ABRATES

Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

NOTE

Journal of Seed Science, v.45, e202345027, 2023

An alternative approach to conducting germination tests on chemically treated and untreated stored cotton seeds

Nasma Henriqueta Da Sorte Cossa[1,2*], Everson Reis Carvalho[1], Juliana Maria Espíndola Lima[1], Luiz Fernando de Souza Moraes[1], Felisberto Venâncio Chicamasso Miquicene[1,3], Ivan Natividade Júlio Zevo[1,4]

http://dx.doi.org/10.1590/2317-1545v45266896

ABSTRACT: The objective of this study was to assess the impact of incorporating vermiculite into germination tests for both chemically treated and untreated stored cotton seeds. To that end, two independent experiments were conducted using cotton seeds of the cultivar DP1746B2RF under chemical treatment with a mixture of fungicide, insecticides, and nematicide; and both experiments were set up in a completely randomized design with four replications. In Experiment 1, the objective was to determine the ideal water volume for moistening the vermiculite. Four water volumes were used: 1.5, 2.0, 2.5, and 3.0 times the weight of the dry vermiculite. Experiment 2 was conducted in a 2 × 2 × 2 triple factorial arrangement consisting of two substrates (germitest paper and germitest paper + vermiculite), two treatments (chemically treated seeds and untreated seeds), and two storage periods (0 and 150 days). First germination count and germination were evaluated in the two experiments. From the results found in Experiment 1, the water volume corresponding to 3.0 times the weight of the vermiculite provided the best conditions for germination. In Experiment 2, the most appropriate substrate for conducting the germination test of untreated and chemically treated cotton seeds is vermiculite, which led to rapid germination and uniform seedling development.

Index terms: *Gossypium hirsutum* L., phytotoxicity, substrate.

RESUMO: Objetivou-se avaliar o efeito da inclusão da vermiculita no teste de germinação em sementes de algodão não tratadas e tratadas quimicamente. Para isso, foram conduzidos dois experimentos independentes, utilizando-se sementes de algodão da cultivar DP1746B2RF, submetidas ao tratamento químico com mistura de fungicida, inseticidas e nematicida, ambos, instalados em delineamento inteiramente casualizado com quatro repetições. No Experimento 1, objetivou-se determinar o volume de água ideal para o umedecimento da vermiculita. Foram utilizados quatro volumes de água: 1,5; 2,0; 2,5 e 3,0 vezes o peso da vermiculita seca. O Experimento 2 foi conduzido em esquema fatorial triplo 2 x 2 x 2 sendo: dois substratos (papel germitest e papel germitest + vermiculita), dois tratamentos (sementes tratadas quimicamente e sementes não tratadas) e dois períodos de armazenamento (0 e 150 dias). Avaliou-se nos dois experimentos, a primeira contagem de germinação e a germinação. Pelos resultados encontrados no Experimento 1, o volume de água correspondente a 3,0 vezes o peso da vermiculita proporcionou as melhores condições para germinação. No Experimento 2, o substrato mais apropriado para condução do teste de germinação de sementes de algodão não tratadas e tratadas quimicamente, é a vermiculita, tendo proporcionado rápida germinação e desenvolvimento uniforme das plântulas.

Termos de indexação: *Gossypium hirsutum* L., fitotoxidade, substrato.

***Corresponding author**
nasmadasorte@gmail.com

Received: 08/15/2022.
Accepted: 07/19/2023.

[1]Departamento de Agricultura, Setor de Sementes, Universidade Federal de Lavras, Lavras, MG, Brasil.

[2]Departamento de Extensão, Faculdade de Ciências Agrárias, Universidade Lúrio, Sanga, Moçambique.

[3]Departamento de Agricultura, Instituto Agrário de Chimoio, Vanduzi, Moçambique.

[4]Instituto de Investigação Agrária de Moçambique, Departamento de Agronomia e Recursos Naturais, Maputo, Moçambique.

INTRODUCTION

Brazilian legislation requires a minimum of 70% germination for the basic seed category and 75% for the certified seed category for trading cotton seeds (Brasil, 2013). These percentages are provided by the official germination test, whose rules for seed analyses recommend the use of germitest (germination testing) paper or sand as a substrate (Brasil, 2009).

Nevertheless, test lots of cotton seeds have become inviable when germitest paper was used, due to the presence of fungi on the seed coat, which, when associated with the conditions of the paper substrate, create an environment favorable to fungal proliferation, interfering in the germination process (Oliveira et al., 2016).

Another impediment is in the use of sand as a substrate for the standard test, because there may be a negative correlation of the test with the emergence of seedlings in the field, especially if the seeds manifest fungal infestation (Schuab et al., 2006).

In addition to performing analyses with untreated cotton seeds, chemical treatments have been used, with the aim of minimizing problems caused by pathogens, ensuring the protection and/or eradication of pathogens associated with the seeds (Chitarra et al., 2009; Kubo et al., 2012; Braghin et al., 2014). However, some products (insecticides and fungicides) can cause phytotoxicity to the seeds and seedlings at the time of the germination test (Pereira et al., 2018; Del Bem-Junior et al., 2020; Tunes et al., 2020).

In addition, various studies have been developed confirming the phytotoxic effect of the products used in the chemical treatments of seeds at the time of the germination test using germination test paper (Baldini et al., 2018; Abati et al., 2020; Alvarenga et al., 2020).

However, in order to minimize the effects of phytotoxicity, some authors propose the use of vermiculite between papers as an alternative substrate for conducting the germination test in treated seeds of sweet corn (Bersch et al., 2021); rice (Xavier et al., 2021), and soybean (Rocha et al., 2020). Moreover, for cotton, there is little information in this respect.

Thus, both paper and sand substrates used for germination test exhibit inefficiencies in meeting the germination requisites of both untreated and chemically treated cotton seeds. That leads to the need to study alternative substrates that provide specific conditions in which the test can allow expression of the real physiological potential of cotton seeds without negative interference from the substrate.

Therefore, including vermiculite between towel papers as an alternative substrate is proposed for conducting the germination test of chemically treated and untreated cotton seeds, for the purpose of inhibiting the phytotoxic effects and the occurrence of fungi, optimizing maximum expression of germination potential.

In addition, vermiculite is a substrate that has been used for production of ornamental plants (Santos and Castilho, 2018) and in production of seedlings of forest species (Silva et al., 2017), and it might come to be recommended for conducting the standard germination test in seed analysis laboratories. Its characteristics confer advantages for better support of seeds and seedlings, such as low density, porosity, water holding capacity, chemical composition, and uniform particle size (Martins et al., 2009).

Thus, the aim of this study was to evaluate the performance of vermiculite as a substrate between papers to conduct the germination test of treated and untreated cotton seeds, as well as to establish the ideal volume of water for moistening this substrate and preparing the test setup.

MATERIAL AND METHODS

The study was developed in the Central Seed Analysis Laboratory of the *Universidade Federal de Lavras* (UFLA), Lavras, MG, Brazil. Seeds of the cotton cultivar DP1746B2RF from the 2019/20 crop year were used, provided by the J&H Sementes company.

The cotton seeds were chemically delinted using concentrated sulfuric acid (98%) at the proportion of 1 L for every 7 $kg.seeds^{-1}$. The delinting process lasted eight minutes, followed by washing with running water (one minute) and neutralization with calcium hydroxide / hydrated lime ($Ca(OH)_2$) diluted in water at the concentration of 10% (one minute). After the neutralization process, the seeds were washed in running water (one minute) and dried at ambient temperature for 72 hours.

The lot of delinted seeds was divided into two samples: one remained without treatment and the second was treated with a mixture of chemical products, described in Table 1. The L5K device from *Momesso Arktos Laboratório* was used for the chemical seed treatment, which simulates the industrial treatment of seeds in batches, with calibration of 15 hertz in the inverter of the device.

The seeds were placed in multiwall Kraft type paper bags for each period of evaluation and storage in a conventional warehouse without climate (temperature and relative humidity) control. The tests were conducted soon after the seed treatment and at 150 days of storage.

The moisture content (w.b.) was determined before and after storage using the oven method at 105 °C ± 3 °C for 24 hours, in which two replications of 4.5 g of seeds were used (Brasil, 2009). The results were expressed in percentage based on wet weight.

Experiment 1

The experiment was conducted in a completely randomized experimental design with four replications and one factor, which was four volumes of distilled water: 1.5, 2.0, 2.5, and 3.0 times the dry weight of the fine expanded vermiculite (90% to 100% of the particles from 0.30-0.50 mm), using chemically treated cotton seeds.

The towel paper was moistened at the proportion of 2.5 times the weight of the dry paper, according to the Rules for Seed Testing (*Regras para Análise de Sementes*) (Brasil, 2009). The vermiculite was moistened separately at the proportions of water volume cited above (1.5, 2.0, 2.5, and 3.0 times the weight of the dry vermiculite).

After the moistening procedures were carried out, the germination test was set up using three sheets of towel paper, in which two sheets served as a base, placing a 100-mL volume of vermiculite and distributing it in a single uniform layer (Figure 1A). After that, the seeds were sown with the aid of a perforated plate (Figure 1B) and closure was made with the third sheet; and rolls were then formed (Figure 1C).

To prevent the rolls from toppling over, the vermiculite layer was distributed up to the fold of the paper, leaving free space only on the opposite side of the paper and at the upper part, as shown in Figure 1A. One sheet of towel paper was wrapped around every four rolls to hold them together (Figure 1D). In place of germitest paper, rubber bands can be used, placing them at the two ends of the rolls as an alternative.

First germination count was evaluated at four days after sowing and final germination count at twelve days. The results were expressed in percentage of normal seedlings (Brasil, 2009).

Table 1. Doses of the commercial products used in the treatment of cotton seeds.

Commercial product	Type of product 1	Dose of the commercial product (mL)	Active ingredient (mL)	Dose applied (mL)
Avicta 500 FS	N+I	3	Abamectin 500	6
Cruiser 350 FS	I	400 - 600	Thiamethoxam 350	12
Dynasty	F	100 - 300	Azoxystrobin 75 Metalaxyl-M 37.5 Fludioxonil 12.5	6
Fortenza 600 FS	I	300 - 400	Cyantraniliprole 600	6
Biocroma	P	400 - 600		8
Biogloss	DP	400 - 600		12 (g)
Grafite	G	100 - 200		4 (g)

[1]N = Nematicide, I = Insecticide, F = Fungicide, P = Polymer, DP = Drying powder, G = Graphite

Figure 1. Methodology of setting up the alternative germination test with vermiculite, showing adequate distribution of the vermiculite (A), sowing (B), arrangement of the rolls (C), and closure of the rolls (D) (Experiment 1).

Experiment 2

A completely randomized design was used in a 2 × 2 × 2 triple factorial arrangement (two substrates: towel paper and towel paper + fine expanded vermiculite × two seed treatments: chemically treated seeds and untreated seeds × two storage periods: 0 and 150 days).

The methodology of the germination test available in the Rules for Seed Testing (*Regras para Análise de Sementes*) (Brasil, 2009) was used for the towel paper substrate, which stipulates moistening at the proportion of 2.5 times the weight of the dry paper. The substrate composed of the towel paper + vermiculite, in turn, was prepared as described in Experiment 1, using the water volume corresponding to 3.0 times the weight of the vermiculite.

The results were evaluated by first germination count at four days after sowing and final germination count at twelve days, according to Brasil (2009). Results were expressed in percentage.

The Shapiro-Wilk test was used on the data of the two experiments, and after confirmation of the normality of the data, analysis of variance was carried out by the F test ($p < 0.05$), with comparison of the means by Tukey's test

($p < 0.05$) and regression analysis for Experiment 1. The SISVAR 5.6 statistical software was used (Ferreira, 2014), and graphics were prepared on the SigmaPlot 10.0 software.

RESULTS AND DISCUSSION

The moisture content of the cotton seeds after drying at ambient temperature was from 11.7% to 12% after 150 days of storage. These values are considered adequate for germination capacity of the species. The determination of seed moisture content is fundamental for the official seed quality tests because of its effect on physiological quality, where seeds with non-standard moisture content may be of lower quality (Sarmento et al., 2015).

The use of different volumes of water for moistening vermiculite significantly (*p*-value = 0.01%) affected the first germination count and germination variables, and the seeds tended to exhibit an increase in the percentage of first germination count and germination as a result of an increase in water volume in the vermiculite substrate.

In evaluation of first germination count (Figure 2A), the water volume of the treatment of 3.0 times the weight of the dry vermiculite was statistically superior to the other treatments, showing 99% germination, indicating that probably the greatest volume of water (three times) favored rapid imbibition of the seeds, due to the greater water availability of the substrate. This corroborated what was described by Carvalho and Nakagawa (2012), who consider the greater availability of water for the seed as a factor that makes for greater absorption, thus accelerating the germination process and, consequently, providing for greater vigor expression.

The other treatments – 1.5, 2.0, and 2.5 times the weight of the dry vermiculite – were statistically inferior compared to the threefold (3.0) water volume, with differences of 35, 23, and 14 percentage points, respectively. Those water volumes (1.5, 2.0, and 2.5 times) did not favor vigor expression in first germination count, which could lead to a false-negative result.

These results showed how important providing adequate water to cotton seeds is in germination tests, because it prevents erroneous results. Reduction in the percentage of first germination count can be explained by the fact of the cotton seeds having a thicker seed coat compared to other species, such as soybean. Therefore, there is the need for a greater supply of water for cotton seeds.

Nevertheless, the response of species to excess or scarce water may vary according to the characteristics of their seed coat, as observed by Gordin et al. (2015), who found that in Niger seed, greater availability of water in the substrate favored first germination count and germination. The contrary was observed by Azeredo et al. (2010),

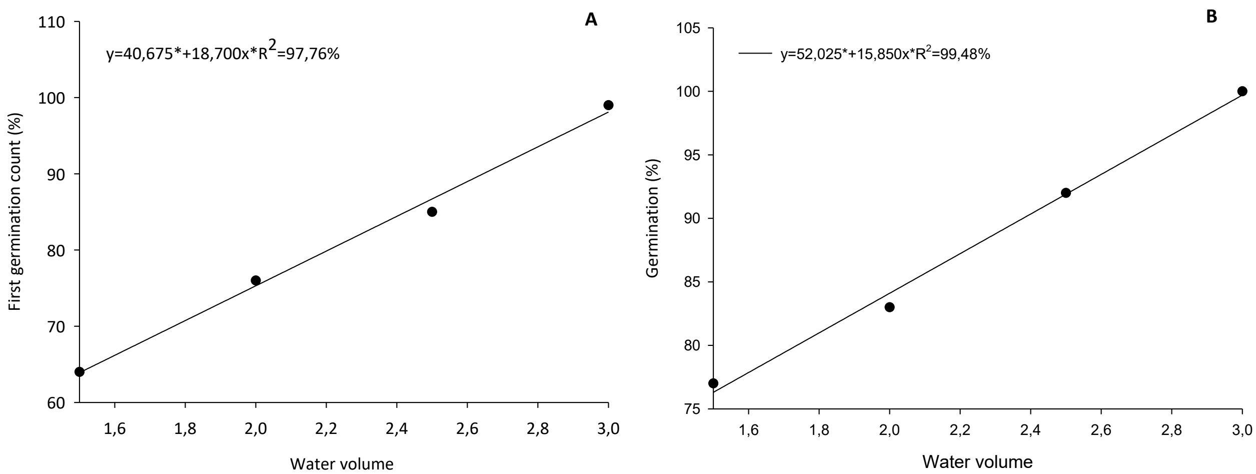

Figure 2. Results of first germination count (A) and germination (%, ●) (B) of treated cotton seeds, as a function of different water volumes (1.5, 2.0, 2.5, and 3.0 times the weight of the dry vermiculite) (Experiment 1). *: Significant at $P < 0.05$.

who found that in Cabbage seeds, water volumes greater than or equal to three times the weight of the dry paper reduce germination.

An increasing linear curve was observed in the germination results (Figure 2B), and the highest germination percentages were at the water volumes corresponding to 3.0 times (100%), followed by 2.5 times (92%), though the threefold water volume was superior (Table 2). The other volumes (2.0 and 1.5 times) exhibited germination below 90%, which did not reflect the real physiological potential of the cotton seeds.

In addition to exhibiting lower germination percentages, the volumes of water below 3.0 times the dry weight required repetition of moistening after first germination count, which showed the ineffectiveness of these water volumes in maintaining the substrate moist over the test period of 12 days. Moistening the substrate after first germination count can lead to variation in the germination results, since it is not uniform moistening, and its ineffectiveness was visibly observed in the results (Figure 2B).

The substrate should be moistened in quantities satisfactory for each type of substrate that is to be used, taking into consideration that each substrate has specific characteristics, such as particular particle sizes, aggregation, water holding capacity, porosity, and contact surface with the seed (Martins et al., 2011).

These water deficit conditions during the germination test confirm that described by Marcos-Filho (2015), who considers the lack of water availability as a condition that reduces germination, up to the point of embryo death.

Therefore, the germination test using fine expanded vermiculite between towel paper can be conducted with vermiculite moistened at 3.0 times its dry weight. Under these conditions, there was sufficient water retention to favor cotton seed germination without the need for additional moistening of the substrate.

The results found in first germination count (Table 2) confirm that the percentage of normal seedlings for untreated seeds was statistically higher in the substrate composed of towel paper + vermiculite (80%) compared to that obtained in the substrate of the towel paper, which exhibited 69%, a difference of 11 percentage points at zero storage time. That suggests that with the addition of vermiculite in towel paper, there was an increase in seed performance already at first germination count, due to the particle size of the vermiculite retaining sufficient moisture and maintaining good aeration, creating optimal and favorable conditions for rapid germination (Martins et al., 2011).

Table 2. Mean results of first germination count and germination of cotton seeds of the cultivar DP 1746 B2RF treated with a mixture of fungicides, insecticides, and nematicides, stored for 150 days, as a function of two substrates (germitest paper and germitest paper + vermiculite).

Substrate	Storage period (days)			
	0		150	
	Treatment			
	Untreated seeds	Treated seeds	Untreated seeds	Treated seeds
First germination count				
Germitest paper	69 bB^{A}	84 bA^{A}	55 bB^{B}	74 bA^{B}
Germitest paper + Vermiculite	80 aB^{A}	100 aA^{A}	70 aB^{B}	83 aA^{B}
CV (%) 1.06				
Germination				
Germitest paper	77 bB^{A}	90 bA^{A}	67 bB^{B}	85 bA^{B}
Germitest paper + Vermiculite	88 aB^{A}	100 aA^{A}	80 aB^{B}	93 aA^{B}
CV (%) 1.05				

*Means followed by the same lowercase letter in the column, uppercase letter in the row, and uppercase superscript letter do not differ from each other by Tukey's test at P < 0.05.

These results corroborate those found by Martins et al. (2011) on the germination performance of *Stryphnodendron* seeds with different substrates, where they observed that the use of vermiculite may be favorable for an increase in normal seedlings in first germination count, as long as particle size and moisture ideal for the species are observed.

Still at the same evaluation time (day 0) for treated seeds, the first germination count percentage was higher in the substrate of towel paper + vermiculite, with 100% normal seedlings, while the towel paper substrate showed 84% normal seedlings, a difference of 16 percentage points between the substrates.

After storage (150 days), there was reduction in the percentage of first germination count (Table 2); the untreated seeds in towel paper substrate resulted in 55% normal seedlings, a reduction of 14 percentage points in comparison with the initial value of 69% at day zero.

That probably occurred as a result of storage without control of temperature and relative humidity. Under those conditions, seed metabolism remains active, and the seed uses up its reserves. In addition, there is an increase in fungi that feed on cotyledons. These factors in combination accelerate seed deterioration.

In the substrate of towel paper + vermiculite, in turn, untreated seeds resulted in 70% first count germination, 10 percentage points below the value at day zero (80%). Although the seeds were not treated, better performance was seen, which was promoted by the use of vermiculite, as it inhibited secondary contamination from infected seedlings to healthy seedlings.

At 150 days of storage, treated seeds sown in the substrate of towel paper + vermiculite showed 83% in first count germination, 9 percentage points different from the seeds treated in the substrate of towel paper alone (74%). These results show the positive correlation of the chemical treatment with the vermiculite-based substrate. The chemical treatment acted to preserve and protect the physiological and sanitary quality of the seeds; vermiculite, in turn, constituted a favorable structure between the paper for germination of treated cotton seeds.

The germination results (Table 2) showed that in the first storage time (day 0), the untreated seeds of the germitest paper substrate had germination of 77%. In the substrate of towel paper + vermiculite, the germination was 88% (Table 2).

There was a difference of 11 percentage points in the performance of the seeds from one substrate to another. In addition, it can be seen that the vermiculite provided for greater capacity of development of cotton seedlings than that of the towel paper substrate alone, as shown in Figure 3.

In that same evaluation time (day 0), the treated seeds in towel paper substrate showed 90% germination, and in the substrate of towel paper + vermiculite, 100% germination, an increase of 10 percentage points from one substrate

Figure 3. Cotton seedlings in the final count of the germination test by the traditional method (A) and alternative method with vermiculite (B) (Experiment 2).

to the other. This result leads us to consider the inclusion of vermiculite in conducting the cotton seed germination test through its plasticity in meeting the requirements of treated seeds; it expressed the maximum potential of the seed lot.

The same did not occur in the towel paper substrate, because it concealed the true physiological quality of the cotton seeds. This lower germination may be the effect of greater concentration of the chemical product around the seed in the paper substrate, leading to phytotoxicity for the seeds and reducing germination potential.

The phytotoxicity that can occur in the standard germination test is due to the area of contact of the seed with the chemical product on the germitest paper, which can bring about an average concentration 3,500 times greater than in emergence in the field (Alvarenga et al., 2020).

In contrast, the response of the treated seeds to the addition of vermiculite was positive because the vermiculite favored germination and spread the chemical product around the seeds, preventing a possible situation of phytotoxicity.

The benefits of the use of vermiculite to improve the performance of treated seeds have already been reported by other authors, such as Xavier et al. (2021) in rice seeds. They observed that the addition of vermiculite between the paper was effective in evaluation of the germination test of treated seeds. Tunes et al. (2021) obtained a higher percentage of germination in chemically treated soybean seeds using vermiculite between the paper; and Bersch et al. (2021), working with maize seeds treated with different products, observed that vermiculite between the paper is a viable alternative substrate for evaluation of germination.

In the second germination evaluation time, at 150 days of storage, the untreated seeds in the towel paper substrate obtained 67% germination, and in the substrate of towel paper + vermiculite, 80% germination, a difference of 13 percentage points. The treated seeds in towel paper substrate obtained 85% germination and in the substrate of towel paper + vermiculite, 93%.

These results suggest that the chemical treatment of cotton seeds was indispensable in maintaining their physiological quality, especially when the intention is to store them. It is also an ally in conducting the germination test and in expression of the physiological potential of cotton seeds.

Therefore, the results observed while conducting the experiment confirmed that vermiculite favors better performance of cotton seeds, whether they are chemically treated or untreated. Vermiculite can be considered an alternative substrate for conducting the standard germination test in seed analysis laboratories.

CONCLUSIONS

The volume of water ideal for moistening the vermiculite for conducting the germination test is 3.0 times the weight of the dry vermiculite.

Utilizing the towel paper + vermiculite substrates is a practical approach for conducting the germination test, enabling the assessment of the physiological quality of both chemically treated and untreated cotton seeds, regardless of whether they have been stored for 150 days or not.

Employing vermiculite effectively minimizes seedling contamination by common fungi during the germination test, when compared to the use of germination papers and, moreover, it helps mitigate potential phytotoxic effects arising from chemical seed treatments.

ACKNOWLEDGMENTS

Our thanks to the *Universidade Lúrio* and to the African Development Bank for granting scholarships. To the J&H Sementes and the Syngenta Seed Care Institute companies for providing seeds and products for carrying out the study. To the *Universidade Federal de Lavras* – Department of Agriculture, Seed Sector (Seed Analysis Laboratory), for making infrastructure, equipment, and material available for carrying out the study.

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