
# Influence of fungi incidence in germination on seeds of plantain (*Plantago major* L) in different periods of storage

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Received: August 08, 2016; Revised: 30 de Outubro de 2016

## Abstract

It was evaluated, percentages and germination speed, in seeds of *Plantago major* L., at different periods of storage. The seeds were placed in a sealed paper bag and then stored at room temperature for 30, 90, 240 and 570 days. The freshly collected seeds were exposed on a stand in shade conditions, for a period of 24 hours. After plating, seeds were exposed to a photoperiod of.of 12h light for 12 days until exteriorization and development of fungi. The experimental design was completely randomized. The analysis of health revealed the presence of four genera of fungi associated with seeds of plantain. Regarding the the germination speed index, the seeds stored for 30 days had higher rates than the fresh seeds. It was found that the seeds did not germinate after 30 days of storage. The high incidence of the *Alternaria* and *Drechslera* fungi did not interfere in seed germination of the specie.

**Key-words:** Medicinal plant, *Plantago major* L, sanitary quality, germination.

# Incidência fúngica e germinação em sementes de tanchagem (*Plantago major* L) em diferentes períodos de armazenamento

## Resumo

Avaliou-se a incidência fúngica, a porcentagem e o índice de velocidade de germinação, nas sementes de *Plantago major* L., em diferentes períodos de armazenamento. As sementes foram acondicionadas, em saco de papel lacrado e posteriormente, armazenadas em condições de temperatura ambiente, durante 30, 90, 240 e 570 dias. As sementes recém - colhidas foram expostas em uma bancada em condições de sombreamento, por um período de 24 horas. Após plaqueamento, as sementes foram incubadas em temperatura ambiente e fotoperíodo de 12h de luz durante 12 dias até exteriorização e desenvolvimento dos fungos. O delineamento experimental utilizado foi o inteiramente casualizado. A análise de sanidade revelou a presença de quatro gêneros de fungos associados às sementes de tanchagem. Em relação ao índice de velocidade de germinação as sementes armazenadas por 30 dias apresentaram maior índice que as sementes recém-colhidas. Verificou-se que as sementes não germinaram a partir de 30 dias de armazenamento. A alta incidência dos fungos *Alternaria* e *Drechslera*, não interferiram na germinação das sementes da espécie.

**Palavras-chave**: Planta medicinal, *Plantago major* L, qualidade sanitária, germinação.


## Introduction

In Brazil, medicinal plants and the herbal sector are considered of great importance to the economy due to the creation of 100 of thousands of jobs and, it is estimated that the sector is responsible to generates about one billion of reais per year (ABIFISA, 2009). Given the economic relevance of these plants, the need arises to optimize the research of herbal medicines in Brazil.

Among the many plants used by the population in various regions of Brazil, stands out the species *Plantago major* L. (Plantaginaceae), popularly known as plantain, common plantain, Plantago, cow's tongue. The leaves of this specie have flavonoids (allantoin), tannin, mucilage, saponins, organic acids, potassium salts, vitamins. Are used as diuretic, anti-diarrhea, expectorant, haemostatic and healing, is also commonly used against upper respiratory tract infections, chronic bronchitis and assist in the treatment of peptic ulcers (SHAMELZE & GURIB-FAKIM, 2008).

The seeds have anthraquinones and are used against conjunctivitis and eye irritation due to trauma. Besides its medicinal value, the specie has nutritional relevance due to high levels of vitamin A and C, calcium and phosphorus, characteristics that give the plant a great commercial potential (KAWASLITY et al. 1994; HOLETZ et al., 2002).

Most species of medicinal plants, aromatic herbs and are propagated by seeds. For the species *P. major* propagation is only through seeds (LIMA, 2008), however, in studies previously carried were verified that the germination index of seeds from the nursery of medicinal plants is very low, the low germination verified in the seeds may be due to fungal infection, this being one of the factors that most influence the germination (SILVA et al., 2007).

The seeds are considered one of the most effective mean of dissemination and transmission of pathogens to plants and it allows the introduction of diseases on crop areas, causing losses in agricultural production (SENEME, et al., 2010).

The incidence of in seed fungal can also cause changes in the metabolism of plants and compromise their therapeutic properties (KRUPPA & RUSSOMANNO, 2008). During storage, some fungi cause


physical and chemical changes in tissues of the seeds, causing loss of lipids, carbohydrates, proteins and increased fatty acid, and directly affect seed germination (BARROCAS & MACHADO, 2010)

Fungi that attack the seeds or grains can occur even in the field, depending on the presence of excessive moisture, but it can also develop also in storage low temperature (PUTZKE & PUTZKE, 2002).

The longer the period of storage of seeds, greater care should be taken, mainly on seeds moisture content and the room temperature storage (TAKAHASHI et al., 2006).

The storage time of seeds can influence not only the incidence of fungi, as well as the germination percentage or the germination speed index. However, knowledge of the physiological and sanitary quality of seeds as a function of storage time is still scarce.

Thus, the knowledge the fungi present in seeds at different storage periods and immediately after harvest is essential to the appropriate management of plants and proper storage of the seeds. However, studies investigating the incidence of fungal seeds of *P. major*, at different periods of storage, as well as the influence of fungi and the storage time on germination percentage and germination speed index is still incipient, with further studies needed.

Therefore this work aimed to evaluate and correlate the fungal incidence in seeds with germination and the germination speed index of *Plantago major*, at different periods of storage

## Material and methods

The seeds of *P. major*, were collected in beds of medicinal plant nursery, located in the municipality of Jacareí - SP.

After harvesting, the seeds were stored in sealed paper bag and then stored at room temperature for 30, 90, 240,570 days. The freshly collected seeds were exposed on a stand in shade conditions, for a period of 24 hours. Each storage period was considered a treatment.

Five lots of 200 seeds from each storage period were distributed in Petri, covered with three sheets of filter paper, previously sterilized and moistened in sterile water.

The experimental design was completely randomized. After plating, seeds were incubated at room temperature


and photoperiod of 12h light for 12 days until exteriorization and development of fungi.

Seed vigor was determined by comparing the germination percentage (GR) together with the germination test, following the recommendations contained in the Rules for Seed Analysis (BRASIL, 2009). The evaluations were performed daily from the day they occurred the first seeds germinated, the evaluations were performed daily from the day the first germinated seeds occurred, until the 15th day after the seeding.

The germination speed índex (GSI) was calculated using the formula for the speed of germination (MAGUIRE, 1992), IVG = $(G_1/N_1) + (G_2/N_2) + (G_3/N_3) + ... + (G_n/N_n)$, on what:

IVG = germination speed index, $G_1$, $G_2$, $G_3$,..., Gn = Number of seeds germinated in the first, second, third and last count

$N_1$, $N_2$, $N_3$, ..., Nn = Number of days of sowing to the first, second, third and last count.

The mycoflora associated to the grains was evaluated by counting and identification of fungi by examining each individual seed in a stereomicroscope. Identification was confirmed by visualization of morphological structures of fungi in an optical microscope. The results were expressed as percentages of infected seeds for each condition evaluated. The data were subjected to analysis of variance (ANOVA) by the Instat statistical system and the means were compared by Tukey test ($p \leq 0.05$).

**Results and discussion**

The analysis of health revealed the presence of only four genera of fungi associated to the seeds of *P. major*, and the genera *Alternaria* and *Drechslera* had the highest incidence (Table 1), however the high incidence of *Alternaria* was observed only in newly harvested and stored seeds for 30 days, *Drechslera*, species were observed at lower frequency in seeds stored for a period exceeding 30 days, although there was a high incidence of this genus in seeds stored for 90 days (Table 1).


Table 1: Fungal Incidence (%) in seeds of plantain in different periods of storage

| Storage | Days | Fungi | | | |
|---|---|---|---|---|---|
| | | *Alternaria sp* | *Drechslera sp* | *Fusarium sp* | *Penicillium sp* |
| Freshly harvested seed | 0 | 19,5 b | 86,0 a | 0 ,0 b | 0,0 b |
| Period 1 | 30 | 86,0 a | 98,5 a | 0,0 b | 0,0 b |
| Period 2 | 90 | 0,0 c | 52,5 b | 18,0 a | 0,5 b |
| Period 3 | 240 | 0,0 c | 1,0 c | 2,0 b | 0,0 b |
| Period 4 | 570 | 0,5 c | 4,0 c | 2,0 b | 1,0 a |
| C.V. (%) | | 8,32 | 16,15 | 62,65 | 66,70 |

Means followed by the same letter in columns do not differ by Tukey test ($p \leq 0.05$).

During storage, the seeds had have water contents of 12 to 13%, providing the pathogen an adverse environment for mycelial growth, (REIS & CASA, 2004), to genres *Alternaria* and *Drechslera* fungi found associated with seeds of plantain, this interruption of mycelial growth was sufficient to eliminate these pathogens from seed.

The reduction or inactivation of inoculum in seeds due to the increase of storage time was observed in several studies (CROCHEMORE & PIZA, 1994; HUANG et al., 1994; VERZIGNASI *et al.*1997)

The genus *Alternaria* has been found associated with seeds of various species of medicinal plants, being reported as the causal agent of disease in rosemary (PERELLO & BELLO, 1995), lemon grass (MACHOWICZ-STEFANIAK et al., 2002, 2004), (MENDES et al., 1998), thyme (SURVILENE & DAMBRAUSKIENE, 2006) and plantain (KRUPPA & RUSSOMANO, 2008), however the genre *Drecheslera* is not normally associated with species of medicinal plants.

Seeds are considered as the main sources of inoculum of pathogens, since infected seed introduces the plant pathogenic fungus when a culture is installed, however it was found that the incidence of fungi associated with seeds of this species decreased with storage time, this reduction in the incidence of fungal pathogens of plants, during storage of seeds, could constitute an effective method for reducing the inoculum, since, from 90 days of storage the mean percentage

incidence of fungal genera *Alternaria* and for *Drecheslera* associated the seeds of this species was very low, however, the average percentage of germination after 30 days of seed storage is null, this result discards the possibility of the use of storage in the elimination of fungi associated with seeds.

The high incidence of the *Alternaria* and *Drechslera* fungi, did not affect seed germination not because there was a positive correlation between the high incidence of these fungi and seed germination. Probably, fungi was only associated to external surface of the seed and did not reach the embryo.

The genus *Fusarium* was observed in seeds only after 90 days of storage, with an incidence of 18%, the germination speed index also was null this storage period, this result might suggest that this fungus was associated internally to seeds, and possibly reached the embryo, influencing the germination process, however this result may explain the reduction in germination seeds but not justified the total loss of germination capacity after 30 days of storage.

Information on the index of germination in seeds of medicinal species are still incipient, however, a study conducted with chamomile (*Chamomilla recutita* L.) showed that the germination percentage was significantly reduced after two years of storage (SOUZA et al. 2007).

It was observed that germination was also reduced during storage in fresh seeds of three poppy cultivars (*Papaver orientale* Linn), stored at room temperature for 18 months (VERMA et al., 1996). The species *Kielmeyera coriaea* and *Mikania* spp have totally lost their germination capacity when stored for a period of 18 months at a relative humidity of 70%, temperature 20 º C, and the species *Libertia edulis* and *Himatantus oboata* showed small decreases in the germination percentage (ALBURQUERQUE et al., 2007).

It can be assumed that different species of medicinal plants may have different responses in relation to fungal incidence and the germination percentage in different storage conditions, so more studies are needed on the ideal conditions for the storage of seeds of this species.

Regarding the speed of germination (Table 2), seeds stored for 30 days had


higher rates than the fresh seeds, probably due to the maturation of seeds during the storage period, this result suggested that planting the seeds of common plantain soon after harvest is not indicated, since both the percentage of germination and germination speed index, have been augmented with a short storage period, however this storage time should not exceed 30 days, when seeds are stored under environment.

Table 2: Germination percentage (G%), germination speed index (GSI) in seeds of plantain in different periods of storage

| Storage | Days | G% | GSI |
|---|---|---|---|
| Freshly harvested seed | 0 | 35,0 a | 4,80 a |
| Period 1 | 30 | 86,0 a | 9,50 a |
| Period 2 | 90 | 0,0 b | 0,0 b |
| Period 3 | 240 | 0,0 b | 0,0 b |
| Period 4 | 570 | 0,0 b | 0,0 b |
| C.V. (%) | | 18,37 | 14,58 |

Means followed by the same letter in columns do not differ by Tukey test ($p \leq 0.05$)

## Conclusions

The seeds of common plantain, stored for a period exceeding 30 days completely lose its germinating power. Few genera of fungi were associated with the seeds of plantain, but there was a high incidence of the *Alternaria* and *Drechslera* fungi, but these fungi did not affect the germination of seeds because there was no positive correlation between high fungal incidence and seed germination.

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