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Treatment of bean seeds with plant extracts for controlling *Zabrotes subfasciatus* and its effects on physical and physiological quality during storage

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Bean crop occupies a prominent place in medium and large farming units in Brazil. Most of the time, the success of this crop depends, among other factors, on the use of good quality seeds at sowing; which requires strict quality control, harvesting, and storage. The objective of this study is to evaluate the effect of leaves and shells extracts of *Aspidosperma pyrifolium* Mart., *Anadenanthera colubrina* and myrtle (*Licania rigida* benth) in controlling *Zabrotes subfasciatus* associated with bean seeds and to evaluate the seeds treated and stored in pet bottles for a period of 180 days. The experiments were conducted in the Agricultural Products Storage and Processing laboratory of the Federal University of Campina Grande. It was observed that for leave extracts, germination decreased with storage time for all the extracts and the best germination percentage (95.00%) occurred in 5 ml of *A. pyrifolium* Mart. leaves extract. These results found for the bark extract *A. pyrifolium* Mart. showed that germination was 94.00% in the seeds treated with 5 ml dose of this extract. On the other hand, the extracts that had lower efficiency on germination were the extract of myrtle leaf and bark which showed 64.00 to 65.40% germination in 180 days of storage. It is concluded that low percentages of infestation show the efficiency of the extracts tested in the control of insects. The efficiency of these extracts may be due to the action of their secondary metabolites; especially the extracts of *A. colubrina's* leaves and bark.

Key words: Bioactive plants, bean weevil, stored seeds.

INTRODUCTION

In recent years, Brazil is the world's third leading producer of beans (*Phaseolus vulgaris* L.), the

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largest producer is Myanmar, followed by India (Fao, 2013). In the scenario of the northeastern agricultural production, given the infrequent rainfall, beans, because of their short cycle and tolerance to water stress, holds special relevance in the food supply and the composition of family income. Due to the observation, that there is lower occurrence of losses and hand labor during seasonal periods, as in the case of cultivation in a second season crop, because of the use of wet or irrigated lowlands (Frota and Pereira, 2000).

Beans can lose their physical and physiological quality after harvest, especially if incorrectly stored. This can happen due to fungal contamination, insect infestation and/or metabolic processes that reduce germination and vigor, causing, among other defects; seed coat darkening (especially the Carioca group); due to oxidation of phenols in the presence of oxygen (Lazzari, 2005). Therefore, the adoption of conservation practices is critical to the maintenance of seed quality during storage, to prevent loss in germination and vigor, as well as pest attack.

The presence of insects causes qualitative and quantitative losses. The insects consume the substrates and the respiration of the insects creates hot spots. These hot spots cause increase in temperature and inter granular humidity which create an environment for fungal growth (Lazzari, 1997; Rupolho et al., 2006). The losses of grains and seeds, resulting from contaminated storage in Brazil, are 10% (Viebrantz et al., 2016).

The grains and seeds can rot and become useless for consumption due to the appearance, smell and taste. According to Lorini et al. (2002), reducing water content of the beans up to 13% by drying, helps reduce losses due to fungal attack. Although without chemical control, this does not prevent the presence of insect pests during storage. In this context, extracts of plants can be used as an alternative to control insects and may also be used together with other pest control practices. The advantages are their rapid biodegradation which reduces the environmental contamination risk; and they are easy to obtain.

Given the unavailability of technical information on the control of *Zabrotes subfasciatus* L. using natural insecticide produced by plants, this work aims to evaluate the effect of leaves and barks extracts (*Aspidosperma pyrifolium* Mart. and *Anadenanthera colubrina*) and myrtle (*Licania rigida* benth) in the control of *Z. subfasciatus* associated to seed bean. It also aims to evaluate the seed treated during storage in pet bottles for a period of 180 days.

MATERIALS AND METHODS

The experiment was conducted in Agricultural Products Storage and Processing Laboratory) of the Academic Unit of Agricultural Engineering (UAEA) at the Federal University of Campina Grande (UFCG) Paraíba. Bean seeds were obtained from producers of Belo Jardim, PE, Brazil and seeds were obtained in the 2010/2011 crop season.

The initial quality of the seeds was evaluated by the purity analysis and the determination of moisture content, germination, vigor and infestation percentage of beans by *Z. subfasciatus*.

The moisture content was conducted in accordance with the Rules for Testing Seeds (Brazil, 2009). Moisture content was determined with two replications of 10 g of seeds and by using the oven method at 105 for 24 h. After treatment, the seeds were packed in pet packaging and stored in laboratory conditions without temperature and relative humidity control for a period of 180. They were evaluated every 45 days for moisture content, germination and first count of germination infestation, and weight loss.

The procedures of germination were carried out according to the Rules of Seed Analysis (BRASIL, 2009), but 200 seeds were used per treatment instead of 400. In this case, four replicates of 50 seeds were used per treatment (leaf and bark extracts and doses of 0, 1, 2, and 3 ml). The seeds were placed on germitest paper and the paper rolls were kept in a growth chamber at 25°C. The first count (vigor test) was performed together with the germination test by counting the normal seedlings found on the fifth day after sowing. Final germination was evaluated at 9 days (Brasil, 2009).

The infestation of *Z. subfasciatus* present in the stored beans was evaluated by observing the seeds per 100 g. The damaged seeds were then separated from the intact ones and were calculated over the total number of samples. The calculation was performed using the equation suggested by Almeida and Villamil (2000).

The percentage of beans' weight loss under storage conditions was conducted using 100 g of intact seeds and 100 of damaged seeds, and then calculated by the equation suggested by Almeida and Villamil (2000).

$$PP = (I - D/I) \times 100$$

where PP is the weight loss (%), I is the seeds loss integrity (g), and D is the weight damaged seeds (g).

The experiment of the extracts of leaves were separated, weighed and dried in an oven with forced air circulation for 72 h. Then, the plant materials were ground separately and placed in amber glass container containing 8 L of ethanol at 70% for maceration under occasional shaking. The extracted liquid passed through a first filtration filter paper under reduced pressure and a second filtration by gravity to hopper closed with cotton. Therefore, the filtrate was concentrated on evaporator under reduced pressure at a temperature of ± 80°C, for separating ethanol. The obtained solution was placed in water bath at a constant temperature of 65°C to evaporate the water and obtain solid material (Costa et al., 2011).

The hydro alcoholic extracts were used in doses that are sufficient to kill the insects, within seven days of life. The extracts were applied by the pipetting method directly on the mass of beans. Homogenizing was done by manual agitation and one plot was used for control. Then, the seeds were spread on polyethylene trays for a period of 24 h at room temperature in order to increase extract absorption for the seed mass.

After this time, seeds were distributed in plastic pet containers, with capacity of 500 g which were infested with 30 adult insects of *Z. subfasciatus* each. Insect were collected from bean seeds which are stored in plastic bottles. They were multiplied after selection, separation and identification using a microscopic. Insects were inoculated into a mass of beans, previously purged, inside a glass container of 300 ml capacity, sealed at the top with organdy cloth to allow natural ventilation.

The same procedure was used for untreated seeds. They were then stored in laboratory without temperature conditions and relative humidity control, for a period of 180 days. After this period, the percentage of infested seeds, weight loss, germination and the moisture content were performed. The control did not receive

Table 1. Control of infestation (adult emergency) of *Zabrotes subfasciatus* using hydroalcoholic extracts of leaf and bark of *A. pyrifolium* mart, *A. colubrina* and *L. rigida* in inoculated and non-inoculated bean seeds after 180 days of storage in pet packaging.

Extracts	Doses (ml)			
	0	1	3	5
A. pyrifolium (leaves)	9.00^{bB}	13.68^{aA}	6.82^{aC}	9.22^{abB}
A. colubrina (leaves)	9.09^{bA}	7.11^{bB}	6.48^{aB}	7.45^{bcB}
L. rigida (leaves)	9.30^{bB}	13.46^{aA}	2.88^{bD}	7.07^{cC}
A. pyrifolium (barks)	11.99^{aA}	7.00^{bB}	3.96^{bC}	7.22^{bcB}
A. colubrina (barks)	9.39^{bA}	6.69^{bB}	6.34^{aB}	8.79^{abcA}
L. rigida (barks)	9.60^{bAB}	6.56^{bC}	7.95^{aBC}	10.64^{aA}

DMS in the columns = 2.13; DMS in the lines =1.92; Classific.c/lower case; Classific.c/capital letters.

treatment and was not infested (they did not received any hydro alcoholic solution)

The experiment was conducted in a completely randomized design, in which the experiments were arranged in a factorial design with four replications and the means were compared by Tukey test at 1 and 5% probability. Effects of quantitative factors were analyzed using regression analysis: linear and quadratic equations for all variables and their interactions were examined. The data were evaluated with the software ASSISTAT version 7.6 (Silva and Azevedo, 2009).

RESULTS AND DISCUSSION

The seeds were initially stored with 13.29% moisture content and 96% germination was observed. The results of the evaluation of the initial quality of the seeds prior to storage with respect to moisture content was in the range suggested for storage by Delouche et al. (1976), Popinigis (1985) and Carvalho and Nakagawa (2000).

According to the results shown in Table 1, it appears that the infestation reduced from 9.49 (control) to 6.82, 6.48 and 2.88% with a dose of 3 ml of the leaves extracts of *A. pyrifolium* Mart., *A. colubrina* and *L. rigida*, respectively and similar behavior was observed in extracts from barks of the same species. It can be noted that the 3 ml dose was the best for controlling insect infestation and extracts from *L. rigida* leaf and bark of *A. pyrifolium* Mart. showed the best results in controlling the infestation of *Z. subfasciatus* present in seed mass of stored beans. It was also observed that extracts of *L. rigida* (2.88) and *A. pyrifolium* Mart. barks (3.96) in 3 ml dose, showed no statistic difference in controlling insect infestation.

The low percentages of insect infestation in controlled bean seeds, reveal the efficiency of the extracts used in the control of insect infestations. This efficiency is probably due to its secondary metabolite action, especially in *Anadenanthera colubrine* extracts (bark or leaf); which it does by leaving a thin protective waterproof film. This thin lubricant layer serves as a barrier to insect. According to Silva et al. (2013), repellent effects are observed in insect sensory system when they are exposed to undesirable substances.

Costa (2011) studying *Z. subfasciatus* infestation in a mass of beans, treated with extracts of jackfruit (*Artocarpus heterophyllus* L.) and *Chenopodium ambrosioides* L. verified reduction of infestation by 90% using doses of 6 to 8 ml, during 120 days of storage. Ali et al. (2011) observed that the amount of damage to grains caused by weevils increased with storage time and this devalued the product sale.

Garcia et al. (2000) noted that the treatment of beans with ground black pepper with doses 4 and 6 g per kg^{-1} had absolute control on *Z. subfasciatus* during the storage period. It is noted that, it is feasible to use alternative plant extracts in order to control insect infestations in seeds and grains during storage.

For interaction with extracts procedure as shown in Table 2, it is noted that when inoculated with *A. colubrina* and myrtle leaves extracts or *A. pyrifolium* Mart. and myrtle shells, no statistic differences were observed. The best results were observed in extracts from *A. pyrifolium* Mart. leaves and *A. colubrina* barks. However, uninoculated beans showed better control of *Z. subfasciatus* with *A. pyrifolium* Mart. leaves and myrtle bark extracts which did not differ statistically; this result was followed by *L. rigida* leaves (Silva et al., 2013).

When the bean seeds were inoculated with *A. colubrina* and myrtle leaves extracts, no statistical differences were observed.

The low percentage of infestation demonstrates the efficient action of the extracts in the study. However, we noticed an emollient action on stored seeds treated with myrtle extracts, this action promoted a strong bond between the seeds. These residues are probably due to the chemical compounds (saponins) present in the myrtle extract, indicating that these seeds should not be stored for more than 180 days under these conditions, when treated with myrtle extracts.

Garcia et al. (2003) studying the bioactivity of *A. colubrina* and myrtle extracts, observed results which show that the extract had active substances against insects (mortality). Barbosa et al. (2000) observed resistance associated with species of bean arcelin *Z. subfasciatus* protein, implying that some substances

Table 2. Efficiency (mortality) of hydro alcoholic extracts of leaf and bark *A. pyrifolium* Mart., *A. colubrina* and *L. rigida* applied in bean seeds inoculated and non- inoculated with *Z. subfasciatus* in 180 days of storage in pet type packaging.

Extracts	Treatments	
	Inoculated	Non-inoculated
A. pyrifolium (leaves)	10.84^{aA}	8.77^{aB}
A. colubrina (leaves)	8.73^{bA}	6.54^{bcB}
L. rigida (leaves)	8.92^{bA}	7.53^{abcB}
A. pyrifolium (barks)	9.02^{bA}	6.07^{cB}
A. colubrina (barks)	9.51^{abA}	6.15^{cB}
L. rigida (barks)	9.30^{bA}	8.02^{abB}

DMS para columns= 1.51; DMS in the lines = 1.03; Classific.c/lower case; Classific.c/capital letters.

Table 3. Mean values (%) of the infestation procedure of bean seeds, inoculated and non-inoculated with *Zabrotes subfasciatus* and treated with plants extracts in pet packaging, for a period of 180 days.

Procedure	Periods (days)			
	45	90	135	180
Inoculated	4.61^{aD}	6.66^{aC}	11.22^{aB}	15.04^{aA}
No Inoculated	3.57^{bD}	5.50^{bC}	8.32^{bB}	11.33^{bA}

DMS in the columns = 0.84; DMS in the lines = 1.11; Classific.c/lower case; Classific.c/capital letters.

Table 4. Mean value (%) of Zabrotes subfasciatus infestations in inoculated and non-inoculated been seeds, treated with different doses of plant extracts, stored in pet containers, in a period of 180 days.

Doses (ml)	Periods (days)			
	45	90	135	180
0	3.83^{aD}	6.55^{bC}	10.27^{bB}	15.68^{aA}
1	2.10^{bC}	2.28^{cC}	6.66^{cB}	10.91^{cA}
3	5.06^{aC}	6.24^{bC}	9.88^{bB}	12.41^{bcA}
5	5.38^{aC}	8.27^{aB}	12.26^{aA}	13.73^{bA}

DMS in the columns = 1.57; DMS in the lines = 1.26; Classific.c/lower letters; Classific.c/capital letters.

derived from the secondary metabolism of plants can affect the biology of insects.

Analysis of the data contained in Table 3, exhibits equal statistics of doses in controlling *Z. subfasciatus* present in beans paste stored at room condition for 180 days in pet packaging. However, for procedures within each dose (column), the dose of 3 ml showed the best control of *Z. subfasciatus,* where the infestation after 180 days of storage was 5.73% (average). These results are due, probably, to the quantity of the dose as secondary plant constituents present in the extracts; this dose operates with higher efficiency due to better distribution upon its application to the seeds.

According to Costa (2011), when the extracts *A. heterophyllus* and *C. ambrosioides* were used to control the infestation of *Z. subfasciatus,* in *P. vulgaris*, for a storage period of 120 days, equal statistical result was observed in both the inoculated and non-inoculated procedure with doses of 6 and 8 ml.

As shown in Table 4, for interaction with the procedure time, there is increased infestation of *Z. subfasciatus* in seed stored in the mass, with the passage of storage time, gradually, in both procedures with superiority in the inoculated seeded process. It appears also that after three months (90 days) of storage, the percentage of infestation was 6.66% for the mass of seeds infested with *Z. subfasciatus*. It can be seen that during storage the extracts were loosing their bioactivity due to the effects of their volatile constituents, which might explain the increased infestation with time. The insecticide effect of plant extracts was also analyzed by Pessoa (2004) for corn seed storage for popcorn with positive results in controlling infestation of *Sitophilus zeamais*. Santos et al. (1998) and Costa (2011) also controlled infestation of *Z.*

Table 5. Mean value (%) of Zabrotes subfasciatus infestations in inoculated and non-inoculated been seeds, treated with different doses of plant extracts, stored in pet containers, in a period of 180 days.

Extracts	Time (days)			
	45	90	135	180
A.pyrifolium (leaves)	5.05^{aD}	8.07^{aC}	11.16^{aB}	14.94^{abA}
A. colubrine (leaves)	2.91^{cD}	4.88^{bC}	9.16^{bB}	13.58^{bcA}
L. rigida (leaves)	4.14^{bD}	6.36^{abC}	9.53^{bB}	12.87^{bcA}
A.pyrifolium (bark)	4.21^{bB}	5.53^{bB}	10.12^{aA}	10.31^{dA}
A. colubrine (bark)	4.47^{bC}	6.25^{abC}	9.03^{bB}	11.56^{cdA}
L. rigida (bark)	3.78^{bC}	5.40^{bC}	9.62^{bB}	15.85^{aA}

DMS in the columns = 2.13; DMS in the lines = 1.92; Classific.c/lower case; Classific.c/capital letters.

Table 6. Germination percentage (%) of bean seeds treated with hydroalcoholic extracts of leaves and barks of *Aspidosperma pyrifolium* Mart., *A. colubrina* and *L. rigida*, inoculated and not inoculated with *Zabrotes subfasciatus* for 180 days of storage packaging pet.

Extracts	Procedures	
	Inoculated	Not inoculated
A. pyrifolium (leaves)	91.00^{abA}	92.00^{aA}
A. colubrina (leaves)	88.00^{bcB}	90.00^{aA}
L. rigida (leaves)	78.00^{dB}	93.00^{aA}
A. pyrifolium (bark)	92.00^{aA}	92.00^{aA}
A. colubrine (bark)	88.00^{cB}	92.00^{aA}
L. rigida (bark)	80.00^{dB}	91.00^{aA}

DMS in the columns = 2.91; DMS in the lines= 2.00; Classific.c /lower case; Classific.c/capital letters.

subfasciatus in bean seed with plant extracts.

Silva Junior (2011) working with hydroalcoholic extracts of sugar apple and black pepper on the infestation of corn with *S. zeamais,* inoculated the seed mass; after 180 days in storage, he observed lower infestation for the highest levels of these extracts, which partly agree with this research.

From the results obtained in Table 5, it appears that the behavior of infestation by *Z. subfasciatus* is progressive and increases with time regardless of the dose. It was observed that the best controls were those with higher doses of 1 and 3 ml, respectively (line), with a dose of 1 ml, the infestation of *Z. subfasciatus* was controlled more efficiently within the time (column) when compared to the dose of 3 ml. It appears that the lower volume of extract was the most effective in controlling infestation, probably by the interaction of its chemical constituents in the form of application and speed of exposure of extracts on the seeds.

Resende et al. (2008) studied bean storage, observed after 84 days, a significant increase of 91.67% in grain population of insect pests stored in a mass of seeds treated with plant extracts.

In this study, the maximum infestation of *Z. subfasciatus* after 180 days of storage was 13.73% with a 5 ml dose compared to 10.91% with a dose of 1 ml. Therefore, these two doses are confirmed as being the most efficient for the control of weevil in beans seeds.

Harborne (1980) stated that the leaves of angiosperms accumulate flavonoids which serve as insect repellant. A similar result was found in *A. colubrina* and *A. pyrifolium* Mart. which are present in the extracts of the leaves and flavonoids of the barks.

The *A. colubrina* leave extract was also more effective in the control of *Z. subfasciatus* at the rate of 2.91, 4.88 and 9.16% in 45, 90, and 135 days, respectively. The bark extracts from myrtle was also found to be more efficient, it showed a result of 3.78 and 5.40% infestation in 45 and 90 days of storage, respectively. This level of effectiveness is followed by *A. pyrifolium* Mart. extract.

As shown in Table 6, there is infestation of *Z. subfasciatus* in seed mass, during storage and the extracts of *A. colubrina* leaves showed higher results in infestation control at all times. This result is followed the by *A. colubrina* bark.

The percentage of infestation of leaf extracts was statistically equal for all times, however, there was no statistical difference for all leaves and bark extracts analyzed during the 180 days of storage. There was a lower infestation occurrence in *A. pyrifolium* Mart. bark with 10.31%, followed by *A. colubrina* bark with 11.56%. Neves et al. (2000) used botanical products to control *Z. subfasciatus* in bean seeds. Assessments were performed at 30, 60 and 90 days after infestation and

Table 7. Average germination values (%) of interaction in bean seeds treated with doses of leaves hydro alcoholic extracts ,bark of *Aspidosperma pyrifolium* Mart., *A. colubrina* and *L. rigida*, inoculated and not inoculated with *Zabrotes subfasciatus* for 180 days in storage with packaging pet.

Extracts	Doses (ml)			
	0	1	3	5
A.pyrifolium (leaves)	88.00 ^{aC}	90.00 ^{bcBC}	92.00 ^{aAB}	95.00 ^{aA}
A. colubrina (leaves)	88.00 ^{aA}	87.00 ^{cA}	90.00 ^{aA}	91.00 ^{bA}
L. rigida (leaves)	85.00 ^{aB}	91.00 ^{abcA}	84.00 ^{bB}	82.00 ^{cB}
A.pyrifolium (bark)	88.00 ^{aB}	94.00 ^{aA}	93.00 ^{aA}	94.00 ^{abA}
A. colubrine (bark)	87.00 ^{aB}	93.00 ^{abA}	93.00 ^{aA}	84.00 ^{cB}
L. rigida (bark)	88.00 ^{aA}	89.00 ^{bcA}	84.00 ^{bB}	81.00 ^{cB}

DMS in the columns = 4.12; DMS in the lines = 3.71; Classific.c/lower case; Classific.c/capital letters.

they had the best results with a mixture of neem oil with oil *Piper hispidinervum*. The result was a 100% reduction in the numbers of eggs and damaged grains, at 90 days.

Navickiene et al. (2007) after testing organic extracts from the seeds, leaves and stalks of *Piper tuberculatum*, ascertained that these extracts showed potential insecticidal activity, showing a process of rapid poisoning against *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) causing 80% mortality when doses greater than 800 insect^{-1} cg were administered.

According to Torres and Marcos Filho (2001), the susceptibility of plants to insects extracted from plant allelochemicals, depends on the organ and plant species, form of extraction and insect species. According to Chagas et al. (2003), a range of different compounds can be isolated depending on the solvent used to obtain the extract.

In the analysis of germination percentage of stored bean seeds (Table 7) and their interaction with extract products, it appears in the inoculated procedure that the highest percentage of germination was given to the shell *A. pyrifolium* Mart. extract (92.00%) followed by *A. pyrifolium* Mart. leaf extract (91.00%), then the *L. rigida* bark (80.00%) and the lowest for the *L. rigida* leaf extract (78.00%).

In the non-inoculated procedure, all extracts showed the same efficiency in maintaining the viability of seeds, this is revealed by the average germination rate which was 91%. The efficiency showed by all extracts was with the exception of the *A. pyrifolium* Mart. extracts sheet and *A. pyrifolium* Mart. shell, which though showed the same germination in the non-inoculated procedure, the seeds remained higher than that of the inoculated procedure. This shows that the presence of *Z. subfasciatus* caused damage to the beans seeds.

According to Olanda et al. (2011), when the leaf extract of *Casearia sylvestris* Sw. was used to treat bean seeds in a concentration of 0.78%, the germination percentage of 12.5 was observed but from 25 to 50%, complete death of the seeds was observed. Thus, a high concentration of *Echinodorus macrophyllus* (Kunth) Micheli bark extract showed phytotoxicity on the bean seeds. However, when applied in lower concentrations, the extract showed a beneficial effect on germination; thus, demonstrating the potential of *E. macrophyllus* (Kunth) Micheli in bean seed treatment.

The addition of millet extracts reduced the percentage of germination rate in bean seeds (Farias et al., 2009). Karunakaran et al. (2001) viewed fungal growth in wheat seeds stored with 19% moisture content at temperatures between 20 and 35°C, only after the germination of the seeds have reduced the percentage below 90%.

Matioli et al. (1978) found that in corn seeds infested by *Sitophilus oryzae*, there was increased insect population and reduced weight and seed germination. Garcia et al. (2000) found effects of ground black pepper extract at a dose of 4 g/1 kg of seeds, as the most efficient treatment in the control of *Z. subfasciatus* in bean seeds.

It can be seen that seed germination was not influenced by *A. pyrifolium* Mart leaf and *A. colubrina* bark extracts. The percentages of germination of bean seeds were statistically similar at doses of 1, 3 and 5 ml. There was statistical similarity in the germination of bean seeds with doses 1, 3 and 5 ml. In general, germination was more affected by *L. rigida* leaf and bark extracts, which was statistically similar at doses of 3 and 5 ml, but within each dose (column) the 3 ml dose proved to be the overall best in the treatment of the seed in order to obtain seed quality. Whereas, the following *A. pyrifolium* Mart. with *A. colubrina* leaf and *A. pyrifolium* Mart. with *A. colubrina* bark resulted in a higher percentage of seed germination (92.00%) (Table 8).

It is noted that in the procedures, equal statistic results were observed, except for the dose of 1 ml in the inoculated procedure which has the highest percentage of germination. It is also observed that there is difference in 3 and 5 ml doses with higher germination percentage for the non-inoculated procedure (Table 9).

In summary, it can be stated that germination was not affected in seeds treated with doses of these extracts. It is also observed through graphic representation that there is high coefficient and quality. This validates the use of equations in the conditions under which the study did; when you want to estimate intermediate points of the

Table 8. Average values (%) of germination of the interaction procedure with dose in bean seeds treated with hydroalcoholic extracts of leaves and bark of *Aspidosperma pyrifolium* Mart., *A. colubrina* and *L. rigida*, inoculated and not inoculated with *Zabrotes subfasciatus* for 180 days of storage packaging pet.

Procedure	Doses (ml)			
	0	1	3	5
Inoculated	85.00^{bB}	90.00^{aA}	86.00^{bB}	83.00^{cB}
Non inoculated	89.00^{bA}	92.00^{aA}	93.00^{aA}	92.00^{aA}

DMS in the columns = 2.14; DMS in the lines= 1.63; Classific.c/lower case; Classific.c/capital letters.

Table 9. Average values of germination percentage (%) for interaction of extract with bean seeds treated with hydroalcoholic extracts of leaves and bark *Aspidosperma pyrifolium* Mart., *Anadenanthera colubrina*, and *L. rigida* leaves inoculated and not inoculated with *Zabrotes subfasciatus* in pet type storage packaging for 180 days.

Extracts	Periods (days)			
	45	90	135	180
Aspidosperma pyrifolium Mart. leaves	98.00^{aA}	92.00^{aB}	92.00^{aB}	83.00^{bC}
Anadenanthera colubrina leaves	97.00^{aA}	94.00^{aA}	86.00^{bcB}	79.00^{cC}
L. rigida leaves	95.00^{aA}	93.00^{aAB}	90.00^{aB}	64.00^{dC}
Aspidosperma pyrifolium Mart. barks	98.00^{aA}	93.00^{aB}	89.00^{abC}	88.00^{aC}
Anadenanthera colubrina barks	97.00^{aA}	92.00^{aB}	90.00^{abB}	80.00^{bcC}
L. rigida barks	98.00^{aA}	94.00^{aB}	85.00^{cC}	65.00^{dD}
Inoculated	96.00^{aA}	93.00^{aB}	87.00^{bC}	67.00^{bD}
No Inoculated	98.00^{aA}	92.00^{aB}	90.00^{aC}	86.00^{aD}

DMS in the columns = 4.12; DMS in the lines= 3.71; Classific.c/lower case; Classific.c/capital letters.

Table 10. Percentage of germination (%) for the interaction procedure in bean seeds treated with hydroalcoholic extracts of leaves and bark of *Aspidosperma pyrifolium* Mart., *Anadenanthera colubrina* and *L. rigida* inoculated and not inoculated with *Zabrotes subfasciatus* in storage packaging type pet for 180 days.

Procedures	Periods (days)			
	45	90	135	180
Inoculated	96.00^{aA}	93.00^{aB}	87.00^{bC}	67.00^{bD}
No Inoculated	98.00^{aA}	92.00^{aB}	90.00^{aC}	86.00^{aD}

DMS in the columns = 1.63; DMS in the lines = 6.51; Classific.c/lower case; Classific.c/capital letters.

experiment.

Brito (2010) found that *Mimosa tenuiflora* (Wild) extract negatively influenced all the variables studied mainly in corn germination, supporting the thesis that corn can be used as a model in bioassays in tests on allelopathy (Macias et al., 2000).

Silva Junior (2011) reported the interaction of inoculated and uninoculated procedures in the treatment of corn seeds within a period of 180 days, showing a different result on germination. There was a record of 72% germination at the end of the storage period for inoculated seeds. This shows the efficiency of the extracts.

It appears that time was decisive in the germination results of stored bean seeds; those that were observed between 45 and 90 days showed similar results for the extracts. That is, significant effects of extracts on seed germination were only observed after 90 days of storage (column). However, after 180 days of storage, the *A. colubrina* leaf extract which showed 64.00% result and myrtle bark which showed 65.00% showed the worst germination result. This is followed by *A. colubrina* leaf extract (79.00%) (Table 10).

L. rigida bark extracts (85.00%) and A. colubrina leaf (86.00%) were the least efficient, with the former being statistically, in the maintenance of seed germination after 135 days of storage. However, it was observed that the tough *A. pyrifolium* Mart. extract (88.00%) kept seed germination to the end of the storage period. It was also observed that the *A. pyrifolium* Mart. leaf extract (92.00%) and *L. rigida* leaf (90.00%) showed similar results at 135 days of storage.

Table 11. Percentage (%) of germination for dose interaction in bean seeds treated with hydroalcoholic extracts of leaves and bark of *Aspidosperma pyrifolium* Mart., *Anadenanthera colubrina* and *L. rigida*; inoculated and not inoculated with *Zabrotes subfasciatus* in pet packaging storage for 180 days.

Doses (ml)	Periods (days)			
	45	90	135	180
0	94.00^{bA}	91.00^{bB}	87.00^{bcC}	77.00^{bD}
1	98.00^{aA}	94.00^{aB}	86.00^{cC}	85.00^{aC}
3	98.00^{aA}	95.00^{aB}	90.00^{abC}	76.00^{bD}
5	99.00^{aA}	92.00^{abB}	92.00^{aB}	69.00^{cC}

DMS in the columns = 3.03; DMS in the lines = 3.03; CV% = 6.51; Classific.c/lower case; Classific.c/capital letters.

This result is due to the presence of natural substances with potential insecticide, present in the hydro alcoholic extracts used. This potential is revealed in the phytochemical study, where steroids and tannins showed bioactive effects on *L. rigida* and *A. colubrina* extracts.

Medeiros et al. (2007) evaluated dry and green leaves of neem on the quality of cowpea seeds and they concluded that the extracts affected seed germination; the treatments which received neem seed powder differed significantly from the control. Silva (2007) observed reduced germination in corn seeds treated with extracts of *M. tenuiflora* (Wild Poiret). Haven given effect to the woody part, it was observed that germination of seeds of other grasses was not affected when treated with leaf extract of *M. tenuiflora* (Wild) Poiret).

Silva and Aquila (2006) verified the effect of *Erythroxyhum argentinum* extracts, *Luehea divaricata*, *Mysine guianensis* and *Ocotea peberula* on the initial germination of lettuce. They observed that the treatment showed significant difference from the control group despite that those species presented allelopathic potential. Lima et al. (2007) observed that aqueous extracts of the aerial part of *Crotalaria juncea, Canavalia ensiformes* (L.) DC. and *Sesamum indicum* reduced the final germination of *Bidens pilosa* in a concentration of 20%.

It was also observed that there was reduction in seed germination with advance deterioration during storage time for both the inoculated and non-inoculated, statistically, equal to the procedures within 45 times and 90 days.

But in the final two times (135 and 180 days), the inoculated procedure (87.00 and 67.00%) was lower than the uninoculated procedure (90.00 and 86.00%), respectively (Table 11).

Almeida et al. (2008) found that *Croton sonderianus* of aqueous extracts also promoted a reduction in the percentage of germination of *Cassia tora*.

Germination decreased within storage period of 45 and 90 days (column), there were no significant statistical differences, but seed germination showed different behavior at 135 and 180 days. In addition, at 180 days, there was lower seed germination for the dose of 5 ml 69.00% (Table 12).

This result shows that the doses tested for this test are effective in controlling *Z. subfasciatus* and did not inhibit the germination of seed bean.

Promising results in the control of *Z. subfasciatus* were verified by Queiroga (2010) with vegetable oils in the treatment of stored beans. The insects were controlled and after 150 days of storage, there was an observation of 57.00% seed germination.

Almeida (2003) studied the effects of plant extracts in the control of *Callosobruchus maculatus* and its effects on the bean *Vigna unguiculata*. From the research, it was concluded that the vigor and germination of seeds treated with *Conospermum caeruleum* extracts and *Piper nigrum* were positive, while this decreased over storage time. While the best extract in preserving the seed was *P. nigrum*.

Conclusions

Plant extracts showed insecticidal activity against *Z. subfasciatus*, killing them and/or inhibiting their development. In the treatment of bean seeds, the dose of 3 ml was the best to control the incidence of *Z. subfasciatus*, with better results from the use of *L. rigida* leaf extracts and *A. pyrifolium* Mart. bark. The procedures adopted in seed treatment with the extracts of plant species were efficient in maintaining the viability and did not affect germination during 180 days of storage. Germination was affected by insufficient storage time (less than 180 days), and the doses 1 and 3 ml were the most efficient for the maintenance of seed germination.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Almeida FAC, Villamil JMP (2000). Insetos plagas de los granos

almacenados, Apostila de almacenamiento de granos. Madrid/UPM.
Almeida GMA, Albuquerque MB, Santos RC, Melo Filho PA (2008). Avaliação do potencial alelopático do *Croton sonderianus* em sementes de *Cassia tora*. In: JORNADA EM ENSINO, PESQUISA E EXTENSÃO DA UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO, 8., Recife-PE, Anais… Recife:UFRPE. 1 CD-ROM.
Almeida SA (2003). Extratos vegetais no controle do *callosobruchus maculatus* e seus efeitos na conservação do feijão V*igna unguiculata (*L. walp.) Dissertação (Mestrado engenharia agrícola) – Universidade Federal da Paraíba, Campina Grande.
Barbosa FR, Siqueira KMM, Souza EAde, Moreira WA, Haji FNP, Alencar JAde (2000). Effect of chemical control of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) on the incidence of Bean Golden Mosaic Virus in common beans and on its yield. In: INTERNATIONAL CONGRESS OF ENTOMOLOGY, 21. Foz do Iguassu. Resumos... Londrina: Embrapa Soja 327 p.
Brasil (2009). Ministério da Agricultura e Reforma Agrária. Regras para análise de sementes. Brasília: SNAD/DNDV/CLAV 365 p.
Brito ICA (2010). Alelopatia de Espécies Arbóreas da Caatinga na Germinação e Vigor de Sementes de Feijão Macaçar e de Milho. Patos, PB: UFCG, 53f. (Dissertação – Mestrado em Zootecnia – Sistemas Agrosilvipastoris no Semi-Árido).
Carvalho NM, Nakagawa J. (2000). Sementes: ciência, tecnologia e produção. 4.ed. Jaboticabal: Funep. 588 p.
Chagas ACS, Passos WM, Prates HT, Leite RC, Furlong J, Fortes ICP. (2003). Efeito acaricida de óleos essenciais e concentrados emulsionáveis de *Eucalyptus* spp em *Boophilus microplus*, J. Vet. Res. Anim. Sci. 39(5):247-253.
Costa ART, Amaral MFZJ, Martins PM, Paula JAM, Fiuza TS, Resvenzol LMF, Paula JR, Bara MTF (2011). Ação do óleo essencial de *Syzygium aromaticum* (L.) Merr. & L.M. Perry sobre as hifas de alguns fungos fitopatogênicos. Rev. Bras. Plantas Med. 13(2):240-245.
Costa GV da (2011). Avaliação da bioatividade de dois extratos vegetais no controle do *Zabrotes subfasciatus* isolado e inoculado em uma massa de feijão *Phaseolus vulgaris* L. 85f. Dissertação de Mestrado. Campina Grande.
Delouche JC, Still TW, Raspet M, Lienhard MO (1976). Teste de tetrazólio para viabilidade da semente. Brasília. AGIPLAN 103 p.
FAO-Food and Agriculture Organization of the United Nations (2013). Feijão. Disponível em: < http://faostat.fao.org/default.aspx>. Acesso em: 05 sept. 2016.
Farias SGG de, Freire AL de O, Santos DR dos, Bakke IA, Silva RB. (2009). Efeitos dos estresses hídrico e salino na germinação de sementes de gliricidia (*Gliricidia sepium* (Jacq.) Steud.). Rev. Caatinga 22(4):152-157.
Frota AB, Pereira PR (2000). Caracterização da produção de feijão-caupi na região Meio-Norte do Brasil. In: CARDOSO, M. J. (Org.). A cultura do feijão-caupi no Meio-Norte do Brasil. Teresina: Embrapa Meio-Norte pp. 28-40.
Garcia J, Kamada T, Jacobson TKB, Curado MA, Oliveira SM. (2000). Superação de dormência em sementes de pimenta-do-reino (*Piper nigrum* L.). Pesqui. Agropec. Trop. 30(2):51-54.
Harborne JB (1980). Plant phenolics. In: E.A.Bell & B.V. Charlwood (eds.). Secondary Plant Products. Encyclopaedia of Plant Physiology. Berlin, Springer- Verlag. A. Pirson & M.H. Zimmermann (orgs.) 8:329-395.
Karunakaran C, Muir WE, Jayas DS, White NDG, Abramson D. (2001). Safe storage time of niger moisture wheat, J. Stored Prod. Res. 37(3):303-312.
Lazzari FA (1997). Umidade, fungos e micotoxinas na qualidade de sementes, grãos e rações. 2. ed. Curitiba: Ed. do Autor 134 p.
Lazzari FN (2005). Controle de *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera, Chrysomelidae, Bruchinae) e qualidade do feijão (*Phaseolus vulgaris* Linnaeus, 1753) tratado com terra de diatomácea..69f. Dissertação (Mestrado em Ciências Biológicas) – Curso de Pós-graduação em Ciências Biológicas, Universidade Federal do Paraná, Curitiba, PR.
Lima C, Pereira LM, Mapeli MC (2007). Potencial alelopático de crotalária, feijão-de porco e gergelim na germinação e vigor de picão-preto. Resumos do V CBA - Manejo de 47 Agroecossistemas Sustentáveis. Rev. Bras. Agroecol. 2(2):1175-1178.
Lorini I, Morás A, Beckel H (2002). Pós inertes no controle das principais pragas de grãos armazenados. Boletim de pesquisa e desenvolvimento da Embrapa 8:1-35.
Macias FA, Castellano D, Molinillo JMG (2000). Search for a standard phytotoxicbiossay for allelochemicals. Selection of standard target species. J. Agric. Food Chem. 48(6):2512-2521.
Matioli JC, Almeida AA, Matioli CH (1978). Efeitos da infestação do *Sitophilus oryzae* (L., 1763) sobre a germinação de sementes de milho armazenado. Rev. Bras. Armazenamento 3(4):15-18.
Medeiros DC, Andrade NRC, Figueira LK, Nery DK, Maracajá PB (2007). Pó de folhas secas e verdes de nim sobre a qualidade das sementes de feijão caupi. Rev. Caatinga 20(2):94-99.
Navickiene HMD, Debonsi MJE, Bortoli AS, Kato MJ, Bolzani VS, Furlan M (2007).Toxicity of extracts and isobutyl amides from *Piper tuberculatum*: potent compounds with potential for the control of the *Velvetbean caterpillar*, *Anticarsia gemmatalis*. Pest Manage. Sci. 63:399-403.
Neves BP, Fageira NK, Oliveira IP, Guimarães CM (2000). Eficiência de alguns produtos de origem vegetal no controle de *Zabrotes subfasciatus* em sementes de feijoeiro. In: CONGRESSO BRASILEIRO DE DEFENSIVOS AGRÍCOLAS NATURAIS, 1. Fortaleza. Anais... Fortaleza: Academia Cearense de Ciênc. 75 p. Olanda GB, JOB RB, Cavalheiro VBD, Bevilaqua GAP, Olanda RB, Müller LE. (2011). Efeito do extrato alcoólico da folha de erva de bugre no desempenho de sementes de feijão. In. XVII Congresso Brasileiro de Sementes, Natal/RN.
Pessoa EB (2004). Controle de *Sitophillus zeamais* em milho de pipoca nas fases adulta imatura com extratos vegetais, Campina Grande: UFCG. 57f. Dissertação de Mestrado,.
Popinigis F (1985). Fisiologia da semente. Brasília: AGIPLAN 289 p.
Queiroga MFC (2010). Bioatividade de óleos fixos na manutenção da qualidade física e fisiológica de sementes de feijão *Phaseolus vulgaris* armazenadas e no controle do caruncho *Zabrotes subfasciatus*.Campina Grande: UFCG. 63f. Dissertação de Mestrado. Resende O, Corrêa PC, Faroni LRD'A, Cecon PR (2008). Avaliação da qualidade tecnológica do feijão durante o armazenamento. Ciênc. Agrotéc. 32(2):517-524.
Rupolho G, Gutkoski LC, Martins IR, Elias MC (2006). Effects of grain moisture and hermetic storage on fungi contamination and mycotoxin production in oats. Ciênc. Agrotec. 30 (1):118-125.
Santos MVF, Farias I, de Andrade LM, Maria M, do Nascimento A, dos Santos DC, Filho JJT (1998). Colheita a palma forrageira (*Opuntia ficus indica* Mill.) cv. gigante sobre o desempenho de vacas em lactação. Res. Soc. Bras. Zootec. 27(1):33-39.
Silva FAZ, Azevedo CAV (2009). Versão do programa computacional Assistat para o sistema operacional Windows, Rev. Bras. de Prod. Agroindustriais 4(1).
Silva FM, Aquila MEA (2006). Contribuição ao estudo do potencial alelopático de espécies nativas. Ver. Árvore 30(4):547-555.
Silva JF, Melo BA, Pessoa EB, Figueiredo Neto A, Leite DT (2013). Extratos vegetais para o controle do caruncho do feijão Zabrotes subfaciatus. Rev. Verde Agroecol. Desenvolv. Susten.l 8(3):01-05.
Silva Junior PJ (2011). Medidas de controle do *Sitophilus zeamais* em sementes de milho armazenadas com extratos de *Piper negrum* e *Annona squamosa* L. 57f. Dissertação (Mestrado em Engenharia Agrícola).Universidade Federal de Campina Grande, Campina Grande, PB.
Silva WA (2007). Potencial alelopatico de extratos do cumaru(Amburana cearensis A.C.Smith) e da jurema-preta (*Mimosa tenuiflora* (Willd.) Poir) na germinação e crescimento do sorgo(Sorghum bicolor L.), milho (Zea mays L) e feijão guandu (*Cajanus cajan* L.). 62f. Dissertação (Mestrado em Sistemas Agrosilvipastoril) - Universidade Federal de Campina Grande, Patos.
Torres SB, Marcos Filho J (2001). Teste de envelhecimento acelerado em sementes de maxixe (*Cucumis anguria* L.). Rev. Bras. de Sementes 23(2):108-112.
Viebrantz PC, Radunz LL, Dionello RG (2016) Motality of insects and quality of maize grains in hermetic and non-hermetic storage. Rev. Bras. De Engenharia Agríc. Ambient. 20(5):487-492.