage and germination or even

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permanently damaging the embryo (Pinã-Rodrigues *et al.*, 2015). The occurrence of empty seeds is a common characteristic that is caused by embryo abortion, leaving only the outer tissues of the seed (Baskin & Baskin, 2019). Both, empty and damaged seeds, are common physical problems that decrease quality in forest seeds (Oliveira *et al.*, 2018).

Seed dormancy can be classified into physiological, morphological, physical or combined; Morphological dormancy is defined by the undifferentiated or underdeveloped embryo, which remains in the development phase even after dispersal; Physiological dormancy occurs due to the action of chemical inhibitors that impede the germination process; Physical dormancy is caused mainly by an integumentary or pericarp obstruction that prevents the process of diffusion of water and/or gases to the embryo (Baskin & Baskin, 2014).

Combined dormancy occurs due to more than one cause of dormancy in the seed usually when there is non-deep physiological dormancy together with physical dormancy, where germination depends on overcoming both. This class of dormancy is usually observed in seeds that do not germinate even when physical dormancy is overcome (Araujo *et al*., 2018).

Although dormancy is considered as a strategy for the perpetuation of many species, preventing all the seeds from germinating at the same time, in adverse, the environmental conditions can compromise the establishment of the species in a given environment (Araujo *et al*., 2018), this mechanism can impose restrictions when the production of seedlings is desired. In this sense, studies on the dormancy in seeds of a species are essential, which allows the selection of methods to overcome them.

The definition of problems related to the germination and inhibitory characteristics of forest species provides technical and scientific knowledge that assists in the production of seedlings. In this way, this study aimed to determine the main causes of low germination of *Zanthoxylum rhoifolium* (Rutaceae) seeds through studies related to physical and physiological qualities and the cause (s) of dormancy.

MATERIAL AND METHODS

The research was carried out in two stages: Stage I - Physiological (germination and tetrazolium tests) and physical evaluations (weight of a thousand seeds, water content, presence of full, empty and damaged seeds) were conducted; and Stage II- presence of physical dormancy (water soaking curve and methylene blue), physiological (Germination test with scarification of the coat)) and morphological (immature or undifferentiated embryos), in addition to histochemical analyzes and bioassay to check water-soluble and alcohol-soluble inhibitors.

Stage I

For seed lots of *Z. rhoifolium* harvested and sold in three Brazilian states (Santa Catarina, Paraná and São Paulo) were used. The seeds used, from all lots, were dark brown color (Munsell 2.5YR ½), characteristic of ripe seeds (Carvalho, 2006).

The seeds of Dois Vizinhos, Paraná state, were collected in a fragment of Semideciduous Forest in regeneration stage, in a natural area. The average altitude of the place is 409 meters (Ipardes 2019b), and the typical climate is classified as humid subtropical 'Cfa' (Alvares *et al.*, 2017). The harvest happened in February 2018, when the fruits were semi-open, using a trimmer. After harvesting, the seeds were dried in the shade on paper towels and processed with the aid of a sieve to remove fragments and then storage for 20 days in cold chamber (10 ± 2 ºC).

The remaining lots were purchased (without information on drying, storage and processing), of the localities: Penápolis, São Paulo state - average altitude of 416 meters (Daep, 2019), and predominant climate classified as high altitude tropical "Cwa" (Alvares *et al.*, 2017); Apucarana, Paraná state - altitude of 988 meters (Ipardes, 2019a) and climate classified as humid subtropical "Cfa" (Alvares *et al.*, 2017); and Lages, Santa Catarina state - average altitude of 990 meters with humid temperate "Cfb" climate (Alvares *et al.*, 2017).

Physical analysis

The weight of a thousand seeds was carried out following the recommendations of the Rules for Seed Analysis (Brasil, 2009). The average, the variance, the standard deviation and the coefficient of variation were calculated from the values resulting from the weighings. The water content was determined using the drying oven method at 105°C ± 3°C, for 24 hours, calculated based on wet weight.

In order to verify the physical quality of the seeds, four repetitions of 25 seeds per lot were sectioned longitudinally with a scalpel, parallel to the hilum (Baskin & Baskin, 2014). The internal visualization of the seeds was performed using a Zeiss stereo microscope model Stemi 305. The open seeds were classified as full, empty and damaged; and the results were expressed as a percentage.

a) Full seeds: well-formed embryo, white/slightly yellow color and firm tissues.

b) Damage seeds: damaged embryo, flaccid texture and/or black or brown color. Seeds contaminated internally with pathogens were also classified as damaged.

c) Empty seeds: They did not have embryo.

Physiological analyses

The germination test was performed in a Biological Oxygen Demand (B.O.D) chamber, regulated at 25ºC, in the presence of constant white light with four repetitions of 25 seeds per lot. The seeds were treated in 1% sodium hypochlorite solution and put to germinate in transparent plastic boxes of the gerbox type, using as a substrate two germitest paper moistened to 2.5 times its weight in deionized water. The evaluations were carried out daily from the 4th day until the 120th day of assembly of the test and the criteria adopted was the formation of a normal seedling (Brasil, 2009).

For the tetrazolium test, four repetitions of 25 seeds per lot were soaked in water for 12 hours at 25ºC, and cut laterally, parallel to the hilum, to expose the endosperm. The seeds were submerged in a 0.05% tetrazolium solution (methodology defined by pre-tests), where they were kept at a temperature of 25ºC for 48 hours in the absence of light. Seeds with embryonic axis and more than 50% of colored cotyledons were considered viable.

The experiments were carried out in a completely randomized design and the data set was submitted to the normality and homogeneity test, as the results were subjected to analysis of variance (F test), at the level of 5% probability. Comparisons between means were obtained using the Tukey test at 5% probability, using the Sisvar 5.6 statistical program (Ferreira, 2019).

Stage II

Seeds from the four lots were used to assess the presence of morphological dormancy and only seeds from the Dois Vizinhos lot for physical and physiological dormancy tests, due to the greater viability shown in the tetrazolium test.

Morphological Dormancy

To assess the presence of morphological dormancy (immature/undifferentiated embryos), 100 mature seeds (dark brown colour) from the four lots were opened and the embryos were observed with the aid of a stereomicroscope. The embryos considered mature and well-formed were those that presented two full cotyledons, hypocotyl/root axis, white color and firm texture (Silva & Paoli, 2000).

Physical dormancy

To assess the presence of physical dormancy, the seeds were subjected to soaking in water and methylene blue. The water soaking curve was conducted with four repetitions of 25 seeds between moistened paper and placed in a gerbox, at a constant temperature of 25ºC, in B.O.D. The weighing started before the imbibition, and after every hour until the first eight hours. After that, the weighing was carried out every 24 hours until the seed mass stabilized, and then evaluated every 48 hours.

To evaluate the imbibition in methylene blue, the seeds were immersed in a 1% solution. The seeds were treated in 1% sodium hypochlorite solution Two seed preparations and a without preparation were used:

Preparation A: lateral section, parallel to the hilum, with total removal of the integuments (testa + tegmen), showing the endosperm; B) Preparation B: lateral section, parallel to the hilum, with the removal of the testa, exposing the tegmen; C) Whole seeds. After 1, 3, 5 and 24 hours immersed in the dye, the seeds were sectioned longitudinally with a scalpel, and were observed in stereomicroscopic Zeiss model Stemi 305.

Physiological dormancy

In order to verify the presence of physiological dormancy, germination test was perfomed with the scarification of the coat. With the aid of a scalpel, the seeds from the Dois Vizinhos lot were cut laterally, parallel to the hilum, evidencing the endosperm. Then four repetitions of 25 seeds were put to germinate in gerbox (B.O.D at 25º C), with two germitest paper moistened to 2.5 times its weight in deionized water. The evaluations were carried out daily from the 4th day until the 120th day of assembly of the test. The criteria adopted was the formation of a normal seedling.

Hystochemical

Anatomical sections of twenty *Z. rhoifolium* full seeds were performed (Penápolis and Dois Vizinhos lots) to analyse characteristics related to the presence of physical and physiological dormancy. In this way, the testa was removed and the seeds fixed in 70% FAA for 24 hours and washed with 70% alcohol and then subjected to the dehydration process in alcoholic series (70% to 100%). The material followed the pre-infiltration, infiltration and inclusion processes (Historesin Embedding Kit Leica ™). The samples were sectioned in a Leica microtome (SM 2010R), with a thickness between 5 to 7 µm.

The sections were stained with: Toluidine blue-basic fuchsin, ferric chloride and Toluidine blue 0,05% for the determination of the phenolic compounds, mucilage and cellulose; Sudam III for lipids, cutin and suberin. The slides were observed in a Leica 2500 photomicroscope, with the ZEISS system, coupled to a digital camera (Leica ICC50 HD model) and a microcomputer.

Stage III

Bioassay of inhibitors

To evaluate the existence of germination inhibitors, bioassays were carried out with aqueous and hydroalcoholic extracts, obtained from seeds of *Z. rhoifolium* (Silveira *et al.*, 2013). The extracts were applied in a germination test on lettuce (*Lactuca sativa*), carrot

(*Daucus carota*) and tomato (*Lycopersicon esculentum*) commercial seeds (97 – 99% germination index), which are sensitive to allelochemicals (Gui Ferrera & Aquila, 2000). To obtain the aqueous extract, 50 g of *Z. rhoifolium* whole seeds were dried in an oven at 105ºC, milled and mixed with 500mL of hot distilled water (Coelho *et al*., 2011). The concentrated macerate (100%) obtained was filtered and diluted in three concentrations (10%, 25%, 50% and control (distilled water only)), with distilled water.

The methodology for hydroalcoholic extract was carried out with the macerate of 300g of dry seeds (oven 30°C for 24 hours) of *Z. rhoifolium* diluted in 1,9L of ethanol (92.8%). The resulting material was subjected to ethanol extraction for two hours cold. The extracts were filtered and evaporated in a rotary evaporator with the addition of 1:1 Tween 80 (1 %) and diluted in distilled water at different concentrations (10%, 25% and 50% and control (distilled water only)). The germination tests with extract were conducted in Petri dishes with two leafs of germitest paper with 2.5 times its weight in deionized water. The experiment, remained in a B.O.D germination chamber under constant light and at 20ºC for seeds of lettuce and carrot, and 25ºC for tomato (Brasil, 2009).

Four repetitions of 25 seeds per species were tested in each type and concentration of extract. The evaluations were carried out daily for 14 days, with seeds showing 2 mm of root protrusion being considered germinated. The germination percentages and germination speed indexes (GSI) were calculated.

The experiment was carried out in a completely randomized design. The data were tested about its normality and homogeneity and the results were subjected to analysis of variance (F test) at 5% probability. The averages were compared using the Tukey test at 5%, using the statistical software Sisvar 5.6 (Ferreira, 2019).

RESULTS

Stage I

Physical and physiological analyses

Regarding physical quality, the lots of *Z. rhoifolium* showed similar characteristics, with most seeds with well-formed embryo and endosperm, except in the Apucarana lot, where only a grayish mass with a sandy aspect and yellow spots were observed inside the seeds (Table 1; Figure 1B).

Seeds with a higher weight than the other lots were found in the Apucarana lot, even with a higher percentage of damage (Tables 1 and 2). It should be noted that contamination by fungus (unidentified) was verified during the germination test.

Regarding the germination test, after the 120-day period, only 1% was verified for the Apucarana and Dois Vizinhos lots, while seeds from the Penápolis and Lages lots did not show germination. The germinated seed of the Apucarana lot had 2 mm of radicle, in this period. However, the attack by pathogen occurred, which prevented its subsequent development for seedling. The presence of physical and physiological dormancy may have been the cause to low germination, since viability (15 to 88%) was verified by the tetrazolium test in the Penápolis and Dois Vizinhos lots respectivily (Table 2).

Stage II

Morphological, Physical and Physiological Dormancy

The seed is bitegumented, dividing into two portions: testa, dark brown in color; and tegmen, a light brown layer (Silva & Paoli, 2000). Internally, it has an endosperm and embryo. The internal characteristics showed that 98% of the seeds of *Z. rhoifolium* had mature and well-formed embryos according to the morphological characteristics.

In relation to studies on physical dormancy, an increase in mass was observed, in the first hour, around 2% per hour until the eighth hour (Figure 2). Around the 56th hour, the increase in mass reached its peak, characterizing the imbibition process, and from then on there were variations until stabilization (56th to 941th hour) (Figure 2). From the 941st hour, there was a decrease in the mass of the seeds, possibly due to the scaling of the tegument (Figure 2), however, weighing was completed and germination was not verified.

Although there was an increase in the mass of the seeds, probably due to soaking in water, the seeds immersed in blue methylene showed different characteristics according to the preparation used. The seeds of Preparation 1 showed imbibition in the first hour, endosperm and the embryo were already completely pigmented with blue (Figure 3).

The seeds submitted to Preparation 2 did not show imbibition, the dye pigmented the exposed surface of the testa; however, without being able to penetrate through the tegmen layer, a similar result occurred with the whole seeds, which did not show imbibition (Figure 3).

The germination test with the removal of the coat did not show germination (0%) during the evaluation period. The seeds deteriorated at the end of the 120th day test.

Histochemical analyzes showed the anatomical constitution of *Z. rhoifolium*, formed by teguments, endosperm and embryo (embryo-axis and cotyledons) (Figure 4A). The tests with Toluidine blue-basic fuchsin and Ferric chloride did not indicate the presence of phenolic compounds in the seed coat (testa and tegmen). However, the presence of cellulose and suberin in the cell wall of the tegmen was observed (Figure 4B, 4C, 4D) by the reaction of this compound with Toluidine blue 0,05% and Sudan III.

Stage III

Bioassay of inhibitors

In bioassays, the addition of aqueous extract did not inhibit the germination of lettuce, tomato and carrot seeds in any concentration. When compared to the control, there were variations only in the germination speed of tomato seeds (Table 3).

In the bioassays with hydroalcoholic extract of *Z. rhoifolium*, there was a significant influence on the germination percentage and germination speed index in lettuce, tomato and carrot seeds (Table 4). It was showed as the doses of the extract increased, germination and the germination speed index decreased in all seed species.

DISCUSSION

Physical and physiological analyses

Forest seeds can present problems related to physical quality, such as damage that can affect the health quality of the seed, serving as an entrance to the attack of fungi (Baskin & Baskin, 2014). In general, pathogens associated with seeds can increase the respiration rate of seeds and in storage, lead to rapid deterioration of seeds, and consume all seed reserves (Oliveira *et al*., 2018). A problem also reported for the genus *Zanthoxylum* is the presence of damaged seeds in *Z. giletti* (Okeyo *et al.*, 2011).

Regarding the mass of seeds, although the larger size or density is related to the higher physiological quality, the seeds from the Apucarana lot don't have embryo and showed higher mass (Table 2). This may be related to the environmental variables to which the seeds were exposed, like predation, fungus or embryo abortion (Oliveira *et al*. 2018).

The water content provides parameters related to the physiological quality of the seeds (Oliveira *et al*., 2018). The Penápolis, Lages and Apucarana lots may have shown low viability due to the high water content (19.6%, 15.8 and 16.1%, respectively), which allows, among others, the action of pathogens and physiological disorders that affect seed tissues. The reduction in the water content for the Dois Vizinhos lot (10.8%) corroborates the statement by Baskin & Baskin (2014), where the drastic reduction of water in seeds that reach physiological maturity causes a decrease in metabolism and allows the preservation of viability for orthodox seeds, such as *Z. rhoifolium* (Corrêa *et al.*, 2021).

The variation between seeds from different places has been reported as an adaptation of the species for survival, and it can also be used to provide a theoretical basis for selection and conservation of high quality germplasm resources, especially for *Z. rhoifolium* (Corrêa *et al.*, 2021).

Morphological, Physical and Physiological dormancy

The results of the present study differ from the statement by Carvalho (2006), who reported a high number of malformed embryos for this species. Thus, there was no morphological dormancy in *Z. rhoifolium*.

Z. rhoifolium cells on the testa has a thick cuticle combined with cells filled with lipid content and phenols, which delays or prevents the passage of water to the

Table 1: Physical analysis of seeds of four lots of *Zanthoxylum rhoifolium*

Lot	Full seeds (%)	Damaged seeds (%)	Empty seeds (%)
Penápolis	95	3	1
Dois Vizinhos	94	3	3
Apucarana	0	100	0
Lages	94	4	2

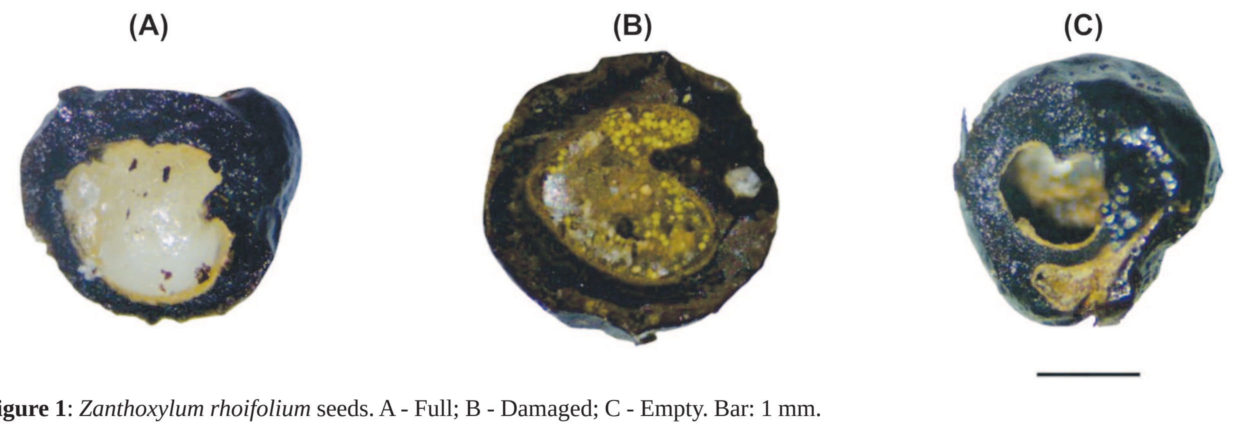

Figure 1: *Zanthoxylum rhoifolium* seeds. A - Full; B - Damaged; C - Empty. Bar: 1 mm.

internal tissues due to its hydrophobic character (Silva & Paoli, 2000). However, the results observed with methylene blue and demonstrate the imbibition in this structure occurred in seeds of *Z. rhoifolium*.

Likewise, the inner part of the testa is impregnated with phenolic compounds that prevent the absorption of water by the seed (Silva & Paoli, 2000). However, in the present work, the tests with toluidine blue and ferric chloride did not indicate the presence of phenolic compounds in the seed tissues. About this, a specific analysis of the testa is recommended with a greater variety of dyes that demonstrate the presence of structural and non-structural phenolic compounds.

The methylene blue test indicates that the tissue that block imbibition was the tegmen (internal tegument), this was verified with the histochemical analysis that indicates cellulose and suberin in the composition of the tegmen. Cellulose acts as a structural element in plant tissues that guarantees consistency to the cell wall. This compound does not act as a waterproofing agent for the tegument; however, it gives rigidity to the thick tegmen cell wall (Appezzato-da-Gloria & Carmello-Guerreiro, 2012; Bewley *et al.*, 2013). The suberin is characterized as a lipid substance, present in plant tissues that give impermeable character to water and gases (Appezzato-da-Gloria & Carmello-Guerreiro, 2012) and the presence of this substances in seed teguments is indicative of physical dormancy, due to the hydrophobic character that gives this coating (Bewley *et al.*, 2013). These results corroborate with the statement by Silva & Paoli (2000) for *Z. rhoifolium*, who indicated that the tegmen consists of a tissue with thick palisade cells of the tracheoidal type with the presence of oils that prevent the passage of water.

The embebition curve test demonstrates that the phase I of germination occurs (rapid water uptake), when the first signs of metabolism reactivation and increase in respiratory activity are noted (Figure 2). The phase II occurs whith the stabilization of embebition, probably because of the interval for the mobilization of substances to the embryo. However, the drop in imbibition and the absence of phase 3 of germination suggest the occurrence of physical+physiological dormancy, as it was observed that the embryo was completely formed and absorbed water when the coat/tegument (physical barrier (tegmen+testa)) was removed, but did not germinate (Baskin & Baskin, 2014; Araujo *et al.*, 2018).

Some species have chemo-anatomical characteristics like those observed in *Z. rhoifolium*, such as *Bixa Orellana* L. (Picolotto *et al.*, 2013), *Chaetostoma armatum* (Spreng.) Cogn. (Ribeiro *et al.*, 2015) and *Sapium sebiferum* (L.) Roxb. (Shah *et al.*, 2018). In fact, some cell layers with thickened walls and formed by impermeable compounds

Table 2: Weight of one thousand seeds, water content, viability (tetrazolium) and germination for the different lots of *Zanthoxylum rhoifolium*

Lot	Weight of one thousand seeds (g)	Water content (%)	Viability (TZ) (%)	Germination (%)
Penápolis	1.03 d	19.6 a	15 b	1 a
Dois Vizinhos	1.51 b	10.8 a	88 a	1 a
Apucarana	2.21 a	16.1 a	0 c	0 a
Lages	1.3 c	15.8 a	0 c	0 a
C.V	4.01	35.48	17.35	28.08

Means followed by the same letter in each column are not significantly different at Tukey test ($p < 0.05$).
C.V = Coefficient of variation

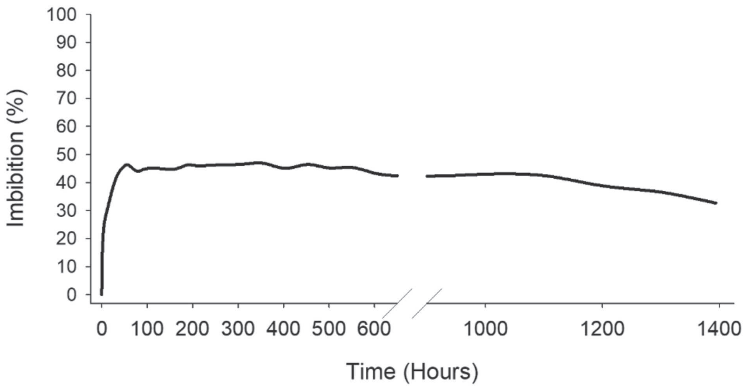

Figure 2: *Zanthoxylum rhoifolium* seed imbibition curve (%) over 1349 hours.

are not restricted to the mechanical protection function, but may also have a waterproofing function to water and oxygen, protection against the entry of microorganisms or water storage (Appezzato-da-Gloria & Carmello-Guerreiro, 2012).

The ecological aspect of the seeds of *Z. rhoifolium* seems to be related to the causes of their dormancy. The tegument composed of inhibitory substances has the presence of oil (Silva & Paoli, 2000), thick tegmen and is filled with cellulose and suberin. It can be deduced that this is a dispersion strategy, since the seed is consumed by birds (Carvalho, 2006). The rupture of the tegument must be conditioned to the exposure to acids present in the digestive tract of these animals, allowing the propagules to be non-dormant in contact with the soil after dispersion (Baskin & Baskin, 2014). The use of acids for this species has already been suggested (Souza Junior & Brancalion, 2016), which manifests some interaction of this substance to overcome dormancy.

The peeling of the tegument demonstrates that abiotic environmental factors, such as rain, light and temperature, may be responsible for breaking its physical barrier, providing water entry and subsequent germination. Besides that, some species with physiological dormancy adopt this same strategy to guarantee the survival of the seed over time, with rain as its main leaching agent of the inhibitory substances (Bewley *et al.*, 2013).

The results about dormancy presented in this research, can be compared with the other works with the genus *Zanthoxylum*, as in seeds of *Z. gilleti* (Okeyo *et al.*, 2011) and *Z. zanthoxyloides* (Neya *et al.*, 2017) that have physical dormancy due to the tegument. In fact, the presence of physical dormancy is a constant in several species of the genus *Zanthoxylum*, as reported by Baskin & Baskin (2014).

Zanthoxylum dissitum Hemsl. has seeds with a very low germination rate. Previous investigations suggested some causes for inhibiting germination, like seed wax–rich and composed of a hard, thick horny outer–layer, which reduces the permeability of water and air through the seed coat, besides some germination inhibitors (Sun *et al*., 2019). In a similar way, *Z. armatum* presents low germination due to hard seed coat, improper seed setting, seed predation by insects, and presence of oily seed coat, requiring pre sowing treatment and stratification (Purohit *et al*., 2015; Patade *et al*., 2019). In natural landscapes, those characteristics are interesting for plant adaptation, however, for seedlings production and restoration programs, germination needs to be fast and uniform. Thus, the understanding of low seed germination causes improves seed management and seed use efficiency (Kildisheva *et al.*, 2020).

From the results obtained, it can be seen that the low germination of *Z. rhoifolium* seed lots produced/commercialized may be due to the presence of damaged seeds, with high water content and combined dormancy. Some recent research suggests the existence of combined dormancy for the genus *Zanthoxylum*. Bodede *et al.*

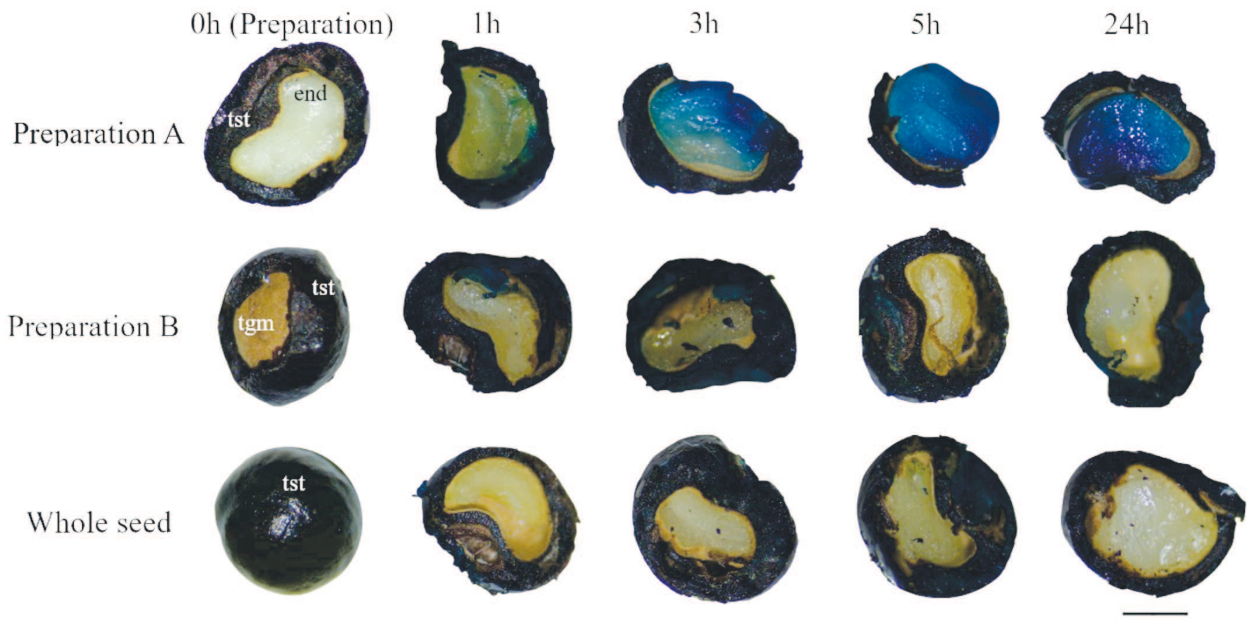

Figure 3: *Zanthoxylum rhoifolium* seeds, before and after exposure to 1% methylene blue. Seeds from times 1 h, 3 h, 5 h and 24 h were sectioned for visualization of color absorption. Preparation A - lateral section, parallel to the hilum, with total removal of the teguments (testa + tegmen), showing the endosperm. Preparation B - lateral section, parallel to the hilum, with the removal of the testa, exposing the tegmen. Bar: 1 mm. Where: tgm= tegmen; tst= testa; end = endosperm.

(2015), with research in *Z. capense* and Datt *et al.* (2017), with *Z. armatum*, reported that the seeds of these species have a combined physical-physiological dormancy. These African species apparently share the same germinative impediments as *Z. rhoifolium*, including the presence of damaged seeds.

A variety of tropical species has combined dormancy as a way of maintaining viability (Baskin & Baskin, 2014). The inclusion of *Z. rhoifolium* in this group of species with combined dormancy, together with the presence of damaged seeds, allows dormancy overcoming methods to be developed, beneficiation and sanity to promote the germination of its seeds.

Bioassay of inhibitors

Some seeds may not have water-soluble germination inhibitors (Souza Filho *et al.*, 2011), but with hydro-alcoholic solutions. Thus, it can be deduced that the behaviour of the seeds of *Z. rhoifolium* is similar of the species *Canavalia ensiformis* DC. (Fabaceae) seeds, which hydroalcoholic extracts showed inhibitory effects in *Ipomoea grandifolia* (Dammer) O'Donell and *Commelina benghalensis* L. (Mendes & Rezende, 2014). The plant species of medicinal importance generally have inhibitory effects on the germination of other species (Dorneles *et al.*, 2015). Some studies, engage extracts from other organs of *Z. rhoifolium*, prove that the species has intense allelopathic activity, such as a variety of extracts obtained from the leaf, fruit (Krause *et al.*, 2013) and the hydroalcoholic extract of the shell (Turnes *et al.*, 2014). This characteristic may possibly be present in the seeds, suggesting that this component affecting the germination of the other seeds in the vegetal community. However, it is difficult to specify which specific compound is responsible for the inhibitory activity (Souza Filho *et al.*, 2011).

It is recommended that future studies with *Z. rhoifolium* seeds describe the substances present in their tissues and whether there is any relationship with the low germination of the seed itself.

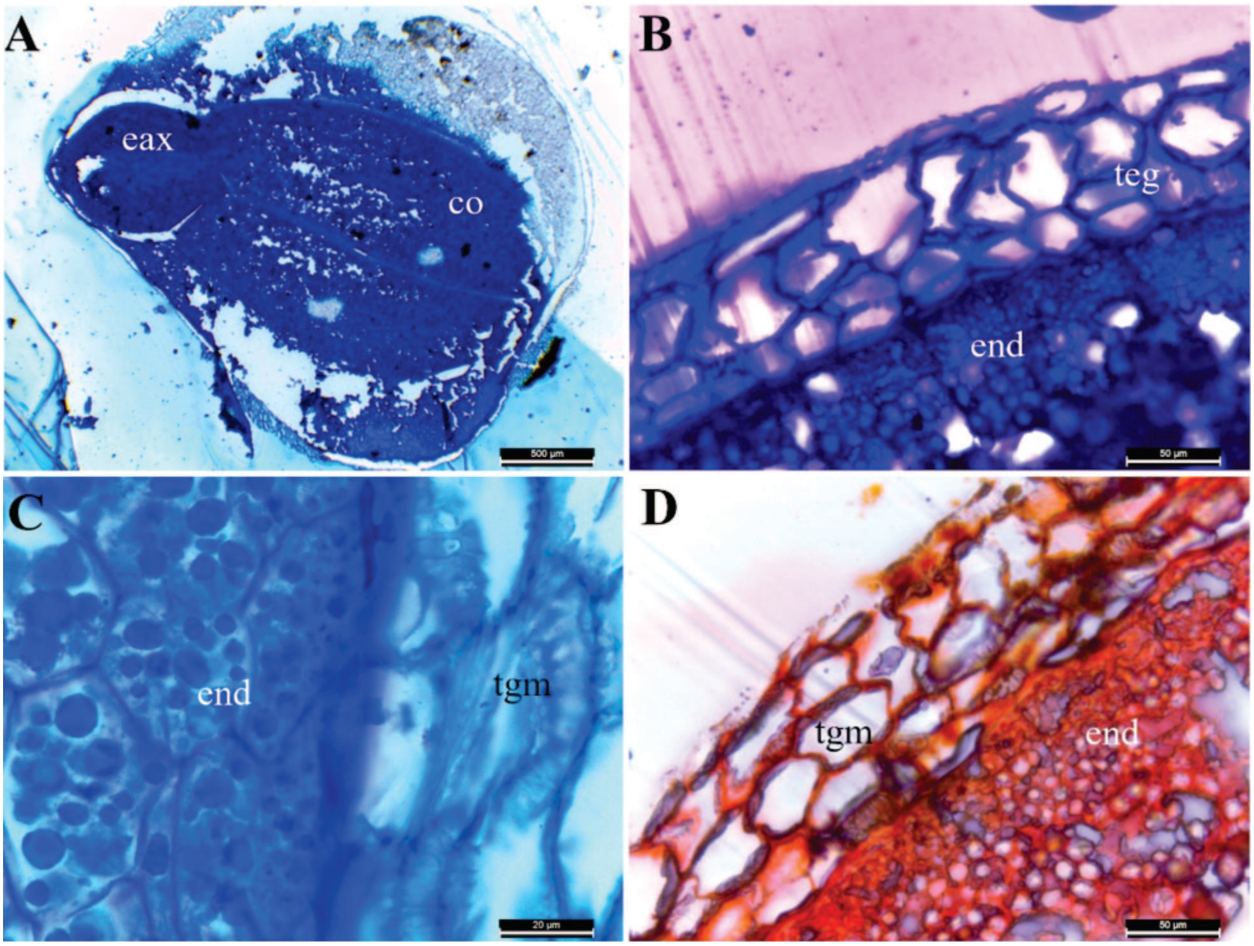

Figure 4: Anatomical sections of seeds of *Zanthoxylum rhoifolium.* A. B. - seeds (10x 40x and 100x) coloring with Toluidine blue/basic fuchsin; C - Toluidine Blue 0,05% (100x); D - (40x) Coloring with Sudam III. Where: eax = embryo axis, co = cotyledons, tgm = tegmen, end=endosperm.

Table 3: Germination percentage (G%) and Germination Speed Index (GSI) in bioassay with aqueous extracts of *Zanthoxylum rhoifolium* in lettuce, tomato and carrot seeds

Treatments	Lettuce		Tomato		Carrot	
	G%	GSI	G%	GSI	G%	GSI
Control	99 a	21.3 a	97 a	5.95 a	81 a	3.43 a
10%	99 a	22.4 a	89 a	3.69 b	94 a	4.03 a
25%	96 a	17.9 a	94 a	4.29 b	86 a	4.01 a
50%	94 a	20.6 a	93 a	4.41 b	86 a	4.05 a
C.V. %	1.79	13.81	5.66	12.38	14.69	16.04

Means followed by the same letter in each column are not significantly different at Tukey test ($p < 0.05$).
C.V = Coefficient of variation

Table 4: Germination percentage (G%) and Germination Speed Index (GSI) in bioassay with hydroalcoholic extract of *Zanthoxylum rhoifolium* in lettuce, tomato and carrot seeds

Treatments	Lettuce		Tomato		Carrot	
	G%	GSI	G%	GSI	G%	GSI
Control	100 a	12.5 a	97 a	5.95 a	72 a	3.11 a
10%	16 b	0.55 b	45 b	1.37 b	39 b	1.08 b
25%	8 c	0.21 c	14 c	0.40 c	16 c	0.40 bc
50%	0 d	0 c	0 d	0 c	0 c	0 c
C.V. %	11.78	3.02	18.84	17.77	34.26	39.86

Means followed by the same letter in each column are not significantly different at Tukey test ($p < 0.05$).
C.V = Coefficient of variation

CONCLUSION

The main causes associated with low germination of *Z. rhoifo*lium seeds are attributed to damaged seeds and combined dormancy; physical dormancy by imbibition block caused mainly by the suberin and cellulose present in the tegmen and some non-specific physiological dormancy. The seeds also contain inhibitory substances insoluble in water.

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