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Article

Effect of Treatment with Grounded Bay Leaf on the Physiological Quality of Bean Seeds Stored at Room Temperature

Ana Cristina Siewert Garofolo [1], **Tatiana Santos Vidal** [2], **Marcelo Antoniol Fontes** [3], **Luiz Fernando de Sousa Antunes** [4]

[1] Doutorado em Engenharia Agrícola Embrapa Agrobiologia Seropédica, Rio de Janeiro . ORCID: 0000-0002-4207-8178. E-mail: ana.garofolo@embrapa.br

[2] Bióloga Embrapa AgrobiologiaSeropédica, Rio de Janeiro . ORCID: 00009-0005-5285-7959. E-mail: tatiana.guimaraes@embrapa.br

[3] Analista da Embrapa Embrapa Agrobiologia. ORCID: 0000-0002-9651-8802. E-mail: marcelo.fontes@embrapa.br

[4] Doutor em Fitotecnia. Universidade Federal Rural do Rio de Janeiro. ORCID: 0000-0001-8315-4213. E-mail: fernando.ufrrj.agro@gmail.com

ABSTRACT

Quality seeds are important inputs for agriculture and form the basis of a healthy and productive crop. The practice of seed storage by family farming often takes place in environmental conditions of uncontrolled temperature and humidity and under potential risk of attack by insects and pathogens. In this scenario, the use of plants with insecticidal and fungicidal active principles has acquired great importance in order to maintain sanitary and physiological quality. The objective of this work was to evaluate the effect of bean seed treatment with bay leaf on the physiological and sanitary quality of seeds stored at room temperature. The seeds were treated with concentrations of 1%, 2.5% and 5% w/w (measured in g/kg) and stored at room temperature in multiwall paper bags for a period of 24 months. The results indicate a decrease in germination after 24 months of storage for all treatments studied. The treatment of Manteigão bean at 1% powdered laurel showed better germination maintenance for 24 months and less leaching of potassium, calcium and magnesium ions, showing that this variety is more resistant than Amarelinho bean to storage at room temperature under the conditions of the study.
Keywords: germination; released ions; lipid peroxidation.

RESUMO

Sementes de qualidade são insumos importantes para a agricultura e formam a base de uma cultura saudável e produtiva. A prática do armazenamento de sementes pela agricultura familiar muitas vezes ocorre em condições ambientais de temperatura e umidade descontroladas e sob risco potencial de ataque de insetos e patógenos. Nesse cenário, o uso de plantas com princípios ativos inseticidas e fungicidas tem adquirido grande importância para manter a qualidade sanitária e fisiológica. O objetivo deste trabalho foi avaliar o efeito do tratamento de sementes de feijão com louro na qualidade fisiológica e sanitária de sementes armazenadas em temperatura ambiente. As sementes foram tratadas nas concentrações de 1%, 2,5% e 5% p/p (medidas em g/kg) e armazenadas à temperatura ambiente em sacos de papel multifoliado por um período de 24 meses. Os resultados indicam queda na germinação após 24 meses de armazenamento para todos os tratamentos estudados. O tratamento do feijão Manteigão a 1% de louro em pó apresentou melhor manutenção da germinação por 24 meses e menor lixiviação dos íons potássio, cálcio e magnésio, mostrando que essa variedade é mais resistente que o feijão Amarelinho ao armazenamento em temperatura ambiente nas condições do estudo.
Palavras-chave: germinação; íons liberados; peroxidação lipídica.

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Introduction

Brazil is a large producer of kidney beans, and this crop is the basis of Brazilian people's diet. In this scenario, family farming presents a great diversity of bean types as a result of the activities developed by farmers throughout history (BEVILAQUA *et al*., 2013).

Bean seeds can be subject to attack by numerous pests from emergence in the field to storage. Several pests such as *Zabrotes subfasciatus* (Coleoptera, Bruchidae) (BOIÇA JÚNIOR *et al*., 2002) and *Acanthocelides obtectus* (GALLO *et al*., 2002) can cause reduction of weight, germination power, seed quality, and may cause complete destruction of stored beans (GALLO *et al.,* 2002).

The storage of beans in family farms is usually done in uncontrolled environmental conditions, and the practice of farmers to saving seeds for future crops (BURG *et al.,* 2015). Generally, the seeds are stored in various containers such as plastic containers, glass, paper bags, jute bags, often without proper temperature and humidity control. These storage conditions can cause deterioration of the stored seeds, since these are determining factors for their longevity (SANTOS *et al.,* 2005).

A good storage is one that preserves the initial qualities of the product, avoiding its deterioration, and reducing as much as possible the biochemical reactions that cause the loss of the physiological quality of the seeds. However, when done incorrectly, it can cause loss of germination and the development of fungi (BLACK; BEWLEY; HALMER, 2006).Environments with uncontrolled humidity and temperatures promote damage in cell metabolism such as increased respiratory and enzymatic activity, which will favor the appearance of fungi (Vieira and Yokoyama 2000). Water contents below 13% are adequate for storage and guarantee the maintenance of seed quality for up to 8 months (BEVILAQUA *et al.* 2013), under room temperature conditions.

According to Delouche and Baskin (1973), the first signs of seed deterioration are related to the alteration or loss of integrity of the cell membranes. This disorganization of the membranes results in an increase in the amount of leachates, especially potassium (CUSTÓDIO 2005), during the process of seed soaking and consequent reduction in vigor (MARCOS FILHO *et al.,* 1990). Other changes observed are lipid peroxidation and modifications in enzyme activity, protein synthesis and inability to maintain the electrochemical gradient. These alterations result in a delay or loss of germination capacity, reduced growth and vigor of seedlings, greater susceptibility to pathogenic microorganisms and seed death.

The use of plants with active principles with insecticidal activity to control pests in storage is a practice used by family farming, both for grains and seeds. Much of this practice is associated with the farmer's habit regarding the fact that the use of chemical products to control pests during storage can cause problems for the farmer's health.

The action of active ingredients on plants presents positive and negative effects such as maintaining their health, altering their growth and development, altering their reproduction and may cause mortality (ALMEIDA *et al*., 2005). They can be used in the form of oils, extracts and dry powders and are easy to be manipulated by the farmer (MAZZONETTO; VENDRAMIM 2003). On the other hand, plants that present active principles with insecticidal character present low cost and are easy to prepare and apply. However, regardless of the active principle, they can only be used if they do not affect the physiological quality of the seed.

Bean seeds can be stored with garlic cloves, bay leaves, dried pepper or rock powder (SEEDS OF AGROECOLOGY NETWORK, 2021). Morais (2011) observed that in agro-ecological based settlements the storage of bean seeds was done in plastic containers (PET) using eucalyptus leaves, roll-your-own tobacco, bay leaves and/or ashes to control pests and storage fungi.

The laurel (*Laurus nobilis*) is a species originating from Asia Minor, being distributed in the European Mediterranean. Its leaves can be used both as a condiment, medicines in traditional medicine (Lorenzi and

Matos 2008) due to its stimulant properties, narcotic, fungicidal and bactericidal (OLIVEIRA; VENDRAMIM 1999). It can also be used in the manufacture of cosmetics, soaps and detergents (ALONSO, 2004). Leaves present 1 to 3% of essential oil with the terpenoids cineol (40-44%), eugenol and methyleugenol (17%), sabinene (6.2%), terpineol (5.7%), and pinene (4.7%) as the major compounds, besides other compounds such as flavonoids, tannins, and alkaloids (ALONSO 2004).

Studies have shown that bean seeds stored with bay leaf essential oil at a concentration of 2.5ml/kg showed repellency of 74.6% to *Zabrotes subfaciatus* (OLIVEIRA; VENDRAMIM, 1999). When using bay leaf powder, the authors found a repellency efficiency of 93.7 and 92.9% for concentrations of 2.5 and 5%, respectively. Thanks to its repellent capacity, the bay leaf may become a cheap and environmentally suitable alternative for the management of *Z. subfaciatus* in stored beans, especially in family farming. However, it is necessary to investigate the effect it has on the physiological conditions of seeds stored at room temperature over a period of time.

In view of the above, the present work aimed to evaluate the effect of the treatment of bean seeds with bay leaf powder on the physiological and sanitary quality of seeds stored at room temperature.

Materials and Methods

The present work was developed at the Laboratory of Seeds and Natural Products (LSPN) of Embrapa Agrobiologia located in Seropédica, RJ, during May 2019 and April 2021. The seeds used were beans of the Manteigão and Amarelinho varieties. The Amarelinho beans have a yellow-green color, *round-shaped* grains and a slightly prominent halo, being susceptible to insect-pest attacks. The Manteigão bean stands out for its large grains, with uniformity of size and light brown color. The seeds used in this study were multiplied in the experimental fields of Embrapa. The laurel powder was purchased at a local market.

To evaluate the physiological quality, the seeds under study were submitted to germination tests, radicle length, electrical conductivity, potassium, calcium and magnesium ions and pH released in the soaking water of the conductivity test and lipid peroxidation.

The seeds were treated with bay leaf powder and stored at room temperature in multiwall paper bags for 12 and 24 months. During the trial period, ambient temperature and relative humidity were monitored. Concentrations of 1%, 2.5% and 5% w/w (measured in g/kg) of bay leaf powder were used. These concentrations were experimentally defined in *Zabrotes subfasciatus* repellency studies by Oliveira and Vendramim (1999) and Pacheco *et al.* (2016).

The germinability and root length were measured at the zero time of storage, at 12 months and 24 months when the presence of insects in the stored material was also monitored. The germination test was conducted with three samples with 20 seeds per repetition and readings on the 7th day. The determination of radicle length was conducted together with the germination test, where at the end of the seven days of germination, the radicle length of each seedling was measured and the average results expressed in centimeters (MARCOS FILHO *et al.,* 1990).

The electrical conductivity test was performed on seeds after 24 months of storage according to Vieira and Krzyzanowski (1999) with modifications. 25 seeds were immersed in 75 mL of distilled water for 24h. After this period the conductivity of the leachate was read using a Digimed conductivity meter, model DM - 31 and the results expressed in μ S cm^{-1} g^{-1}. The liquid in which the seeds were immersed was analyzed for potassium (K), calcium (Ca), magnesium (Mg) and pH according to Fessel *et al.* (2010).

Lipid peroxidation was evaluated in seeds after 24 months of storage by determining the thiobarbituric acid index (Araujo 2015). 200mg of dried seeds were ground using liquid nitrogen and homogenized with 2 mL of 0.1% (w/v) TCA. The mixture was centrifuged for 15 minutes at 15000G. After removing the supernatant, 1.5 mL of TBA 0.5% (w/v) + TCA 20% (w/v) were added to 0.5 mL of extract, and incubated at 100°C for 30 minutes, and then placed in an ice bath for 1 minute. After a new centrifugation at 3000G for 4 minutes, it was read in a spectrophotometer at 532 and 600nm, thus obtaining the net absorbance values. The results were expressed in mmols of malonaldehyde (MDA), after absorbance conversion (LEHNER *et al.*, 2008) using as molar extinction coefficient of 155mM/cm to quantify the content.

An entirely randomized design was used with two cultivars and three storage periods and three repetitions. All chemical analyses were done in duplicates. Analysis of variance and Tukey's test for comparison of means were performed using Sisvar (Ferreira 2014) with a significance level of 5%.

Results and Discussion

The maximum and minimum temperatures during the storage period were 34 °C and 16 °C performed between May 2019 and April 2021. The maximum and minimum relative humidity was 100% and 44%. The seeds had an initial water content of 11.4% (Manteigão bean) and 9.92% (Amarelinho bean) and after 24 months of storage at room temperature it was found 9.66% and 9.45% of moisture for Manteigão and Amarelinho respectively. Pacheco *et al.* (2016) observed that bean seeds treated with bay leaf at a concentration of 5 g kg^{-1} did not have their water content, germination and mass altered after 180 days of storage.

The percentages of initial germination were 98% and 100% with initial conductivity of 58.3 and 57.3 mS cm $g^{-1\ -1}$ and 70 and 102µg g^{-1} of released potassium for the varieties Amarelinho and Manteigão respectively. A negative effect of laurel powder on germination was observed at the first contact (time zero), evidencing a concentration x germination relationship of the seeds, however without statistical difference for most treatments and varieties. It was possible to verify changes in germination related to the interaction of laurel powder in different concentrations used according to the storage time and varieties used (Table 1).

Table 1. Effect of different dosages of bay leaf powder on germination and root length of Amarelinho bean and Manteigão bean varieties at 0, 12 and 24 months of storage at room temperature

Bean Variety	Laurel powder (%)	Germination (%)			Root length (cm)		
		0	12	24	0	12	24
Amarelinho	0	98 (ab)	60 (a)	49 (cd)	10.5 (ab)	10,3 (b)	7.9 (ab)
	1	93 (ab)	76 (a)	35 (d)	9.0 (cd)	11.7 (ab)	6.0 (ab)
	2,5	93 (ab)	73 (a)	44 (cd)	8.6 (cd)	11.2 (ab)	4.7 (bc)
	5,0	90 (b)	62 (a)	25 (d)	11,3 (a)	9,3 (b)	2,7 (c)
Manteigão	0	100 (a)	60 (a)	65(abc)	11.1 (ab)	8,4 (b)	8,2 (a)
	1	97 (ab)	65 (a)	75 (a)	9.7 (bcd)	11.62(ab)	8,9 (a)
	2,5	98 (ab)	78 (a)	71 (ab)	8,2 (d)	11.5 (ab)	8,1 (a)
	5,0	95 (ab)	64 (a)	70 (ab)	9.8 (abc)	13,9 (a)	8,6 (a)

Values accompanied by the same letter in the same column show no statistical variation between them by Tukey's test ($p \leq 0.05$).

A good conservation of seeds during storage is directly associated with the maintenance of their physiological characteristics, that is, maintenance of their germinability and vigor. Thus, the biggest challenge to maintain the quality of seed stored at room temperature is to seek to identify physiological parameters that precede the loss of germination capacity (SANTOS *et al.,* 2004). Additionally, it is necessary to follow these parameters when inserting a chemical insecticide active ingredient (natural or synthetic) used for its conservation. It was possible to notice significant differences between the beans Manteigão and Amarelinho for germination at 24 months of storage when all the treatments were compared to each other. This difference between varieties regarding the duration of deterioration time is determined by genetic interaction, degree of humidity and temperature to which the stored seeds are subjected (DELOUCHE, 2002). In this work, the Amarelinho variety of beans was the most susceptible to the deterioration imposed by storage, confirming what was pointed out by Santos *et al.* (2005). In their work, the authors found that different cultivars of beans showed different results regarding the maintenance of physiological quality during storage due to their genetic characteristics.

In the study it was possible to verify a decrease in the length of the seedling roots with the increase in the concentration of bay leaf powder at 24 months of storage for the Amarelinho variety. This fact can be explained by the lower vigor of this variety and an effect of the bay leaf as the concentration used increased. This same deleterious effect of bay leaves for this variety is evidenced when the parameter analyzed is germination at 24 months. For the variety Manteigão it is possible to observe greater uniformity in root length at 24 months, which may be associated with better mobilization of reserves for the development of embryonic tissue.

The determination of vigor obtained by the electrical conductivity test (Table 2) showed few differences at 24 months of storage between the bean varieties studied. Only the control treatment and the treatment with 5% powdered bay leaf for the Amarelinho variety showed significant differences.

Table 2. Effect of different dosages of powdered bay leaf on the electrical conductivity, pH and amount of potassium in the immersion solution of Amarelinho and Manteigão bean varieties after 24 months of storage at room temperature.

Bean Variety	Conductivity ($mS\ cm^{-1}\ g^{-1}$)	pH	Potassium ($\mu g\ g^{-1}$)	Calcium ($\mu g\ g^{-1}$)	Magnesium ($\mu g\ g^{-1}$)
Amarelinho 0%	118,8 (a)	5,83 (a)	300 (bc)	5.4 (cd)	12 (bc)
Amarelinho 1%	118.4 (ab)	5,72 (b)	241 (c)	5,8 (c)	21.4 (ab)
Amarelinho 2.5%	101.8 (ab)	5.75 (ab)	335 (ab)	7.7 (ab)	20,1 (b)
Amarelinho 5%	91,0 (b)	5,73 (b)	402 (a)	8,3 (a)	31,5 (a)
Manteigão 0%	107.3 (ab)	5,66 (b)	252 (c)	5 (cd)	14.4 (bc)
Manteigão 1%	93.3 (ab)	5,68 (b)	227 (c)	3,5 (d)	8,3 (c)
Manteigão 2.5%	103.3 (ab)	5,76 (ab)	235 (c)	6.2 (bc)	12.9 (bc)
Manteigão 5%	95.1 (ab)	5,89 (a)	255 (bc)	5.2 (cd)	12.2 (bc)

Values accompanied by the same letter in the same column show no statistical variation between them by Tukey's test ($p \leq 0.05$).

During the process of seed deterioration, the destructuring of the cell membrane system results in a decrease in the capacity to retain solutes inside the cell. This allows the leaching of cellular components when bean seeds are immersed in water, such as sugars, salts, proteins and amino acids, altering the electrical conductivity of the medium (Santos *et al.*, 2004). The electrical conductivity at 24 months showed no significant difference between treatments for both varieties indicating a deterioration process. There was only a statistical difference between the treatments amarelinho, control and treated with 5% bay leaf.

In the research it was possible to verify an increase in the leaching of potassium ions for the treatments with bay leaf in the Amarelinho variety. In the case of the treatment with 5% bay leaf powder, the increase was significant for all the minerals analyzed. This result, despite not correlating with that observed in the electrical conductivity, ratifies the point made in the literature that the release of ions, especially potassium, is the best indicator of seed deterioration.

When the electrical conductivity is analyzed in this 5% concentration of bay leaf powder, its value is significantly lower than the control. This fact may be correlated to two biochemical processes: aging and consequent disorganization of the seed's membrane system and, secondly, a potential deleterious effect of the bay leaf. For Lin (1990) the decrease in vigor and germination is directly associated with increased electrolytic leaching of cellular solutes from the seeds. Consequently, the lower leaching of ions, especially potassium, in the Manteigão variety evidenced a greater integrity of its membrane system after the 24-month storage period.

Considering the total storage period, it was observed that laurel at a concentration of 5% negatively influenced germination and root length in the Amarelinho variety, indicating a possible allelochemical susceptibility to the active ingredients present. The exudation of cellular constituents was inversely associated with vigor. This decrease in vigor may be associated with loss of membrane integrity and consequent loss of compartmentalization of cellular constituents thus constituting an excellent substrate for the development of microorganisms (PÁDUA; VIEIRA, 2001). In the present study, leaching of minerals, such as potassium and calcium, were the best indicators of seed quality when compared to general indices such as conductivity (CUSTODIO, 2005). Therefore, the observed decline in germination at different concentrations of the active ingredient used may be associated with the disintegration of the membrane system and the loss of cellular compartmentalization. Seeds of Amarelinho beans were more susceptible to lipid peroxidation for all concentrations of laurel powder, differing statistically from Manteigão beans at 24 months of storage (Figure 1).

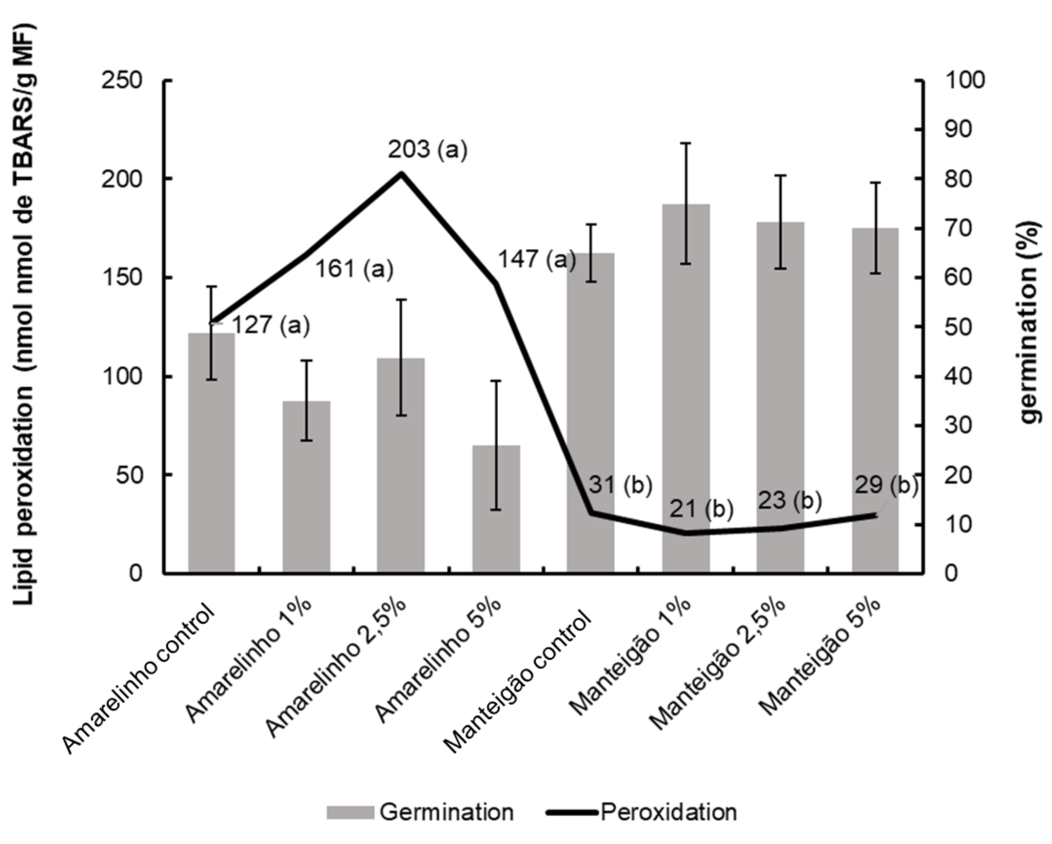

Figure 1. Effect of different dosages of bay leaf powder on lipid peroxidation against the germination rate of seeds of Amarelinho beans and Manteigão beans at 24 months of storage at room temperature. Fonte: elaborado pelos autores

Analyses performed after 24 months of storage at room temperature showed different malonaldehyde contents, especially in the Amarelinho variety. It was possible to observe that the loss of viability observed for the Amarelinho variety correlated inversely with the accumulation of malonaldehyde. On the other hand, higher germination percentages correlated to lower malonaldehyde concentrations in the Manteigão variety.

The loss of seed viability correlates directly with the accumulation of malonaldehyde, a result of this peroxidation. It can be inferred that there was a loss of integrity of the membrane system, reflected in the decrease of germination and vigor of seeds stored at 24 months.

The lower accumulation of malonaldehyde after 24 months shows that the variety Manteigão is more resistant to storage at the concentrations of the active principle used, maintaining greater viability and germinability. These results observed for the varieties under study ratify what was observed by Santos *et al.* (2005), with these characteristics possibly associated with different genetic characteristics.

For the variety Amarelinho with 5% treatment the decrease in the accumulation of MDA compared to that observed in the treatment with 2.5% bay leaf powder may be associated with seed death, with consequent lower metabolism (ATAÍDE *et al.,* 2012). For Paiva *et al.* (2008) among the consequences of seed deterioration, the fall in germination represents one of the last events of physiological decline.

Conclusions

The study allowed, through the treatments applied to both bean varieties, to verify a decrease in germination during storage. Significant differences were observed between the two varieties at 24 months of storage considering germinability and vigor.

Among the parameters evaluated to evaluate vigor, the release of ions, especially the liquid potassium from seed immersion, was the best indicator of seed deterioration under the conditions of this study. In the same way it was possible to correlate the increase in lipid peroxidation with the sharp drop in germination analyzed at 24 months of storage.

Future studies point to the need to elucidate the physiological effect of the active principle present in bay leaf against the enzymatic apparatus involved in the aging process of bean seeds.

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