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Original Article

Influence of essential oils on the quality of *Vigna unguiculata* L. (Walp.) seeds compared by traditional method, image and multivariate analysis

Influência de óleos essências na qualidade de sementes de *Vigna unguiculata* L. (Walp.) comparado pelo método tradicional, análise imagem e multivariada

A. V. da Silva[a], C. M. da Silva[b], M. C. de Azevedo[a], J. H. B. da Silva[a], J. S. Nóbrega[c*], R. T. de Fátima[c], J. T. A Ferreira[c], W. E. Pereira[a] and F. Mielezrski[a]

[a]Universidade Federal da Paraíba – UFPB, Centro de Ciências Agrárias – CCA, Campus Areia, Areia, PB, Brasil

[b]Universidade Federal de Viçosa – UFV, Campus Viçosa, Viçosa, MG, Brasil

[c]Universidade Federal de Campina Grande – UFCG, Unidade Acadêmica de Engenharia Agrícola – UAEA, Campus Campina Grande, Campina Grande, PB, Brasil

Abstract

The extracts of medicinal plants are used for the treatment of seeds in order to reduce the action of phytopathogens and increase the vigor of the seeds. Currently, computerized image analysis has been used to assess the physiological quality of seed lots. The objective was to evaluate the efficiency of the Vigor-S® software in the evaluation of the physiological quality of cowpea seeds treated with essential oils, comparing with a traditional test and the principal component analysis. Two cowpea cultivars were analyzed, BRS Tumucumaque and BRS Guariba, treated with doses of natural extracts of Alfavaca, garlic, horsetail, citronella and pyroligneous acid. The traditional method consisted of evaluations for germination, first germination count, seedling emergence, emergence speed index, accelerated aging, fresh matter and dry matter of seedling and the image analysis for: seedling length, growth index, uniformity index, vigor index, and germination. A Principal component analysis was applied to reduce the number of variables. Horsetail, Alfavaca and citronella extracts were efficient in increasing the physiological quality of the seeds of at least one cultivar. The Vigor-S® software proved to be efficient compared to traditional tests to assess the physiological quality of seeds. Principal Component Analysis is an ally to identify the best extracts and doses to be used. The image analysis method proved to be effective when compared to the traditional method and can therefore be used.

Keywords: plant extracts, principal component analysis, Vigor-S®.

Resumo

Os extratos de plantas medicinais são utilizados para o tratamento de sementes com o objetivo de diminuir a ação de fitopatógenos e aumentar o vigor das sementes. Atualmente, a análise computadorizada de imagens tem sido utilizada para avaliar a qualidade fisiológica de lotes de sementes. O objetivo foi avaliar a qualidade fisiológica de sementes de feijão-caupi tratadas com óleos essenciais, comparado com teste tradicional, análise imagem e a análise de componentes principais. Foram analisadas duas cultivares de feijão-caupi, BRS Tumucumaque e BRS Guariba, tratadas com doses de extratos naturais de alfavaca, alho, cavalinha, citronela e ácido pirolenhoso. O método tradicional consistiu em avaliações de germinação, primeira contagem de germinação, emergência de plântulas, índice de velocidade de emergência, envelhecimento acelerado, matéria fresca e matéria seca da plântula e a análise de imagem para: comprimento da plântula, índice de crescimento, índice de uniformidade, índice de vigor e germinação. Uma análise de componentes principais foi aplicada para reduzir o número de variáveis. Os extratos de cavalinha, alfavaca e citronela foram eficientes em aumentar a qualidade fisiológica das sementes de pelo menos uma cultivar. O software Vigor-S® mostrou-se eficiente em relação aos testes tradicionais para avaliação da qualidade fisiológica de sementes. A Análise de Componentes Principais é uma aliada para identificar os melhores extratos e doses a serem utilizados. O método de análise de imagens mostrou-se eficaz quando comparado ao método tradicional, podendo, portanto, ser utilizado.

Palavras-chave: extratos vegetais, análise de componente principal, Vigor-S®.

*e-mail: jacksonnobrega@hotmail.com
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1. Introduction

Cowpea [*Vigna unguiculata* L. (Walp.)] is a legume used as a staple food, especially in the Brazilian Northeast region, where it is considered one of the main sources of plant protein. In addition to being an important component of the diet of the population of the Northeast, this crop is also an important generator of employment and income for this region, and food item for population (Fontes et al., 2011; Melo et al., 2021).

In Brazil, in the 2020/2021 growing season, cowpea had a planted area of 1.397.6 million hectares and a production of 623.3 tons (Conab, 2022). In the Northeast region, this crop is very important, with more than 76.4% of the 1.068.00 thousand million hectares for sowing in the country in this first harvest of 2020/2021. The State of Paraíba produces cowpea, however it has low representativeness, both in acreage with 66.800 hectares, and in productivity with 289 kg/ha (Conab, 2022).

This low productivity is due to several factors such as the soil and climate conditions of the region, the appearance of pests and diseases (Farias et al., 2022), productive and improved potential of the cultivar and the availability of nutrients (Barros et al., 2011), and also the physiological quality of seeds used in planting, as seedling growth and development depends on the germinative capacity of the seeds (Reed et al., 2022), so it is important to acquire high quality seeds and the analysis of seeds is an effective method for checking seed germination and vigor (Costa et al., 2022).

Seed analysis is an essential process in the seed production system, as it allows to check the status of seeds of a lot or cultivars (or hybrids) that helps in decision-making regarding the management to be adopted during the whole process until planting. This is because beans, especially cowpea, have several types of pathogens that damage seeds and consequently plants in the field.

Unlike what happens with the presence of pests in seeds and crops, which can be solved by observing the egg-laying and insects present before the economic losses, the diseases in cowpea, when present, already represent partial or total loss in production and often when it is noticed it is already too late for control. Thus, knowing the diseases of the crop before its appearance, and applying preventive measures for such diseases minimize the possible losses from the attack of these pathogens (Lima, 2017).

In order to avoid these problems, chemical treatments become an alternative, but these treatments negatively influence seed germination (Fantinel et al., 2017). In turn, there are natural products extracted from plants, which have medicinal functions and represent alternatives to be used in the fight against pathogens (Bardin et al., 2015), because they have phytopathogenic substances or induce resistance in plants and have no negative effect to the environment (Carboni and Mazzonetto, 2013; Radünz et al., 2022).

The distribution of cowpea seeds must follow the criteria established by the rules for seed analysis (RSA) which is the traditional analysis adopted in this research, and for this a more accurate analysis is necessary taking into account the germination and vigor tests, which allows the selection of the best lot for commercial sale and for sowing (Nunes et al., 2019).

In this sense, RSA sets the procedures for seed analysis that are done manually by the traditional test. One of the analyses is the vigor test, which makes it possible to identify which seed lots are in a state of deterioration, enabling the choice of the best lot helping in decision-making in seed production (Wendt et al., 2017). Among the various vigor tests recommended by the Rules for seed analysis, the emergence speed index, first germination count, seedling length, accelerated aging, among others, can be highlighted.

Currently, with the advancement of technology, it is now possible to make this analysis by image. These computerized image analyses allow to quantify the physiological quality of seeds in less time (Marques et al., 2015). Among several softwares on the market, there is the Vigor-S® software, and its use is justified because it is a free program, easy to handle and available (Castan et al., 2018).

These computerized programs have already been used in several crops such as corn (Castan et al., 2018), soybean (Medeiros and Pereira, 2018; Rodrigues et al., 2020), crambe (Leão-Araújo et al., 2017), and cowpea (Rego et al., 2021) among others.

The Vigor-S® software is a system developed in partnership between Embrapa Instrumentação and the Luiz de Queiroz School of Agriculture (USP / ESALQ), both based in the city of São Paulo. The program allows the determination of the vigor index and the length of seedlings with great speed and precision. An advantage of this system is the elimination of possible errors made by humans, adding reliability and allowing the image to be compared to other images later (Rodrigues et al., 2020).

Thus, the objective of this experiment was to evaluate the efficiency of the Vigor-S® software in the assessment of the physiological quality of cowpea seeds, compared with a traditional test and multivariate analysis, taking into account the use of essential oils for seed treatment.

2. Materials and Methods

2.1. Site description and experimental design

The research was carried out at the Seed Analysis Laboratory of the Phytotechnics Department, Federal University of Viçosa, in Viçosa, State of Minas Gerais (20°45'30" S, 42°52'15" W and 648 m), Brazil.

The experiment was conducted in a completely randomized design, with four replications, in a 5 × 5 × 2 + 1 factorial arrangement, corresponding to five natural products (Alfavaca - *Ocimum gratissimum*, garlic - *Allium sativum* L., horsetail (*Equisetum arvense* L.), citronella - *Cymbopogon winterianus* Jowitt, pyroligneous acid extracts – Maggi pyroligneous), in the doses of 0, 2, 4, 6 and 8 g kg^{-1} seeds, two cultivars, BRS Tumucumaque (Cultivar 1) and BRS Guariba (Cultivar 2) and a commercial product (Captan in the dose of 3 mg kg^{-1} of the company ADAMA).

The natural extracts used in this research are commercialized in Brazil for medicinal purposes for human use and have fungicidal compounds: the basil extract has 39.42% Eugenol, 29.15% Thymol, 7.46% 1.8-cineole and 32.97% of Others (not identified); Garlic extract contains 27.82% Allicin, 30.28% Allyl disulfine, 4.25%

Allyl sulfone, 4.82% Allyl tetrasulfide, 2.05% 2-ethylhexyl oxalic acid and 30.78% others(not identified); Horsetail extract has 30.24% Hexadecanoic acid (palmitic acid), 30.02% Plenol, 2,4-bis(1,1dimethylethyl), 12.09% Thymol, 10.74% Cis-Geranyl acetone, 1.72% Propanoic acid, 5.88% Phytol, 16.12% 9, 12, 15-octadecatrien-1-ol and 3.19% Others (not identified); Citronella extract contains 29.35% Citronellal, 10.82% Citronellol, 10.45% Eugenol, 21.22% Geraniol, 5.12% Geranial, 1.02% Terpineol, 8.24% Elemol and 13.78% of Others (not identified); Pyroligneous extract has 35.75% Acetic acid, 8.78% Guaiacol, 13.14% Furfural, 10.22% Syringol and 35.11% Others (not identified).

2.2. Variables analyzed

The tests conducted were: 1) Traditional test - Germination (GT), First germination count (FGC), Seedling emergence (E), Emergence speed index (ESI), Accelerated aging (AA), Fresh matter (FM) and dry matter (DM) of seedlingby the rules for seed analysis of the Ministry of Agriculture in Brazil, Livestock and Supply [18], and 2) Image Analysis - seedling length (SL), growth index (GI), uniformity index (UI) and vigor index (VI) and germination index (GI) per image as follows:

Germination test: performed with four replications of 50 seeds, in rolls of germitest paper moistened with distilled water, equivalent to 2.5 times the weight of non-moistened paper and kept in Biochemical Oxygen Demand (BOD) at 25°C. The final count was performed eight days after the test was installed, and the results are expressed as a percentage of normal seedlings, according to Brasil (2009). First germination count: performed along with the germination test. The percentage of normal seedlings obtained on the fifth day after sowing was calculated (VC Stadium) (Brasil, 2009).

Seedling emergence: performed with four replications of 50 seeds, sown at a depth of 2 cm, in multiple-cell expanded polystyrene trays with one seed sown per cell. The trays were filled with washed, sterilized sand, irrigated daily and kept at 25º C. The count of emerged seedlings was performed on the tenth day after sowing (in the phenological stage V2). The results were expressed as a percentage.

Emergence speed index: The number of seedlings emerged was evaluated daily until no further increase in the number of normal seedlings, considering, in this condition, those with apparent cotyledons, above the substrate level. The seedling emergence speed index was calculated according to Maguire (1962).

Accelerated aging: performed with four replications of 50 seeds per lot, with 220 seeds placed on a stainless-steel screen in the upper third of a plastic gerbox (11 x 11 x 3.5 cm) standardized model for this type of analysis, containing 40 mL water. After closing, the boxes were kept in a BOD chamber, set at 41 ° C for 48 hours. According to the seed analysis rules, the culture of cowpea beans does not require light conditions for the germination test. After that, the germination test determined was carried out on the fifth day after the installation of the test.

Computerized analysis of seedling images (Vigor-S®) - four replications of 20 seeds per treatment were distributed in two rows in the upper third of two sheets of paper towels and covered with a third sheet. All sheets were previously moistened with water in the amount of 2.5 times the weight of the dry paper. The 20 seeds were distributed with the hilum towards the bottom of the paper. After covering with a third sheet of moistened paper, rolls were made, which were then placed in plastic bags and placed vertically in BOD for periods of two days at 25°C. At the end of that period, images of the seedlings were captured by a computerized digitization system (60 cm × 50 cm × 12 cm), with a blue cardboard sheet with an area of 30 cm x 20 cm (size chosen for the proper distribution of the 20 seedlings, 10 at the top and 10 at the bottom) on which the germinated seedlings were placed. The seedlings of each replication were digitized with a scanner (HP, Scanjet 200) adjusted to a resolution of 300 dpi. The images were processed individually by the Vigor-S® software, which provides the following variables: seedling length, growth index, uniformity index and vigor index.

The weight of the hypocotyl and the weight of the primary root were adjusted to 10% and 90%, respectively, to calculate the growth index. The contribution of the growth and uniformity variables used to calculate the vigor index were 70% and 30%, respectively (Medeiros et al., 2019).

Fresh and dry matter of seedlings - were determined together with the computerized analysis of seedling images (Vigor-S®). On the fifth day after mounting the test of imagens, normal seedlings from each treatment were weighed on an analytical balance accurate to 0.0001 g. Subsequently, they were placed on kraft paper bags and taken to a forced air oven set at 65 ± 2 ºC, for 72 hours. Then, they were weighed again and the results expressed in grams per seedling.

2.3. Statistical analysis

After confirming the normal distribution of errors by the Shapiro-Wilk test and homogeneity of variances by the Bartlett test, analysis of variance was applied to the data.

To compare the cultivars and the products, the Tukey's test was applied, while the doses of the products were evaluated by regression analysis. Then, Pearson correlation coefficients (r) were calculated for all combinations between the seed quality assessment tests, in which the significance of the r values was determined by the t-test ($p \leq 0.05$)

Subsequently, the Dunnett test was carried out in order to compare all natural products with the Commercial Captan product by the principal component analysis. Soon afterwards, principal component analysis (PCA) was applied in order to reduce the number of variables. The criterion 0.7/root (lambda) was used to determine the contribution of a variable to a given principal component. All analyses were performed with the aid of the R 3.6 software (R Core Team, 2019).

3. Results

The interaction of products (P) x Dose (D) x Cultivars (C) showed a significant effect for all characters studied. The combined effect of P x D x C was broken down for all variables and the respective regressions were made considering product and cultivar as qualitative data and doses as quantitative data.

For the fresh matter and dry matter of seedlings, the extracts used react differently for the two crops. For fresh matter (A) Table 1, it was observed that with increasing doses of pyroligneous acid extract and horsetail extracts, the fresh matter increased linearly.

The highest value of fresh matter was found for horsetail extract at dose 8 mg Kg^{-1}, reaching almost 15 g fresh matter. The extracts of Alfavaca and garlic behaved in a quadratic way, in which dose 4 mg Kg^{-1} exhibited the best results. In cultivar 2, citronella and pyroligneous acid extract dose increase had no effect, horsetail and Alfavaca extracts were significant in a linear manner and garlic extract, in a quadratic way, in which the dose of 4 mg Kg^{-1} was more appropriate. The maximun fresh matter value for c2, 15g, was obtained by the extract of Alfavaca at 8 mg Kg^{-1}.

The horsetail extract was more efficient in releasing nutrients to the seed, also acting on seed health, as can be seen in Table 1 (fresh matter and dry matter) of cultivar 1. As for cultivar 2, in addition to the horsetail extract, the extract of Alfavaca also proved efficient in the transfer of reserves as it is observed in Table 1 (fresh matter and dry matter).

In Table 2, citronella, garlic and horsetail extracts do not affect the emergence speed index of cowpea seeds in cultivar 1. In turn, the extract of Alfavaca and pyroligneous acid extract increased the emergence speed, with the highest emergence speed at 8 mg Kg^{-1} for the extract of Alfavaca. For cultivar 2, only the extract of Alfavaca did not affect the emergence speed. The other extracts reduced the seedling emergence speed.

For the percentage of emergence of cultivar 1, none of the extracts tested increased the percentage of seed germination. In cultivar 2, only the extracts of garlic and horsetail increased the percentage of emergence up to the dose of 4 mg kg^{-1}, higher doses reduce the emergence. Alfavaca extract did not affect the emergence and citronella and pyroligneous acid extracts reduced the emergence.

The use of extracts that increase the seedling emergence speed index has the potential to increase crop productivity, since seedlings that emerge with greater speed and uniformity reach the appropriate stand and become more competitive in relation to weeds.

With regard to the first germination count, the best result was found for the extract of Alfavaca with 88% germination at the dose 8 mg kg^{-1} (Table 3) and 85% germination with the same dose for cultivar 2. The horsetail extract behaved quadratically for both cultivars, being efficient up to the dose of 4 mg kg^{-1} for C1 and 5 mg kg^{-1} for C2, higher doses caused the reduction of germination. The extracts of pyroligneous acid, garlic and citronella reduced the first germination count.

For germination, in the traditional method, the extracts of Alfavaca, pyroligneous ac-id and horsetail were performed better than the others (Table 3), with the first two behaving in a quadratic way; 2 mg kg^{-1} was the best dose for the pyroligneous acid extract and 4 mg kg^{-1} for the horsetail extract. The citronella and garlic extracts negatively affected the seed germination.

Table 1. Fitted regression equations for Fresh mass and dry mass under the doses (x) for the respective products, cultivars and the determination coefficients.

Variables	Products	Adjusted equations	Maximum efficiency	Dose (mg kg^{-1})	R^2
C1: Fresh matter (g)	Alfav ext	$Y = 9.7 + 1.175^{**}x - 0.1529^{**}x^2$	11.96	3.85	0.805
	Garlic ext	$Y = 9.52 + 0.347^{**}x - 0.0425x^2$	10.23	4.1	0.603
	Pyrol ext	$Y = 10.97 + 0.149^{**}x$	12.16	8	0.472
	Citron ext	$Y = 9.1 + 0.259^{*}x$	11.17	8	0.883
	Horse ext	$Y = \bar{y} = 11.88$	-	-	-
	Captan Sc	$Y = \bar{y} = 10.25$	-	-	-
C2: Fresh matter (g)	Alfav ext	$Y = 13.44 + 0.229^{*}x$	15.27	8	0.603
	Garlic ext	$Y = 12.27 + 0.534^{**}x - 0.0501^{**}x^2$	13.69	5.33	0.883
	Pyrol ext	$Y = 14.09 + 0.123^{**}x$	15.07	8	0.348
	Citron ext	$Y = \bar{y} = 13.34$	-	-	-
	Horse ext	$Y = \bar{y} = 13.11$	-	-	-
	Captan Sc	$Y = \bar{y} = 12.25$	-	-	-
C1: Dry matter (g)	Alfav ext	$Y = 0.66 + 0.01^{**}x$	0.74	8	0.121
	Garlic ext	$Y = 0.59 + 0.009^{**}x - 0.0018^{**}x^2$	0.60	2.5	0.68
	Pyrol ext	$Y = 0.6 + 0.061^{**}x - 0.0083^{**}x^2$	0.71	3.67	0.747
	Citron ext	$Y = \bar{y} = 0.69$	-	-	-
	Horse ext	$Y = \bar{y} = 0.61$	-	-	-
	Captan Sc	$Y = \bar{y} = 0.60$	-	-	-
C2: Dry matter (g)	Alfav ext	$Y = 0.79 + 0.017^{**}x$	0.93	8	0.643
	Garlic ext	$Y = 0.78 + 0.073^{*}x - 0.0102^{*}x^2$	0.91	3.58	0.994
	Pyrol ext	$Y = \bar{y} = 0.76$	-	-	-
	Citron ext	$Y = 0.76 + 0.064^{**}x - 0.0083^{**}x^2$	0.88	3.85	0.994
	Horse ext	$Y = 0.78 - 0.022^{**}x + 0.0056^{**}x^2$	0.76	1.96	0.933
	Captan Sc	$Y = \bar{y} = 0.77$	-	-	-

Research data. Alfav ext - alfavaca extract; Garlic ext - garlic extract; Pyrol ext - Pyroligneous extract; Citron ext - Citronella Extract; Horse ext - Horsetail Extract. * Significant at 5% of probability. ** Significant at 1% of probability.

Table 2. Fitted regression equations for Emergence speed index and Emergence (%) under the doses (x) for the respective products, cultivars and the determination coefficients.

Variables	Products	Adjusted equations	Maximum efficiency	Dose (mg kg^{-1})	R^2
C1: Emergence speed index	Alfav ext	$Y = 9.43 + 0.299^{*}x$	11.82	8	0.908
	Garlic ext	$Y = \bar{y} = 11.31$	-	-	-
	Pyrol ext	$Y = 9.64 + 0.101^{*}x$	10.45	8	0.464
	Citron ext	$Y = \bar{y} = 11.46$	-	--	-
	Horse ext	$Y = \bar{y} = 10.13$	-	-	-
	Captan Sc	$Y = \bar{y} = 10.25$	-	-	-
C2: Emergence speed index	Alfav ext	$Y = \bar{y} = 10.52$	-	-	-
	Garlic ext	$Y = 10.5 - 0.355^{**}x$	10.5	0	0.774
	Pyrol ext	$Y = 9.71 - 0.424^{**}x$	9.71	0	0.438
	Citron ext	$Y = 10.03 - 0.503^{*}x$	10.03	0	0.985
	Horse ext	$Y = 10.13 - 0.509^{**}x$	10.13	0	0.993
	Captan Sc	$Y = \bar{y} = 9.82$	-	-	-
C1: Emergence (%)	Alfav ext	$Y = \bar{y} = 95.5$	-	-	-
	Garlic ext	$Y = \bar{y} = 93.55$	-	-	-
	Pyrol ext	$Y = 96.4 - 3.612^{**}x$	96.4	0	0.849
	Citron ext	$Y = \bar{y} = 92.95$	-	-	-
	Horse ext	$Y = \bar{y} = 95.75$	-	-	-
	Captan Sc	$Y = \bar{y} = 85.25$	-	-	-
C2: Emergence (%)	Alfav ext	$Y = \bar{y} = 86.45$	-	-	-
	Garlic ext	$Y = 77.43 + 8.021^{**}x - 1.3214^{**}x^2$	89.6	3.03	0.821
	Pyrol ext	$Y = 81.9 - 5.463^{**}x$	81.9	0	0.808
	Citron ext	$Y = 90.6 - 3.913^{**}x$	90.6	0	0.573
	Horse ext	$Y = 82.85 - 2.037^{**}x - 0.2813^{**}x^2$	71.79	3.62	0.868
	Captan Sc	$Y = \bar{y} = 85.25$	-	-	-

Research data. Alfav ext - alfavaca extract; Garlic ext - garlic extract; Pyrol ext - Pyroligneous extract; Citron ext - Citronella Extract; Horse ext - Horsetail Extract. * Significant at 5% of probability. ** Significant at 1% of probability.

Table 3. Fitted regression equations for First germination count (%) and Traditional germination (%) under the doses (x) for the respective products, cultivars and the determination coefficients.

Variables	Products	Adjusted equations	Maximum efficiency	Dose (mg kg^{-1})	R^2
C1: First germination count (%)	Alfav ext	$Y = 76.35 + 1.137^{*}x$	85.45	8	0.684
	Garlic ext	$Y = 81.75 - 1.375^{**}x$	81.75	0	0.773
	Pyrol ext	$Y = 84.65 - 4.638^{**}x$	84.65	0	0.897
	Citron ext	$Y = 83.45 - 2^{**}x$	83.45	0	0.542
	Horse ext	$Y = 78.46 - 0.314^{**}x - 0.1607^{**}x^2$	77.98	1	0.492
	Captan Sc	$Y = \bar{y} = 81.25$	-	-	-
C2: First germination count (%)	Alfav ext	$Y = 74.8 + 1.425^{**}x$	86.2	8	0.891
	Garlic ext	$Y = \bar{y} = 71.6$	-	-	-
	Pyrol ext	$Y = 79.95 - 0.575^{**}x$	79.95	0	0.444
	Citron ext	$Y = 82.1 - 3.55^{**}x$	82.1	0	0.804
	Horse ext	$Y = 75.73 + 3.271^{*}x - 0.3214^{*}x^2$	84.24	5.1	0.722
	Captan Sc	$Y = \bar{y} = 78.25$	-	-	-
C1: Traditional germination (%)	Alfav ext	$Y = 81.75 + 1.075^{*}x$	90.35	8	0.783
	Garlic ext	$Y = 84.95 - 1.237^{**}x$	84.95	0	0.643
	Pyrol ext	$Y = 82.34 - 0.661^{**}x - 0.0268^{**}x^2$	70.15	12.3	0.486
	Citron ext	$Y = 85.45 - 0.912^{**}x$	85.45	0	0.358
	Horse ext	$Y = 81.16 + 5.211^{**}x - 1.0982^{**}x^2$	87.34	2.4	0.963
	Captan Sc	$Y = \bar{y} = 81.52$	-	-	-
C2: Traditional germination (%)	Alfav ext	$Y = \bar{y} = 86.3$	-	-	-
	Garlic ext	$Y = \bar{y} = 78.4$	-	-	-
	Pyrol ext	$Y = 82.61 + 3.298^{**}x - 0.692^{**}x^2$	86.54	2.4	0.916
	Citron ext	$Y = 89.80 - 1.021^{*}x + 0.0714^{*}x^2$	86.15	7.15	0.556
	Horse ext	$Y = \bar{y} = 85.25$	-	-	-
	Captan Sc	$Y = \bar{y} = 83.6$	-	-	-

Research data. Alfav ext - alfavaca extract; Garlic ext - garlic extract; Pyrol ext - Pyroligneous extract; Citron ext - Citronella Extract; Horse ext - Horsetail Extract. * Significant at 5 of probability. ** Significant at 1% of probability.

For cultivar 2, horsetail, garlic and Alfavaca extracts did not affect germination, citronella extract reduced the percentagem of germination and the extract of pyroligneous acid at a dose of 2 mg kg^{-1} increased germination, while higher doses reduced germination.

The extracts of Alfavaca, horsetail and citronella proved to be efficient for increasing the vigor of the seeds through accelerated aging (Table 4), and the horsetail extract resulted in 98% germination with the dose of 4 mg kg^{-1}, the extracts of garlic and pyroligneous acid were beneficial up to a dose of 4 mg kg^{-1}. After the test, the seeds of cultivar 2 treated with horsetail extract showed a higher percentage of germination than the seeds treated with the other extracts. The pyroligneous extract was efficient in increasing the germination of the seeds up to a dose of 4 mg kg^{-1}. Alfavaca and citronella extracts increased germination as the dose increases.

Seedling length was not affected by horsetail, garlic and citronella extracts. The only extract that enhanced in gains in seedling length was the Alfavaca extract up to the dose of 4 mg kg^{-1}. For cultivar 2, the extracts that promoted an increase in seedling length were citronella and pyroligneous acid at a dose of 4 mg kg^{-1} and 2 mg kg^{-1}, respectively. The other extracts did not affect the seedling length.

The extracts used did not increase the seedling vigor index and nor the seedling growth index (Table 5). For cultivar 2, only citronella extract at a dose of 4 mg kg^{-1} increased the seed vigor index. The pyroligneous acid extract decreased the vigor index of the seeds as the doses increased. For the growth index, only the citronella and pyroligneous acid extracts had significant effects with quadratic equations, with a dose of 2 mg kg^{-1} for the pyroligneous extract and 4 mg kg^{-1} for the citronella extract, this latter was shown superior to the other extracts.

The only plant extract that affected the uniformity index was the extract of Alfavaca, in which 2 mg kg^{-1} was more appropriate. Higher doses of this extract decreased the uniformity index (Table 6). The other plant extracts did not affect the uniformity indices evaluated by image analysis method. For cultivar 2, the citronella extract decreased the uniformity index and the other extracts had no significant effects.

The plant extracts did not affect the germination evaluated by the image analysis method for cultivar 1, however, the germination percentage was above 80% for the extracts of Alfavaca, horsetail and citronella. For Cultivar 2, the percentage of germination per image increased as the dose increased and exceedied 80% germination at the dose of 8 mg kg^{-1}. Pyroligneous acid extract at a dose of 8 mg kg^{-1} exceeds 90% germination by the image analysis method. The other extracts had no significant effect.

The simple correlation coefficients (Figure 1) showed that the results generated by the traditional tests and the image system were significantly correlated for some variables, showing that the data obtained by the Vigor-S® software accurately determined the seed quality, as there was a strong dependence between them.

Table 4. Fitted regression equations for Accelerated aging (%) and Seedling length (cm) under the doses (x) for the respective products, cultivars and the determination coefficients.

Variables	Products	Adjusted equations	Maximum efficiency	Dose (mg kg^{-1})	R^2
C1: Accelerated aging (%)	Alfav ext	$Y = 80.15 + 1.165^{**}x$	89.47	8	0.636
	Garlic ext	$Y = 78.09 + 4.039^{**}x - 0.4018^{**}x^2$	88.24	5	0.457
	Pyrol ext	$Y = 75.05 + 7.363^{**}x - 1.0312^{**}x^2$	88.19	3.6	0.788
	Citron ext	$Y = 80.1 + 1.35^{**}x$	90.9	8	0.684
	Horse ext	$Y = 73.43 + 1.996^{**}x - 0.1339^{**}x^2$	80.87	7.4	0.845
	Captan Sc	$Y = \bar{y} = 78.25$	-	-	-
C2: Accelerated aging (%)	Alfav ext	$Y = 72.31 + 3.761^{*}x - 0.2857^{*}x^2$	73.04	6.6	0.756
	Garlic ext	$Y = \bar{y} = 79.8$	-	-	-
	Pyrol ext	$Y = 79.54 + 2.357^{*}x - 0.2321^{*}x^2$	85.52	5	0.428
	Citron ext	$Y = 78 + 1.1425^{*}x$	87.14	8	0.750
	Horse ext	$Y = 78.84 + 5.095^{**}x - 0.8259^{**}x^2$	86.7	3.1	0.757
	Captan Sc	$Y = \bar{y} = 80.20$	-	-	-
C1: Seedling length (cm)	Alfav ext	$Y = 4.82 + 0.266^{**}x - 0.042^{**}x^2$	5.24	3.2	0.912
	Garlic ext	$Y = \bar{y} = 4.67$	-	-	-
	Pyrol ext	$Y = 4.96 - 0.115^{**}x$	4.96	0	0.836
	Citron ext	$Y = \bar{y} = 4.96$	-	-	-
	Horse ext	$Y = \bar{y} = 4.86$	-	-	-
	Captan Sc	$Y = \bar{y} = 3.85$	-	-	-
C2: Seedling length (cm)	Alfav ext	$Y = \bar{y} = 5.07$	-	-	-
	Garlic ext	$Y = \bar{y} = 4.72$	-	-	-
	Pyrol ext	$Y = 4.98 + 0.19^{**}x - 0.0384^{**}x^2$	5.21	2.5	0.932
	Citron ext	$Y = 5.04 + 0.02^{*}x - 0.0058^{*}x^2$	5.06	1.7	0.612
	Horse ext	$Y = \bar{y} = 5.1$	-	-	-
	Captan Sc	$Y = \bar{y} = 3.96$	-	-	-

Research data. Alfav ext - alfavaca extract; Garlic ext - garlic extract; Pyrol ext - Pyroligneous extract; Citron ext - Citronella Extract; Horse ext - Horsetail Extract. * Significant at 5% of probability. ** Significant at 1% of probability.

Table 5. Fitted regression equations for Vigor index and Growth index under the doses (x) for the respective products, cultivars and the determination coefficients.

Variables	Products	Adjusted equations	Maximum efficiency	Dose (mg kg^{-1})	R^2
C1: Vigor index	Alfav ext	$Y = 546.36 - 8.829^{*}x$	546.36	0	0.798
	Garlic ext	$Y = \bar{y} = 531.44$	-	-	-
	Pyrol ext	$Y = 544.56 - 14.593^{**}x$	544.56	0	0.846
	Citron ext	$Y = \bar{y} = 514.65$	-	-	-
	Horse ext	$Y = \bar{y} = 529.61$	-	-	-
	Captan Sc	$Y = \bar{y} = 528.46$	-	-	-
C2: Vigor index	Alfav ext	$Y = \bar{y} = 555.84$	-	-	-
	Garlic ext	$Y = \bar{y} = 524.25$	-	-	-
	Pyrol ext	$Y = 547.22 - 9.299^{*}x$	547.22	0	0.845
	Citron ext	$Y = 547.34 - 10.966^{*}x + 1.3424^{*}x^2$	524.9	4.1	0.468
	Horse ext	$Y = \bar{y} = 540.52$	-	-	-
	Captan Sc	$Y = \bar{y} = 520.45$	-	-	-
C1: Growth index	Alfav ext	$Y = \bar{y} = 434.55$	-	-	-
	Garlic ext	$Y = \bar{y} = 408.65$	-	-	-
	Pyrol ext	$Y = 448.73 - 16.119^{*}x$	448.73	0	0.815
	Citron ext	$Y = \bar{y} = 425.03$	-	-	-
	Horse ext	$Y = \bar{y} = 525.62$	-	-	-
	Captan Sc	$Y = \bar{y} = 420.62$	-	-	-
C2: Growth index	Alfav ext	$Y = \bar{y} = 456.79$	-	-	-
	Garlic ext	$Y = \bar{y} = 427.10$	-	-	-
	Pyrol ext	$Y = 442.65 + 14.163^{*}x - 1.5616^{*}x^2$	474.76	4.5	0.364
	Citron ext	$Y = 435.32 + 16.806^{**}x - 3.4902^{**}x^2$	455.55	2.4	0.810
	Horse ext	$Y = \bar{y} = 458.79$	-	-	-
	Captan Sc	$Y = \bar{y} = 425.62$	-	-	-

Research data. Alfav ext - alfavaca extract; Garlic ext - garlic extract; Pyrol ext - Pyroligneous extract; Citron ext - Citronella Extract; Horse ext - Horsetail Extract. * Significant at 5% of probability. ** Significant at 1% of probability.

Table 6. Fitted regression equations for Uniformity index and Image Germination (%) under the doses (x) for the respective products, cultivars and the determination coefficients.

Variables	Products	Adjusted equations	Maximum efficiency	Dose (Mg kg^{-1})	R^2
C1: Uniformity index	Alfav ext	$Y = 743.23 + 30.391^{**}x - 6.5295^{**}x^2$	778.6	2.3	0.779
	Garlic ext	$Y = \bar{y} = 763.52$	-	-	-
	Pyrol ext	$Y = \bar{y} = 746.2$	-	-	-
	Citron ext	$Y = \bar{y} = 763.25$	-	-	-
	Horse ext	$Y = \bar{y} = 756.67$	-	-	-
	Captan Sc	$Y = \bar{y} = 425.25$	-	-	-
C2: Uniformity index	Alfav ext	$Y = \bar{y} = 778.8$	-	-	-
	Garlic ext	$Y = \bar{y} = 725.59$	-	-	-
	Pyrol ext	$Y = \bar{y} = 752.62$	-	-	-
	Citron ext	$Y = 776.72 - 5.505^{**}x$	776.72	0	0.779
	Horse ext	$Y = \bar{y} = 754.58$	-	-	-
	Captan Sc	$Y = \bar{y} = 720.62$	-	-	-
C1: Image Germination (%)	Alfav ext	$Y = \bar{y} = 86.65$	-	-	-
	Garlic ext	$Y = \bar{y} = 74.9$	-	-	-
	Pyrol ext	$Y = 88.16 - 11.82^{**}x - 1.4509^{**}x^2$	88.16	0	0.969
	Citron ext	$Y = 86.1 - 0.162^{**}x - 0.0313^{**}x^2$	85.5	2.6	0.429
	Horse ext	$Y = \bar{y} = 83.6$	-	-	-
	Captan Sc	$Y = \bar{y} = 80.25$	-	-	-
C2: Image Germination (%)	Alfav ext	$Y = \bar{y} = 85.45$	-	-	-
	Garlic ext	$Y = \bar{y} = 77.65$	-	-	
	Pyrol ext	$Y = 81.41 - 12.357^{**}x + 1.7321^{**}x^2$	93.4	8	0.713
	Citron ext	$Y = 82.6 + 0.05^{*}x$	83	8	0.201
	Horse ext	$Y = \bar{y} = 80.85$	-	-	-
	Captan Sc	$Y = \bar{y} = 80.25$	-	-	-

Research data. Alfav ext - alfavaca extract; Garlic ext - garlic extract; Pyrol ext - Pyroligneous extract; Citron ext - Citronella Extract; Horse ext - Horsetail Extract. * Significant at 5% of probability. ** Significant at 1% of probability.

The variables of the traditional test had a positive correlation with variables of the image analysis test, as can be seen in the Vigor Index of the image test, which was positively correlated with traditional Germination (TG), First Germination Count (FGC), dry matter (DM), among others.

The accelerated aging test (AA) (Table 6), which is considered one of the most sensitive to indicate the best lot and/or cultivar for the vigor test, showed significant positive and negative correlations, respectively, with the variables obtained by image analysis which was performed 2 days after sowing (Figure 1). This indicates the efficiency of the Vigor-S® software in assessing the physiological potential of cowpea seeds compared to the traditionally used tests recommended by RSA.

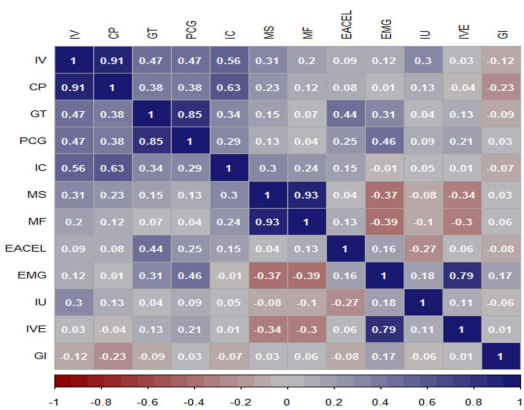

Figure 1. Pearson correlations between the variables studied by the traditional and image methods of physiological analysis of cowpea seeds. Vigor index (IV); Seedling length (CP); Traditional germination (GT); First germination count (PGC); Growth index (IC); Seedling dry matter (MS); Seedling fresh matter (MF); Accelerated aging (EACEL); Emergence (EMG); Uniformity index (IU); Emergence speed index (IVE); Germination by the image analysis method (GI).

The use of the Vigor-S® software for computerized analysis of seedling images allows the evaluation the physiological quality of cowpea seeds taking into account several parameters, which can be explored in a positive way. An example is the seedling length analysis by the Vigor-S® software seen in Figure 2, in which the primary root of each seedling is marked in red and the hypocotyl in blue. The analysis results are presented on the processing screen as shown in the figure and can still be exported to Excel.

Vigor-S® is a system programmed to perform computerized analysis for soybean and corn crops, however, in this experiment, it can be seen that it proved to be efficient for evaluating the physiological seed potential for the cowpea crop in the various parameters proposed by the program, such as size and pixel of hypocotyl and root, total length and the root fipocotyl ratio.

This program can be used to analyze the germination, seed vigor, growth index, uniformity index, medium length as can be seen in the Figure 2 and everything can be verified two days after sowing, which is an advantage.

Searching for relevant information from data from experiments, the pattern recognition methods are highlighted, among them, the principal component analysis (PCA). The first three components explained more than 80% total variability of the samples and were distributed in PC1 (29.1%), PC2 (22.6%) and PC3 (29.1%), as can be seen in Figure 3.

These components have the following representation: PC1 = mean index of PCG, GT, IV, and IC, Related to seed germination; PC2 = IVE, EMG versus MF and MS, Heavier seeds, lower IVE and EMG; and PC3 = EACEl vs UI.

The first component explained 29.1% data variability, while the second explained 22.6% and the third explained 29.1%, adding up to 80.8% of the total samples. In this sense, it can be considered that the two-dimensional plot presented here is adequate to assess the relationships between the variables, since it explains a large part of the data variability – it is usually a sun greater than 50%, in the sum of the components, is considered adequate to use the map (two-dimensional plot).

Figure 2. Screen of the Vigor-S® analysis system software with a cowpea sample (BRS Tumucumaque) two days after sowing the germination test (A); seedling detail identifying the primary root (red) and the hypocotyl (blue) (B).

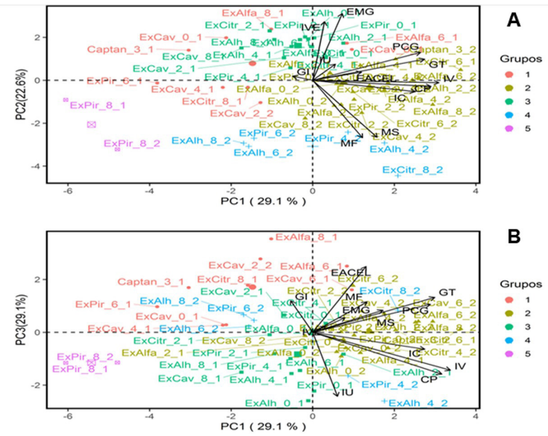

Figure 3. Principal component analysis for the triple interaction Product x Doses x Cultivars in cowpea crop; Garlic extract - ExtAlh; Pyroligneous Acid - ExPir; Alfavaca extract - ExAlfa; Citronella extract - ExtCitr; Horsetail extract – ExCav; Image Germination – GI; Accelerated aging – EACEL; Dry matter – MS; Fresh matter – MF; Traditional germination – GT; First germination count – PCG; Growth index – IC; Vigor index – IV; Seedling length – CP; and Uniformity index – IU.

The germination assessed by image analysis (GI) is negatively correlated with the others, mainly with seedling length, growth index and vigor index. This shows that the lower the IG value, the higher are the values of growth, vigor indices and seedling length that are computerized by image, corroborating that the Vigor-S® software can be used to analyze the vigor of cowpea seeds.

As can be seen in Figure 3, the results were positively correlated when the angle between them is acute, that is, they are less than or equal to 90º. Thus, both in Figure 3A and 3B, the variables obtained by image analysis are correlated, except for germination (IG). In contrast, for the variables of the traditional tests, many of the data are greater than 90º, which means that the correlation between them is very low as ESI and EMG compared with FM and DM (Figure 3A). In Figure 3B, the variables of the traditional test had high correlations with each other.

Regarding the extracts used to improve the physiological quality of cowpea seeds, the principal component analysis conducted the cluster analysis taking into account the correlation between the samples, where they were divided into 5 groups. The leading group is group 1 where it can be seen that the Alfavaca extract is the best product for the analysis of physiological quality of cowpea seeds, in which the best dose was 8 mg kg^{-1} (Figure 3A and 3B), its result was superior to the positive control (Captan®) and the negative control (dose 0).

The extracts of horsetail and Alfavaca, both at the dose of 6 mg kg^{-1}, from the same group (Table 3) had the best results with positive correlations. These results corroborate with the regression graphs for traditional germination, where the best products were the extracts of Alfavaca, in the highest dose, pyroligneous acid at the dose 2 mg kg^{-1} and horsetail at the 4 mg kg^{-1} (Table 3). It is also observed that for germination evaluated by image analysis, where the extracts did not affect germination, but obtained a percentage of germination above 80% for the extracts of Alfavaca, horsetail and citronella and for cultivar 2 (Table 6), the citronella and pyroligneous acid extracts exceed 80 and 90% germination, respectively, corroborating the principal component analysis.

4. Discussion

To improve the physiological quality of seeds, several products are used. Among them, natural products extracted from plants are a viable alternative for this purpose. For the fresh and dry matter variable, the best was the horsetail extract (cultivar 1) and Alfavaca (cultivar 2). Studies show that horsetail extract has the potential to activate the plant defense response in soybean seeds, besides a fungistatic action (Guimarães et al., 2015). Alfavaca extract has biologically active substances that are used naturally as an insecticide, nematicide and fungicide (Pereira and Maia, 2007).

Similar results were reported by Silva et al. (2019), who observed that garlic and citronella extracts did not affect the transfer of reserves from the seed to the seedling. It is worth mentioning that the aforementioned authors analyzed the effect of plant extracts on rice seeds.

Alfavaca extract and pyroligneous extract increased the emergence speed for Cultivar 1 and garlic and horsetail extracts increased the emergence percentage up to a dose of 4 mg kg^{-1} (Table 2). The use of medicinal plant extracts becomes a viable alternative for organic producers, since their performance in improving the physiological quality of seeds is similar to the performance of commercial products that cannot be used in organic crops (Silva et al., 2019; Radünz et al., 2022).

Similar results were reported by Araújo et al. (2019), who evaluated the influence of garlic extract on the physiological quality of seeds of *Chorisia glaziovii* O. Kuntze, and concluded that garlic extract inhibited the development of the root and shoot of the seedlings, negatively affecting the physiological quality of seeds.

In relation to the pirolenhoso extract, the explanation for the best results in the emergency and speed index, are in the effects of these extracts, among them, stimulating effect, insecticide, fungicide, besides acting in the nutritional functions of the vegetable (Castanho et al., 2011).

Garlic extract improves the physiological quality of seeds as it has been used to control pathogens in maize, showing positive results for several pathogens, such as *Aspergillus flavus, Fusarium moniliforme* and *F. proliferatum* (Catão et al., 2013) and with that the percentage of emergence increases.

The citronella extract increased the vigor index and growth (Table 5) of cowpea seed while the Alfavaca extract obtained a better index of uniformity at the dose 2 mg kg^{-1}. This shows that natural extracts of Citronella improves the vigor and growth of the seed because this extract has the function of inhibiting the mycelial growth of several types of fungi such as *Colleotrichum graminicola* DJ Politis (Sarmento-Brum et al., 2013).

As can be seen in Figure 1, there is a positive correlation between the variables of the traditional test and the variables of the image test. The correlation between traditional tests and image analysis tests is extremely important and very valuable for adjusting the model (Gonçalves et al., 2017). Therefore, the positive correlation with the vigor tests and makes it possible to use the indices generated by the image analysis tests to assess the seed vigor (Leão-Araújo et al., 2017).

The Vigor-S® software allows for having several significant vigor variables, which allows a better organization, treatment and interpretation of the results obtained (Medeiros et al., 2019). The analysis of images by the Vigor-S® software (Fig. 2) allows checking the vigor of the seed in record time, as it can be used two days after sowing, demonstrating that the referred program contributes positively by reducing errors that occur in traditional methods due to incorrect handling due to the evaluation of a large number of seed lots (Castan et al., 2018).

Similar fact also observed by Rodrigues et al. (2020) in soybean seeds, where the use of Vigor-S® allowed the determination of lots with greater vigor and physiological potential. As well as, Rego et al. (2021) in cowpea seeds, where the automated analysis provided a positive correlation with the traditional vigor tests. Leite et al. (2020) found that automated analysis with Vigor-S® was effective in determining the physiological potential of melon seeds.

The planning and optimization of the experiments, the comparison of models as is the case of this study that seeks to correlate the traditional model with the computational image analysis, have been studied today in several crops. In this sense, looking for the study of covariance and correlation between data, multivariate analysis can be applied in addition to image processing (Solomon and Breckon, 2011).

The analysis of main components corroborated with the traditional methods and image analysis showing that the two methods have very close results. PCA is a multivariate analysis technique used to analyze the interrelationships between a wide range of data (variables) and apply them in terms of their dimensions (components) with the least possible loss of information and their variations are explained along the axes of the Cartesian plane (Hongyu, 2015).

It was possible to find 80.8% of the sum of the first three main components, which is more than 50% which is recommended. In a study with poultry, Paiva et al. (2010) used 11 production characteristics, and found that only three main components were sufficient to explain 77% total variance of the characteristics. Meira et al. (2013) evaluated 13 morphofunctional characteristics of Brazilian Saddle Horses and obtained 6 main components that explained 78.57% total variance.

With the aid of Principal Component Analysis, it was found that the imaging method was adequate to assess the physiological quality of cowpea seeds as well as the best products used as group I Alfavaca extract. PCA is a multivariate analysis very efficient to check the physiological quality of seeds. Barbosa et al. (2013) and Silva et al. (2017), in studies of seed vigor tests have also found a better adequacy in multivariate analysis, as this analysis is superior to univariate analysis in identifying the most promising vigor tests when a large number of lots and parameters are compared.

5. Conclusions

Horsetail and Alfavaca extracts increased the physiological quality of cowpea seeds of both cultivars. The citronella extract was efficient to increase the seed quality only for the cultivar BRS Guariba.

The computerized image analysis of cowpea seedlings with the Vigor-S® software proved efficient compared to the traditional test to assess the physiological quality of seeds and can be used to assess the quality of cowpea seeds.

Principal Component Analysis proved adequate to identify the best extracts and doses in assessing the physiological quality of cowpea seed, as well as the use of the image analysis method, which in this experiment proved effective when compared to the traditional test and can thus be used.

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References

ARAÚJO, A.K.O., SILVA, M.L.M., GOMES, R.S.S., SANTOS, A.M.S. and NASCIMENTO, L.C., 2019. Sanidade e qualidade fisiológica de sementes de *Chorisia Glaziovii* O. Kuntze tratadas com extratos vegetais. *Ciência Florestal*, vol. 29, no. 2, pp. 649-659. http://dx.doi.org/10.5902/1980509826140.

BARBOSA, R.M., VIEIRA, B.G.T.L., FERRAUDO, A.S., CORÁ, J.E. and VIEIRA, R.D., 2013. Discrimination of soybean seed lots by multivariate exploratory techniques. *Journal of Seed Science*, vol. 35, no. 3, pp. 302-310. http://dx.doi.org/10.1590/S2317-15372013000300005.

BARDIN, M., AJOUZ, S., COMBY, M., LOPEZ-FERBER, M., GRAILLOT, B., SIEGWART, M. and NICOT, P.C., 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Frontiers in Plant Science*, vol. 6, pp. 566. http://dx.doi.org/10.3389/fpls.2015.00566. PMid:26284088.

BARROS, F.R., ANUNCIAÇÃO FILHO, C.J., ROCHA, M.M., NUNES, J.A.R., SILVA, K.J.D., FREIRE FILHO, F.R. and RIBEIRO, V.Q.R., 2011. Potencial genético de progênies de feijãocaupi segregantes quanto ao tipo da inflorescência. *Pesquisa Agropecuária Brasileira*, vol. 46, no. 2, pp. 182-189. http://dx.doi.org/10.1590/S0100-204X2011000200010.

BRASIL. Ministério da Agricultura – MAPA, 2009. *Regras para análise de sementes*. MAPA/ACS, Brasília.

CARBONI, R.Z. and MAZZONETTO, F., 2013. Efeito do extrato aquoso de diferentes espécies vegetais no manejo de *Meloidogyne incognita* em tomateiro em ambiente protegido. *Revista Agrogeoambiental*, vol. 5, pp. 61-66. http://dx.doi.org/10.18406/2316-1817v5n22013496.

CASTAN, D.O.C., GOMES-JUNIOR, F.G. and MARCOS-FILHO, J., 2018. Vigor-S, a new system for evaluating the physiological potential of maize seeds. *Scientia Agrícola*, vol. 75, no. 2, pp. 167-172. http://dx.doi.org/10.1590/1678-992x-2016-0401.

CASTANHO, R.M., BRITO, J.O., and ALMEIDA, R., 2011. Levantamento bibliográfico sobre uso de pirolenhoso na agricultura brasileira. In: *19 SIICUSP – Simpósio Internacional de Iniciação Cientifica*, 24-25 September 2011, Piracicaba. Piracicaba: ESALQ.

CATÃO, H.C.R.M., MAGALHÃES, H.M., SALES, N.L.P., BRANDÃO JÚNIOR, D.S. and ROCHA, F.S., 2013. Incidência e viabilidade de sementes crioulas de milho naturalmente infestadas com fungos em pré e pós-armazenamento. *Ciência Rural*, vol. 43, no. 5, pp. 764-770. http://dx.doi.org/10.1590/S0103-84782013000500002.

COMPANHIA NACIONAL DE ABASTECIMENTO – CONAB, 2022. *Crop 2020/2021 and 2021/2022 Grain production estimate*. Brasília: CONAB.

COSTA, P.S., FERRAZ, R.L.S., DANTAS NETO, J., MARTINS, V.D., VIÊGAS, P.R.A., MEIRA, K.S., NDHLALA, A.R., AZEVEDO, C.A.V. and MELO, A.S., 2022. Seed priming with light quality and *Cyperus rotundus* L. extract modulate the germination and initial growth of *Moringa oleifera* Lam. seedlings. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, no. e255836, pp. e255836. http://dx.doi.org/10.1590/1519-6984.255836. PMid:35507962.

FANTINEL, V.S., OLIVEIRA, L.M., CASA, R.T., ROCHA, E.C., SCHNEIDER, P.F., POZZAN, M., LIESCH, P.P. and RIBEIRO, R.A., 2017. Fungos associados às sementes de *Acca sellowiana*: efeitos na qualidade fisiológica das sementes e transmissão. *Agrarian*, vol. 10, no. 38, pp. 328-335. http://dx.doi.org/10.30612/agrarian.v10i38.4509.

FARIAS, O.R., CRUZ, J.M.F.L., VELOSO, J.S., DUARTE, I.G., BARBOSA, P.R.R., FÉLIX, M.R.F., VARANDA, C.M.R., MATERATSKI, P., OLIVEIRA, M.D.M., and NASCIMENTO, L.C., 2022. Occurrence of Fusarium proliferatum causing vascular wilton cowpea (*Vigna unguiculata*) in Brazil. *Plant Disease,* vol. 106, no. 7, pp. 1773-2006. https://doi.org/10.1094/PDIS-04-21-0839-PDN.

FONTES, L.S.I., PINENT, S.M.J., LIMA, É.F.B. and SUCUPIRA, I.G., 2011. First register of occurrence of *Frankliniella schultzei* (Trybom, 1910) (Thysanoptera: Thripidae) in Cowpea (*Vigna unguiculata* (L.) Walp.) in the state of Piauí, Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 71, no. 4, pp. 1023-1024. http://dx.doi.org/10.1590/S1519-69842011000500023.

GONÇALVES, N.R., CICERO, S.M. and ABUD, H.F., 2017. Seedling image analysis and traditional tests to evaluate onion seed vigor. *Journal of Seed Science*, vol. 39, no. 3, pp. 216-223. http://dx.doi.org/10.1590/2317-1545v39n3160444.

GUIMARÃES, S.S., MAZARO, S.M., FREDDO, Á.R. and WAGNER JÚNIOR, A., 2015. Potencial de preparados de cavalinha (*Equisetum* sp.) na síntese de metabólitos de defesa em cotilédones de soja (*Glycine max* L.) e o efeito sobre o crescimento de *Rhizoctonia solani* Kuhn, in vitro. *Revista Brasileira de Plantas Medicinais*, vol. 17, no. 1, pp. 143-149. http://dx.doi.org/10.1590/1983-084X/12_190.

HONGYU, K., 2015. *Comparação do GGEbiplot ponderado e AMMI-ponderado com outros modelos de interação genótipo × ambiente*. Piracicaba: Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 154 p. Tese de Doutorado em Agronomic Statistics and Experimentation.

LIMA, C.S., 2017. Manejo de doenças. In: J.C. VALE, C. BERTINI and A. BORÉM, eds. *Feijão caupi: do plantio à colheita*. Viçosa: Ed. UFV.

LEÃO-ARAÚJO, E.F., FARIA, J., BARBOZA, C., MARCOS-FILHO, J. and VIEIRA, R.D., 2017. Teste de deterioração controlada e uso do sistema de imagem Seed Vigor (SVIS®) para avaliar o potencial fisiológico de sementes de crambe. *Journal of Seed Science*, vol. 39, pp. 393-400. http://dx.doi.org/10.1590/2317-1545v39n4177911.

LEITE, M.S., TORRES, S.B., GOMES JÚNIOR, F.G., REGO, C.H.Q., PAIVA, E.P. and LEITE, T.S., 2020. Viability of seedling image analysis (Vigor-S) to determine the physiological potential of melon seeds. *Journal of Seed Science*, vol. 42, pp. e202042043. http://dx.doi.org/10.1590/2317-1545v42237826.

MAGUIRE, J.D., 1962. Speed of germination-aid selection and evaluation for seedling emergence and vigor. *Crop Science*, vol. 2, no. 2, pp. 176-177. http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x.

MARQUES, F.R.F., MEIADO, M.V., CASTRO, N.M.C.R., CAMPOS, M.L.O., MENDES, K.R., SANTOS, O.O. and POMPELLI, M.F., 2015. GerminaQuant: uma nova ferramenta para medições de germinação. *Journal of Seed Science*, vol. 37, pp. 248-255. http://dx.doi.org/10.1590/2317-1545v37n3145605.

MEDEIROS, A.D. and PEREIRA, M.D., 2018. SAPL®: um software livre para determinar o potencial fisiológico em sementes de soja. *Pesquisa Agropecuária Tropical*, vol. 48, pp. 222-228. http://dx.doi.org/10.1590/1983-40632018v4852340.

MEDEIROS, A.D., SILVA, L.J., CAPOBIANGO, N.P., FIALHO, C.A. and DIAS, D.C.F.S., 2019. Assessing the physiological quality of common bean seeds using the Vigor-S® system and its relation to the accelerated aging test. *Journal of Seed Science*, vol. 41, no. 2, pp. 187-195. http://dx.doi.org/10.1590/2317-1545v41n2211401.

MEIRA, C.T., PEREIRA, I.G., FARAH, M.M., PIRES, A.V., GARCIA, D.A. and CRUZ, V.A.R., 2013. Seleção de características morfofuncionais de cavalos da raça Manga larga Marchador por meio da análise de componentes principais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, vol. 65, no. 6, pp. 1843-1848. http://dx.doi.org/10.1590/S0102-09352013000600036.

MELO, L.F., SILVA, T.P., PEREIRA, J.S., VALE, J.C. and BERTINI, C.H.C.M., 2021. Selection index for recommendation of cowpea cultivars for green bean production. *Revista Ciência Agronômica*, vol. 52, no. 3, pp. e20207256. http://dx.doi.org/10.5935/1806-6690.20210043.

NUNES, L.R.L., PINHEIRO, P.R. and DUTRA, A.S., 2019. Potencial fisiológico de sementes de feijão-caupi submetidas à pré-hidratação. *Revista de Agricultura Neotropical*, vol. 6, no. 1, pp. 54-59. http://dx.doi.org/10.32404/rean.v6i1.2646.

PAIVA, A.L.C., TEIXEIRA, R.B., YAMAKI, M., MENEZES, G.R.O., LEITE, C.D.S. and TORRES, R.A., 2010. Análise de componentes principais em características de produção de aves de postura. *Revista Brasileira de Zootecnia*, vol. 39, no. 2, pp. 285-288. http://dx.doi.org/10.1590/S1516-35982010000200009.

PEREIRA, C.A.M. and MAIA, J.F., 2007. Estudo da atividade antioxidante do extrato e do óleo essencial obtidos das folhas de alfavaca (*Ocimum gratissimum* L.). *Food Science and Tecnology*, vol. 27, no. 3, pp. 624-632. http://dx.doi.org/10.1590/S0101-20612007000300030.

R CORE TEAM, 2019. *R: A language and environment for statistical computing* [software]. Viena: R Foundation for Satatical Computing.

RADÜNZ, A.L., RADÜNZ, M., BIZOLLO, A.R., TRAMONTIN, M.A., RADÜNZ, L.L., MARIOT, M.P., TEMPEL-STUMPF, E.R., CALISTO, J.F.F., ZANIOL, F., ALBENY-SIMÕES, D., REZENDE, R.S. and DAL MAGRO, J., 2022. Insecticidal and repellent activity of native and exotic lemongrass on *Maize weevil. Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, no. e252990, pp. e252990. http://dx.doi.org/10.1590/1519-6984.252990. PMid:35416848.

REED, R.C., BRADFORD, K.J. and KHANDAY, I., 2022. Seed germination and vigor: ensuring crop sustainability in a changing climate. *Heredity*, vol. 128, no. 6, pp. 450-459. http://dx.doi.org/10.1038/s41437-022-00497-2. PMid:35013549.

REGO, C.H.Q., CICERO, S.M., FRANÇA SILVA, F. and GOMES JÚNIOR, F.G., 2021. Assessing the vigor of cowpea seeds using the Vigor-S software. *Journal of Seed Science*, vol. 43, pp. e202143018. http://dx.doi.org/10.1590/2317-1545v43244858.

RODRIGUES, M., GOMES-JÚNIOR, F.G. and MARCOS-FILHO, J., 2020. Vigor-S: system for automated analysis of soybean seed vigor. *Journal of Seed Science*, vol. 42, pp. e202042039. http://dx.doi.org/10.1590/2317-1545v42237490.

SARMENTO-BRUM, R.B.C., SANTOS, G.R., CASTRO, H.G., GONÇALVES, C.G., CHAGAS JÚNIOR, A.F. and NASCIMENTO, I.R., 2013. Efeito de óleos essenciais de plantas medicinais sobre a antracnose do sorgo effect of essential oils of medicinal plants on the anthracnose of Sorghum. *Bioscience Journal*, vol. 9, pp. 1549-1557.

SILVA, C.B., BARBOSA, R.M. and VIEIRA, R.D., 2017. Accelerated aging as vigor test for sunn hemp seeds. *Ciência Rural*, vol. 47, no. 1, pp. e20151527. http://dx.doi.org/10.1590/0103-8478cr20151527.

SILVA, I.N., CHRIST, A.J., SILVA, S.S., CARVALHO, J.W.P. and PASCUALI, L.C., 2019. Qualidade fisiológica de sementes de arroz tratadas com óleos essenciais e extratos vegetais. *Destaques Acadêmicos*, vol. 11, no. 3, pp. 259-271. http://dx.doi.org/10.22410/issn.2176-3070.v11i3a2019.2333.

SOLOMON, C. and BRECKON, T., 2011. *Fundamentals of digital image processing: a practical approach with examples in Matlab*. Weinheim: Wiley.

WENDT, L., MALAVASI, M.M., DRANSKI, J.A.L., MALAVASI, U.C. and GOMES-JUNIOR, F.G., 2017. Relação entre testes de vigor com a emergência a campo em sementes de soja. *Agrária*, vol. 12, no. 2, pp. 166-171. http://dx.doi.org/10.5039/agraria.v12i2a5435.