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Processing on the sanitary quality of seeds of *Panicum maximum* cv. ‘Tanzânia’

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Key words:

processing
seed pathology
health

ABSTRACT

The aim of this study was to evaluate the sanitary quality of seeds of *Panicum maximum* cv. ‘Tanzânia’, following the several phases of the seed processing process. Seeds were sampled before processing and after leaving the air and screen machine (upper and intermediary screens and bottom); first gravity table (drift, upper and intermediate spouts); second gravity table (upper, intermediate, and lower spouts), and treating machine for dyeing the seeds. The sanitary analyses were conducted according to the filter paper method, with and without superficial disinfection of the seeds, which were incubated at a temperature of 20 ± 2 °C with photoperiod of 12 h for seven days. Some seed processing steps may reduce the incidence of seeds of *P. maximum* cv. ‘Tanzânia’ contaminated with *Phoma* sp. and *Helminthosporium* sp. In general, the processing can increase the incidence of seeds contaminated with *Cladosporium* sp. and *Cercospora* sp. The fungi *Phoma* sp., *Helminthosporium* sp., *Penicillium* sp., *Cladosporium* sp. and *Cercospora* sp. are found inside and outside the seeds and can be disseminated by the processing machines.

Palavras-chave:

processamento
patologia de sementes
sanidade

Beneficiamento na qualidade sanitária de sementes de *Panicum maximum* cv. Tanzânia

RESUMO

Objetivou-se, neste trabalho, avaliar a qualidade sanitária de sementes de *Panicum maximum* cv. Tanzânia durante as etapas de beneficiamento. As sementes foram amostradas antes do processamento e após a saída da máquina de ventilador e peneiras (descarga das peneiras superior, intermediária e fundo), primeira mesa gravitacional (deriva, descarga superior e intermediária), mesa gravitacional (descarga superior, intermediária e inferior) e máquina tratadora para tingimento das sementes. A análise sanitária foi realizada pelo método do papel de filtro com e sem desinfestação superficial das sementes, as quais foram incubadas a 20 ± 2 °C, com fotoperíodo de 12 h durante sete dias. Algumas etapas de beneficiamento podem reduzir a porcentagem de sementes de *P. maximum* cv. Tanzânia contaminadas com *Phoma* sp. e *Helminthosporium* sp. Em geral, o beneficiamento pode aumentar a porcentagem de sementes com *Cladosporium* sp. e *Cercospora* sp. Os fungos *Phoma* sp., *Helminthosporium* sp., *Penicillium* sp., *Cladosporium* sp. e *Cercospora* sp. se encontram alojados nas sementes interna e externamente e podem ser disseminados pelas máquinas de beneficiamento.



INTRODUCTION

The lots of *Panicum* spp. seeds received by the companies contain earth, rocks, straw and empty spikelets and these impurities should be removed by the processing in order to meet the requirements for commercialization (Melo et al., 2016a, 2016b). Processing machines perform sequential operations of cleaning, classification and treatments that allow the improvement of seed quality and disposal of undesired material (Hessel et al., 2012; Melo et al., 2016a, 2016b).

This process, however, can also increase the chances of seed contamination, because the earth existing in the lots is deposited inside the machines and may contain fungal structures that contaminate the subsequently processed lots, as observed by Linares (1999) in bean seeds. Therefore, the processing of forage grass seeds must be enhanced in order to improve its sanitary quality (Marchi et al., 2010).

In seeds of *P. maximum* and *Brachiaria* spp., researchers have detected potentially pathogenic fungi such as the genera *Curvularia*, *Fusarium*, *Phoma*, *Exserohilum*, *Cercospora* and *Helminthosporium*, besides others, such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Nigrospora*, *Penicillium* and *Trichoderma*, (Marchi et al., 2010; Martinez et al., 2010; Mallmann et al., 2013; Marcos et al., 2015). These seeds can be a vehicle of dispersion of pathogens between regions (Vechiato et al., 2010).

The rejects of the processing can also contribute to spreading fungi, because this material is commonly purchased by companies with low technological level from the forage seeds sector to be mixed with the lots intended for less demanding markets (Hessel et al., 2012). Thus, this study aimed to evaluate the influence of the processing on fungal incidence in seeds of *P. maximum* cv. 'Tanzânia'.

MATERIAL AND METHODS

The seeds of *P. maximum* cv. 'Tanzânia' were harvested by ground sweeping in the 2013/2014 season in Jales, SP, processed in a forage grass seed processing unit, passing through an air and screen machine, two gravity tables and one treating machine to dye the seeds. The sequence of processing operations for forage seeds described by Melo et al. (2016b) was adopted.

Eleven treatments were obtained in the various processing steps and different machines, as described below:

T1 - control composed by raw seed, not processed.

Air and screen machine: T2 - after passing through the air and screen machines, the samples were collected from the material retained on the top screen, which has round mesh with diameter of 5.56 mm.

T3 - 20 AWG (0.8118-mm diameter) twisted wire intermediate screen, with square mesh and 30 x 30 mm opening.

T4 - metal plate bottom, which retained the fine material that passed through the screens. The seed company routinely discards the material from treatments T2 and T4, because it contains more than 90% of impurities (Melo et al., 2016b).

First gravity table: T5 - samples were collected from the drift material removed by an aspirator at the inlet of this

machine, also considered as reject by the company, because it contains 97% of impurities in its composition (Melo et al., 2016b). The surface of this device was 2.40 m long and 1.25 m wide, with vibration speed of 1,750 rpm, 17° and 12° of transverse and longitudinal inclination, respectively.

T6 - After passing on the first gravity table, the samples were collected at the upper spout, composed of the fraction of material collected at 35 cm from the highest end of the table's outlet considering its lateral inclination.

T7 - Samples collected at the intermediate spout, composed of material collected in the 60-cm intermediate segment of the table's outlet.

The seeds of the lower spout, composed of the fraction of material collected in the 30-cm segment from the lowest end of the table considering its lateral inclination, were not sampled, but instead passed on a second gravity table.

Seed treating machine: T8 - in the sequence, only the seeds from the intermediate spout of the first gravity table were subjected to dyeing, aiming only to improve their appearance, performed by spraying the green dye of Laborsan Brasil[®] and mechanical movement of the mass in the treating machine Seed Mix VHM- 4/10 T.

Second gravity table: T9 - the seeds were collected after passing on the second gravity table, identical to the first one, but with different adjustments of the spouts, because the upper spout was composed of the fraction of material collected in the 60-cm segment from the highest end of the table's outlet.

T10 - intermediate spout, composed of the material collected in the 45-cm intermediate segment and T11 - lower spout, composed of the material collected in the 20-cm segment of the lowest end. The seed company routinely considers as rejects the materials from treatments T10 and T11, because it contains more than 90% of impurities (Melo et al., 2016b).

The samples collected after the processing steps were reduced in soil splitter to obtain the working sample and the portion of pure seeds was separated for health analysis (BRASIL, 2009).

The health test was conducted at the Seed Pathology Laboratory of the Plant Health Department of the Faculty of Agrarian and Veterinary Sciences - (UNESP), Campus of Jaboticabal, SP, through the filter paper method (Blotter test) without and with superficial disinfestation of the seeds through immersion in NaClO (1%) for 3 min, followed by rinsing with sterilized water and drying at room temperature. For the test, 10 replicates of 10 seeds of each treatment were distributed equidistantly on three sheets of filter paper previously moistened with distilled water and incubated on 9.0-cm-diameter Petri dishes for 7 days, at 20 ± 2 °C and under 12 h of light (Martins et al., 2001).

Then, the seeds were individually analyzed under stereoscopic microscope and the fungi were identified through the morphological characteristics of their structures (Barnett & Hunter, 1998). The results were expressed in percentage of contaminated seeds, for each fungus.

The experiment was evaluated using a completely randomized design, in 2 x 11 factorial scheme (superficial disinfestation x processing steps). The data of fungal incidence

(%) in the seeds were transformed to $(x + 0.01)^{0.5}$ to meet the assumptions of normality and homogeneity tests. The presented means are relative to the original data, which were subjected to analysis of variance by F test. The means of the treatments were compared by Tukey test at 0.05 probability level.

RESULTS AND DISCUSSION

The pathological analysis of *P. maximum* seeds, cv. 'Tanzânia', detected twelve fungal genera: six with higher incidence, above 5%, such as *Phoma*, *Helminthosporium*, *Cladosporium*, *Cercospora*, *Fusarium* and *Penicillium*, and the others with low incidence, inferior to 3%, regardless of the processing step or disinfection procedure, such as *Curvularia*, *Alternaria*, *Epicoccum*, *Rhizopus*, *Aspergillus* and *Rizoctonia* (Figure 1).

Almost all these last six fungi with low incidence in the lot were not affected by the seed processing steps or disinfection, except *Alternaria* sp., which increased from 0.63 to 3.63%, only due to the disinfection procedure (data not shown). Probably, the higher incidence of *Alternaria* sp. in disinfested seeds may have occurred because of the reduction in the fungi of other genera, which competed for survival. This fact was observed for fungi of other genera in studies with seeds of forage grasses (Santos et al., 2014).

The occurrence of some fungi, even at percentages lower than 10%, still represents a risk of loss of seed quality, because these microorganisms have high capacity of multiplication and contamination of the lots in the storage, as observed for *Penicillium* sp. and *Aspergillus* sp. in soybean seeds (Cardoso et al., 2004).

The reduction in the occurrence of the genera *Phoma*, *Helminthosporium*, *Fusarium*, *Penicillium*, *Cladosporium* and *Cercospora* due to the disinfection process (Figure 1) leads to the conclusion that a significant percentage of the structures of these fungi is found on seed surface and they can be disseminated by the machines during the processing, from one lot to another, as observed by Linares (1999) in bean seeds.

As shown in Figure 2, the forage grass seed processing units usually have large amount of dust and earth suspended in the air and the machines are impregnated with these particles, which may contain fungal structures.

On the other hand, the presence of *Phoma* sp., *Helminthosporium* sp., *Penicillium* sp., *Cladosporium* sp. and

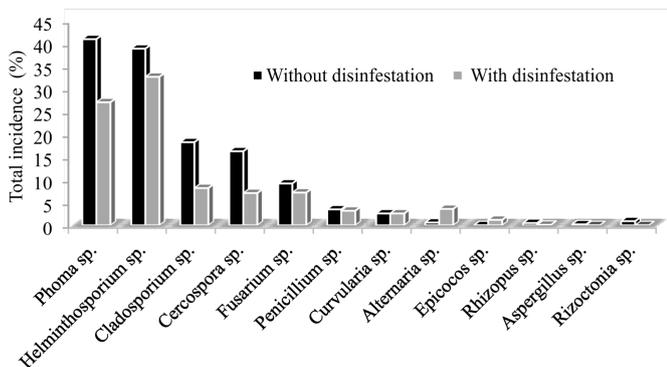


Figure 1. Total incidence of fungi detected during the sanitary test in seeds of *P. maximum* cv. 'Tanzânia', with and without superficial disinfection

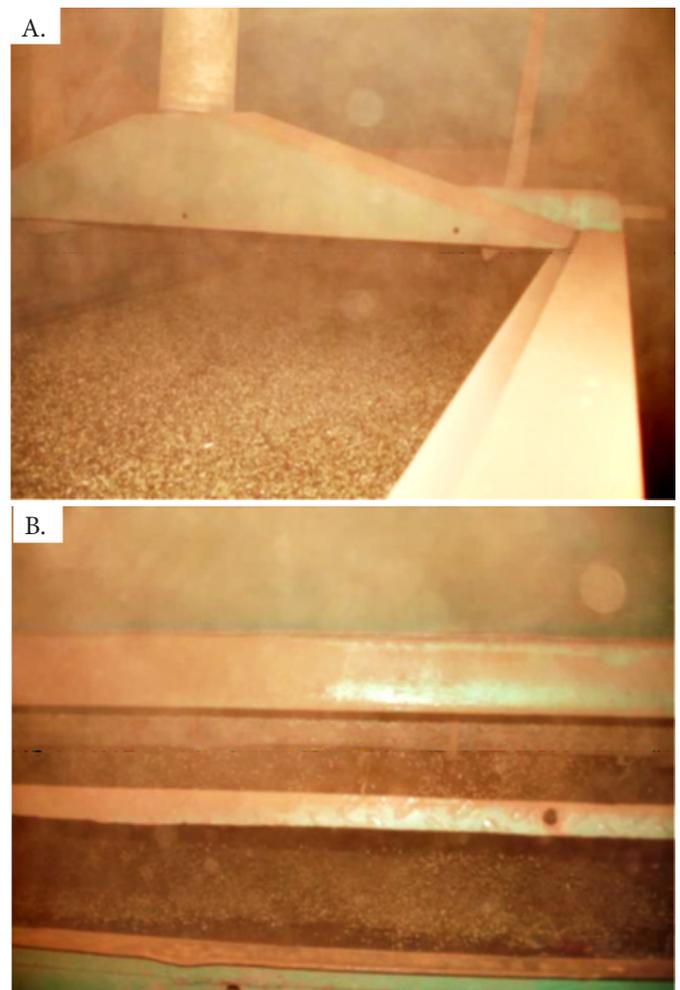


Figure 2. Dust and earth in suspension impregnating the gravity table (A) and the air and screen machine (B) during the processing of *Panicum maximum* seeds

Cercospora sp. also in disinfested seeds allows to infer that the dissemination structures were found inside the seeds of *P. maximum*. Therefore, part of the contamination with these pathogens must have occurred still at the field during seed formation. This fact is possible, because there are reports on the occurrence of these fungi in pastures (Martinez et al., 2010; Mallmann et al., 2013).

Among the studied fungi, the interaction of the seed processing steps and disinfection procedure was only observed on the percentage of incidence of *Phoma* sp., *Helminthosporium* sp., *Fusarium* sp. and *Penicillium* sp. (Table 1).

The disinfection significantly reduced the incidence of *Phoma* sp. in the seeds obtained in almost all processing steps, except in those from the treating machine (T8). In this operation, the seeds were dyed and the procedure may have washed part of the dissemination structures present on seed surface. The seeds without disinfection showed lower percentage of pathogens in comparison to those disinfested and subjected to the treating machine.

For the incidence of *Phoma* sp. in the seeds without superficial disinfection, compared with the control, there were lower percentages of this pathogen in samples from the reject material obtained at the bottom of the air and screen machine (T4), in the upper spout of the first gravity table (T6), after the seed treating machine (T8) and in the lower spout

Table 1. Fungal incidence in seeds of *Panicum maximum* cv. 'Tanzânia' with (WD) and without (WOD) superficial disinfection with sodium hypochlorite, as a function of the processing steps

Processing steps (P)	<i>Phoma</i> sp.		<i>Helminthosporium</i> sp.		<i>Fusarium</i> sp.		<i>Penicillium</i> sp.	
	WOD	WD	WOD	WD	WOD	WD	WOD	WD
T1	65 dB	30 bcA	64 cB	28 aA	6 abcA	11 abA	0 aA	0 aA
T2 ¹	53 cdB	14 abA	23 abA	24 aA	1 aA	10 abB	4 abA	8 bA
T3	46 bcdB	29 bcA	54 cA	46 aA	2 abA	3 aA	0 aA	1 abA
T4 ¹	27 abB	12 aA	45 abcB	20 aA	6 abcA	4 aA	5 abA	5 abA
T5 ¹	48 bcdB	30 bcA	27 abA	27 aA	2 abA	3 aA	1 aA	5 abA
T6	31 abcB	19 abA	42 abcA	31 aA	14 bcA	11 abA	5 abB	0 aA
T7	49 bcdB	31 bcA	21 aA	28 aA	6 abcA	3 aA	2 aA	2 abA
T8	17 aA	30 bcB	30 abcA	35 aA	18 cB	2 aA	1 aA	1 abA
T9	44 bcdA	42 cA	33 abcA	36 aA	9 abcA	20 bB	0 aA	2 abA
T10 ¹	40 bcdA	33 bcA	42 abcA	37 aA	20 cB	5 aA	14 bB	4 abA
T11 ¹	31 abcA	28 bcA	47 bcB	28 aA	17 Cb	7 abA	7 abA	7 abA
F Disinfection (D)	38.12**		6.68*		1.78*		6.68*	
F Processing (P)	6.85**		3.55**		4.45**		3.55**	
F (D x P)	4.66**		2.80**		4.41**		2.80**	
CV (%)	28.37		30.79		90.65		30.79	

**; *Significant at 0.01 and 0.05 probability levels by F test, respectively; Means followed by the same lowercase letter in the column and same uppercase letter in the row do not differ by Tukey test at 0.05 probability level; ASM - Air and screen machine; GTI - First gravity table; GTII - Second gravity table; T1 - Control (not processed); T2 - Upper screen of the ASM (reject); T3 - Intermediate screen of the ASM; T4 - Bottom of the ASM (reject); T5 - Drift GTI (reject); T6 - Upper spout of GTI; T7 - Intermediate spout of GTI; T8 - Treating machine (T7 + dyeing); T9 - Upper spout of GTII; T10 - Intermediate spout of GTII (reject); T11 - Lower spout of GTII (reject); ¹Processing steps that result in reject material by the company, for having more than 90% of impurities in its composition

of the second gravity table (T11). Thus, it demonstrated the importance of the processing to reduce fungal structures of *Phoma* sp. in the seeds of *P. maximum*, cv. 'Tanzânia'.

These results allow to claim that the rejects of the processing can also represent risk of fungal dissemination to other pasture areas, because this material is usually commercialized and mixed with other lots to meet less demanding markets of forage seeds (Hessel et al., 2012; Mallmann et al., 2013).

The percentage of *Helminthosporium* sp. in the seeds was reduced by the superficial disinfection and there was higher incidence of this microorganism in the seeds without disinfection; this event was observed in some treatments, such as seeds not processed (T1), from the bottom of the air and screen machine (T4) and lower spout of the second gravity table (T11).

In the seeds without disinfection, the reject material obtained in the upper screen of the air and screen machine (T2) and drift of the first gravity table (T5) and seeds obtained in the intermediate spout of the first gravity table (T7) showed lower incidence of *Helminthosporium* sp. in comparison to the control. Hence, it highlights the importance of seed processing for the improvement of sanitary quality, corroborating the reports of Fessel et al. (2003) for maize seeds and Mertz et al. (2007) for cowpea seeds.

The processing steps did not affect the incidence of *Fusarium* sp. in the seeds, because none of them significantly altered the occurrence of this fungus, regardless of the disinfection process. The superficial disinfection of the seeds also did not affect the incidence of *Fusarium* sp. along each processing step. This means that the detected fungus was found inside the seeds (Cappelini et al., 2005).

Phoma sp. and *Fusarium* sp. can be considered as the main pathogenic microorganisms associated with forage grass seeds, have rapid, aggressive growth, and can cause seed death and reduction in seedling emergence percentage (Yang et al., 2011; Kuhnem Júnior et al., 2013; Mallmann et al., 2013). Additionally, they were considered by Martins et al. (2001)

and Marchi et al. (2010) as the most common fungi in pastures of *P. maximum*.

In general, *Penicillium* sp. was found with low frequency in the treatments, between 0 and 14%. This is considered by Vechiato et al. (2010) as a fungus of storage and its low incidence in this research can be attributed to the use of recently collected seeds.

There was no interaction between the seed processing steps and disinfection procedure on the occurrence of *Cladosporium* sp. and *Cercospora* sp. (Table 2). However, the disinfection reduced the incidence of these fungi, possibly because they are found on the surface of the seeds.

Table 2. Fungal incidence in seeds of *Panicum maximum* cv. 'Tanzânia' with and without superficial disinfection with sodium hypochlorite, as a function of the processing steps

Processing steps (P)	<i>Cladosporium</i> sp.	<i>Cercospora</i> sp.
	(%)	
T1	5 a	7 a
T2 ¹	9 ab	7 a
T3	3 a	10 ab
T4 ¹	20 b	9 ab
T5 ¹	11 ab	12 ab
T6	18 b	12 ab
T7	15 b	12 ab
T8	15 b	19 b
T9	18 b	15 ab
T10 ¹	18 b	14 ab
T11 ¹	16 b	14 ab
F Disinfection (D)	38.55**	42.10**
F Processing (P)	4.70**	2.33*
F D x P	1.70 ^{ns}	1.61 ^{ns}
CV (%)	69.78	76.95

**; *Significant at 0.01 and 0.05 probability levels by F test, respectively; Means followed by the same lowercase letter in the column and same uppercase letter in the row do not differ by Tukey test at 0.05 probability level; ASM - Air and screen machine; GTI - First gravity table; GTII - Second gravity table; T1 - Control (not processed); T2 - Upper screen of the ASM (reject); T3 - Intermediate screen of the ASM; T4 - Bottom of the ASM (reject); T5 - Drift GTI (reject); T6 - Upper spout of GTI; T7 - Intermediate spout of GTI; T8 - Treating machine (T7 + dyeing); T9 - Upper spout of GTII; T10 - Intermediate spout of GTII (reject); T11 - Lower spout of GTII (reject); ¹Processing steps that result in reject material by the company, for having more than 90% of impurities in its composition

Regarding the effect of processing steps on the incidence of *Cladosporium* sp., it was possible to observe, in comparison to the control, that the reject from the bottom of the air and screen machine (T4) and all samples from the spouts of the two gravity tables (T6, T7, T9, T10 and T11) and from the treater (T8) have seeds with higher percentage of contamination. Therefore, in general, the processing favored the increase in the incidence of *Cladosporium* sp.

Some fungi, such as *Cladosporium* sp., are favored by mechanical damages to the seeds, as observed in soybean by Gomes et al. (2009). In the processing, the successive impacts and falls in the deposits of the machines cause injuries; therefore, these damages are cumulative and favor the access of microorganisms to the inside of the seeds (Carvalho & Nakagawa, 2012). This phenomenon must have favored the incidence of *Cladosporium* sp., because the final steps of the processing contain seeds with higher percentages of this pathogen.

The same phenomenon seems to have occurred with *Cercospora* sp., although it was only possible to observe higher significant incidence of this fungus in comparison to the control, due to the use of the treater to dye the seeds (T8). The increase in incidence of *Cercospora* sp. due to the treater (T8) may have been caused by the greater mechanical damage caused by the machine during the movement of the seeds soaked in the dyeing solution, favoring the access of the fungus.

CONCLUSIONS

1. Some processing steps can reduce the percentage of seeds of *P. maximum* cv. 'Tanzânia' contaminated with *Phoma* sp. and *Helminthosporium* sp.
2. The processing, in general, can increase the percentage of seeds with *Cladosporium* sp. and *Cercospora* sp.
3. *Phoma* sp., *Helminthosporium* sp., *Penicillium* sp., *Cladosporium* sp. and *Cercospora* sp. are found inside and outside the seeds and can be disseminated by the processing machines.

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