Engenharia Agrícola

ISSN: 1809-4430 (on-line)

www.engenhariaagricola.org.br

Doi: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v39n2p234-239/2019

INCIDENCE OF FUNGI IN MOMBASA GRASS SEEDS DURING THE STAGES OF THE SEED CONDITIONING PROCESS

Givanildo Z. da Silva[1*], Cibele C. Martins[2], Lilian F. de Melo[3], Tatiane S. Jeromini[2], Rita de C. Panizzi[2]

[1*]Corresponding author. Universidade Federal de Goiás - Regional de Jataí/ Jataí - GO, Brasil.
E-mail: givanildozildo@hotmail.com | ORCID ID: https://orcid.org/0000-0002-6380-1599

KEYWORDS

seed pathology, processing, seed sanitation, *Panicum maximum* 'Mombasa'.

ABSTRACT

The objective of this study was to evaluate the effect of the seed conditioning process on the sanitary quality of *Panicum maximum* 'Mombasa' seeds. The seeds were sampled before processing and after exiting the seed blower and sieves (discharge from the top and bottom sieves), first gravity separator (intermediate discharge), seed treatment equipment (dyeing the seeds of the intermediate discharge from the first separator), and second gravity separator (upper, intermediate, and lower discharge). Sanitary analysis was performed by the Blotter Test method with and without surface disinfestation of the seeds, which were incubated at 20 ± 2 °C, with a photoperiod of 12 h, for 7 d. The conditioning process increased the incidence of *Fusarium* sp. in the Mombasa grass seeds. In addition, *Cladosporium* sp. and *Alternaria* sp. were found in and on the surface of the seeds and, therefore, can be disseminated by the seed conditioning machines. The percentage of seeds with *Alternaria* sp. decreased after dyeing and after the top discharge of the second gravity separator stage.

INTRODUCTION

With the expansion of pastures and intensification of livestock activity in recent years, several diseases have begun to occur and cause losses in forage grass productivity, especially in central-western and northern Brazil; contaminated seeds are a method of introduction and dispersal of pathogens, causing forage diseases in these regions, and in areas that were previously free of these pathogens (Mallmann et al., 2013).

The pathogens present in seeds can affect germination, seedling development, and the longevity and production of forage plants in the field. Pathogens can also prevent seeds from being traded on the global market, as some countries may have restrictions on seeds with sanitary issues (Santos et al., 2014; Mallmann et al., 2013).

Panicum maximum is an important forage crop but little is known about the dissemination of pathogens by seeds and its effects (Marcos et al., 2015). Some important and potentially pathogenic fungi have been detected in the seeds of *P. maximum* and *Brachiaria* spp. (another forage crop), such as *Curvularia*, *Phoma*, *Fusarium*, *Exserohilum*, *Cercospora,* and *Helminthosporium*. In addition, other fungi such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Nigrospora*, *Penicillium,* and *Trichoderma*, which are considered secondary and storage fungi, have also been detected (Martinez et al., 2010; Pereira et al., 2012; Mallmann et al., 2013; Marcos et al., 2015).

Compared to large crops, whose seeds are harvested directly from the plant, the seeds of tropical forage grasses are harvested by sweeping the soil of the production field after seed dispersal, which makes them more exposed to fungus contamination (Quadros et al., 2012). Thus, seed lots of *Panicum* spp. and *Brachiaria* spp. are received in processing plants mixed with earth, sand, stones, straw, and empty spikelets, which are subsequently totally or partially removed to meet commercialization standards, and to allow for the seeds to be easily sowed (Hessel et al., 2012; Quadros et al., 2012; Melo et al., 2016a, 2016b).

Seed conditioning machines clean, sort, and treat (usually only by dyeing the seeds) seeds for improvement

[2] Universidade Estadual Paulista "Júlio de Mesquita Filho"/ Jaboticabal - SP, Brasil.
[3] Faculdades Associadas de Uberaba/ Uberaba - MG, Brasil.
Received in: 5-17-2015
Accepted in: 1-22-2019

of their physical, physiological, and sanitary quality (Melo et al., 2016a, 2016b; Hessel et al., 2012). The steps of this process allow for the removal of undesirable material, which directly affects the physical quality of the seed lot and indirectly affects the sanitary quality and the physiological potential (Hessel et al., 2012; Pereira et al., 2012), However, these steps may also contaminate the seed lots that will later be conditioned, especially when these machines are not cleaned properly.

With the above in mind, the objective of this study was to evaluate the effect of the seed conditioning process on the sanitary quality of *Panicum maximum* 'Mombasa' seeds.

MATERIAL AND METHODS

The *P. maximum* 'Mombasa' seeds were mechanically harvested by sweeping the soil using a Racing 3500 Hercules harvester during the 2013/2014 harvest in Jatai, GO (Brazil). The seeds were then conditioned using a seed blower and sieves, two gravity separators, and seed treatment equipment. After stabilizing the machines, the seeds were sampled at each stage of the conditioning process, as shown in Figure 1.

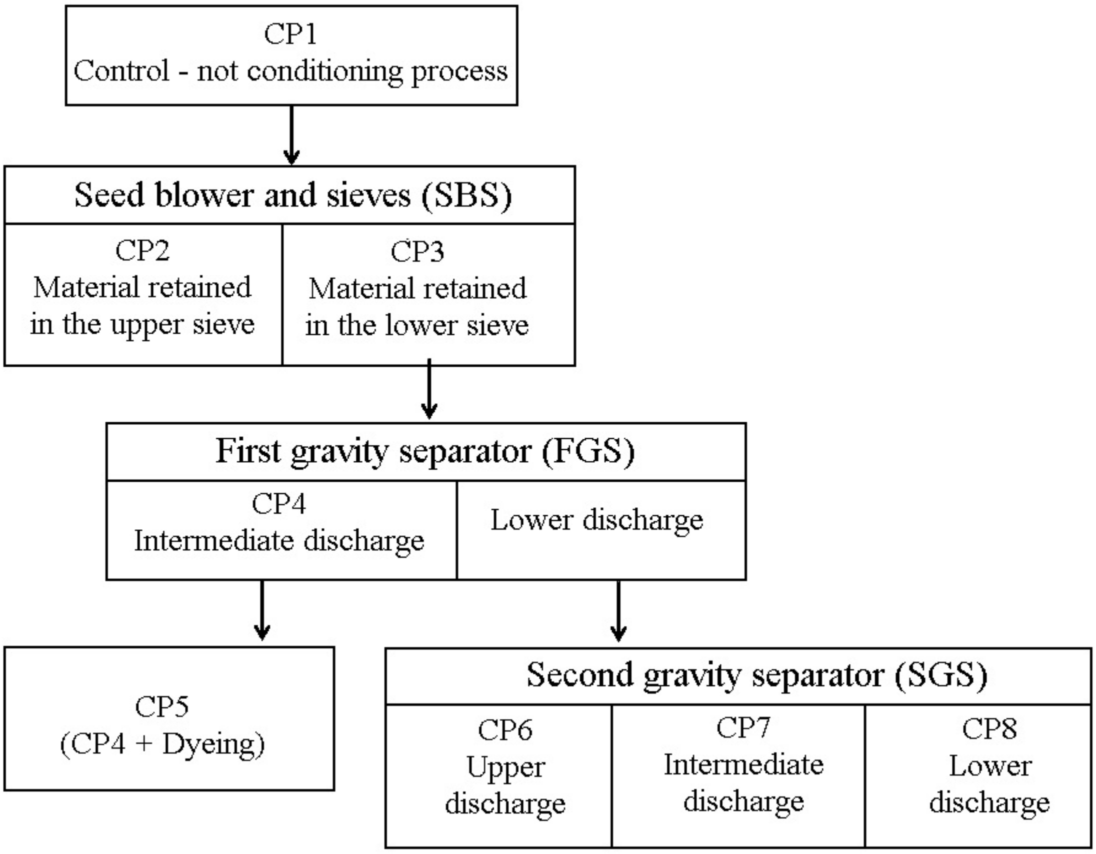

FIGURE 1. Flowchart of the seed conditioning process of *Panicum maximum* 'Mombasa' seeds, showing the sampling points of the conditioning stages (CP1, CP2, CP3, CP4, CP5, CP6, CP7, and CP8).

Eight stages of the conditioning process were evaluated. Seeds sampled for the first stage (CP1) were not subjected to the conditioning process and were considered as the control. The other samples were taken from various stages of the conditioning process, as described below:

Seed blower and sieves: after passing through the seed blower and sieves, the CP2 sample was collected from the discharged material retained in the upper sieve, which was a round sieve with a diameter of 7/32 of inch (5.56 mm). The CP3 sample was collected from the bottom nozzle of the lower sieve, which was a wire mesh sieve of 20 AWG (0.8118 mm diameter) square holes of 30 × 30 mm aperture.

First gravity separator: after passing through the seed blower and sieves, the CP4 sample was collected from the intermediate discharge, which was composed of material collected in the intermediate segment, 60 cm from the table output. The seeds from the lower discharge, composed of a fraction of the material collected 30 cm from the lower end of the separator, were not sampled, but transferred onto a second gravity separator, following the usual seed conditioning process for this species (Melo et al., 2016a). This equipment had an area of 2.40 m × 1.25 m, a vibration velocity of 1,750 rpm, and a transverse and longitudinal slope of 17° and 12°, respectively.

Seed treatment equipment: in the sequence, only the seeds from the intermediate discharge of the first gravity separator were submitted to dyeing, since this is the sample that is directly intended for commercialization. The sole aim of spraying them with the green dye (Laborsan Brasil®) and mechanical handling of the mass in a treatment machine (Seed Mix VHM-4/10 T) is to improve the seed appearance. After this procedure, seeds for the CP5 sample was collected.

Second gravity separator: after passing through the first gravity separator, three samples were collected. The second gravity separator was identical to the first one, but with adjustments to the discharges. Seeds from the upper discharge, composed of the material collected 60 cm from the upper end of the separator exit, were collected for the CP6 sample. Seeds in the intermediate segment, 45 cm from the exit of the table, were collected for the CP7 sample. Seeds from the lower discharge, composed of material collected 20 cm from the lower end, were collected for the CP8 sample.

It should be noted that samples from the bottom of the seed blower and sieves, and from the drift and top discharge of the first gravitational table could not be collected as they did not present seeds. Therefore, the health of seeds at these steps were not evaluated.

Samples from each stage of conditioning were sent to the Laboratory of Seed Pathology of the Department of Phytosanitary of the Faculty of Agrarian and Veterinary Sciences (UNESP), Campus de Jaboticabal/SP, where the pure seeds were manually separated from any impurities and submitted for health analysis. For the analysis, 10 replicates of 10 seeds from each stage, with and without surface disinfestation were tested. Surface disinfestation was performed by immersing the seeds in NaClO (1%) for three minutes, followed by rinsing with sterilized water and drying at room temperature.

The filter paper method (Blotter Test) was used to assess presence of fungi in seeds. The seeds were distributed equal distances apart on three sheets of filter paper previously moistened with distilled water and incubated in 9.0 cm diameter Petri dishes for 7 days at 20 ± 2°C, with 12 h photoperiod. Afterwards, the seeds were analyzed individually under a stereoscopic microscope, and fungi were identified by the morphological characteristics of their structures. The results were expressed as the percentage of seeds contaminated by each fungus (adapted, BRASIL, 2009).

The experiment was evaluated using a completely randomized design, in a 2 × 8 factorial scheme (surface disinfestation × processing steps). The incidence data (%) of fungi in the seeds was transformed using $(x + 0.01)^{0.5}$ to meet the assumptions of normality and homogeneity of variances for tests. The data was subjected to analysis of variance by the F test, and differences in means between stages of the conditioning process were compared by Tukey test ($\alpha = 0.05$). Statistical analyses were performed using AgroEstat software (Silva & Azevedo, 2016). For the interpretation of the results, the averages of the original data are presented.

RESULTS AND DISCUSSION

In the sanitary analysis of *P. maximum* 'Mombasa' seeds, in general, 14 types of fungi were detected. Six were found with relatively high incidence, with values above 5%: *Fusarium*, *Phoma*, *Cladosporium*, *Alternaria*, *Helminthosporium* and *Penicillium* (Figure 2); whereas the others were found with low incidence, with values less than 2% *Cercospora*, *Curvularia*, *Epicoccum*, *Microspora*, *Rhizopus*, *Pyrenochaeta*, *Aspergillus,* and *Rhizoctonia*. The genus *Cladosporium*, *Curvularia*, *Fusarium*, and *Phoma* constitute the most common phytopathogenic fungi in the Mato Grosso do Sul and Mato Grosso seed production fields (Mallmann et al., 2013).

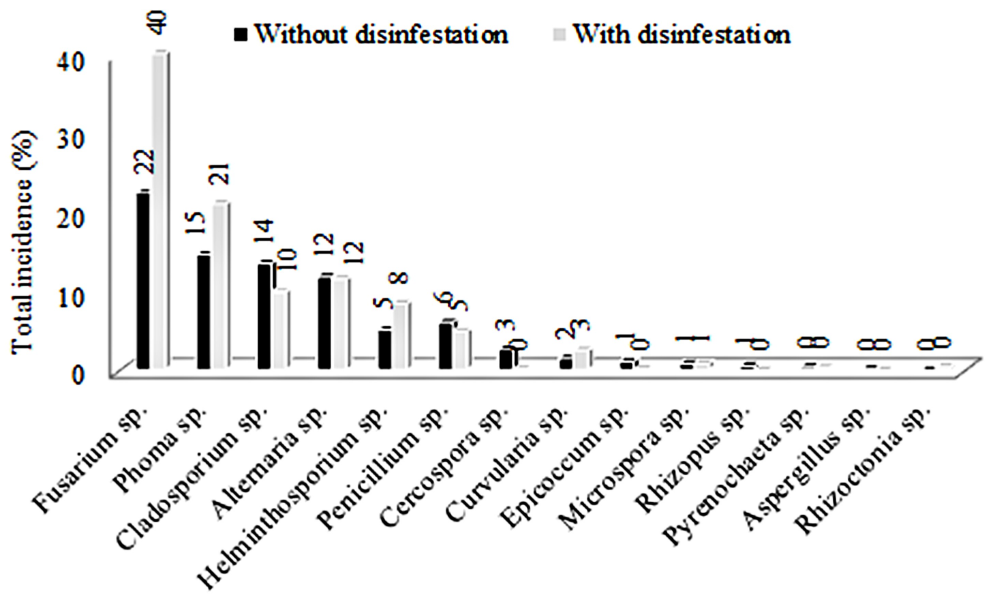

FIGURE 2. Total incidence of fungi detected during the sanitary test of *Panicum maximum* 'Mombasa' seeds, with and without superficial disinfestation.

Almost all the fungi with low incidence in the seed lot were not affected by surface disinfestation, except for *Cercospora* sp. and *Epicoccum* sp. which were controlled by the disinfestation procedure (Figure 2). It is possible that the structures of these fungi were located in the external part of the seeds, so disinfestation of these seeds with NaClO reduced the incidence of these microorganisms. A reduction of *Epicoccum* sp. by disinfestation was previously found in tobacco seeds (Segato & Gabaldi, 2012). It should be noted that the occurrence of some fungi poses a risk even at percentages less than 10%. These microorganisms multiply rapidly and can contaminate seed lots while in storage. This was having been observed in *Alternaria* sp., which was responsible for affecting the physiological quality of fennel seeds, despite being found at an incidence lower than 1% (Gama et al., 2012).

In the present study, it was verified that the incidence of *Cladosporium* sp., *Fusarium* sp., and *Alternaria* sp. was affected by the interaction between the stages of the conditioning process and seed disinfestation (Table 1).

For *Cladosporium* sp., the stages of the conditioning process, in isolation, affected the sanitary quality of the seeds, without taking into consideration disinfestation. In this case, the seeds from the lower sieve of the seed blower and sieve machine (CP3) were less contaminated than those from the intermediate discharge from the second gravitational table (CP7), which were more contaminated. However, seeds from both steps did not differ in percentage infestation from the unprocessed control seeds (CP1).

The reduction in incidence of *Cladosporium* sp. due to seed disinfestation (Figure 2 and Table 1) suggests that a significant percentage of structures of this fungus were lodged superficially in the seeds, similar to what was previously reported for *Cercospora* sp. and *Epicoccum* sp. Considering that contaminated seeds can be responsible for introducing and dispersing pathogens (Mallmann et al., 2013), these fungi could be disseminated by the machines during the conditioning process from one seed lot to another.

On the other hand, from the presence of *Cladosporium* sp., *Cercospora* sp., and *Epicoccum* sp. in disinfested seeds, we can infer that a part of the fungi structures was housed inside the seeds of *P. maximum*, either in tissues of the embryo or endosperm (Figure 2, Table 1). Therefore, attack by these pathogens must have occurred in the field during seed formation. In support of this, there are reports of the occurrence of *Cercospora* sp., *Epicoccum* sp., and *Cladosporium* sp. in pasture areas (Pineda et al., 2002; Mallmann et al., 2013).

TABLE 1. Incidence of fungi *Fusarium* sp., *Cladosporium* sp., and *Alternaria* sp. in seeds of *Panicum maximum* 'Mombasa' with (CD) and without surface disinfestation (SD) with NaClO, at different stages of the conditioning process.

Conditioning process	*Cladosporium* sp. (%)		*Fusarium* sp. (%)		*Alternaria* sp. (%)	
	SD	CD	SD	CD	SD	CD
CP1. Control (not conditioning process)	19 abA	13 aA	18 aA	48 aB	20 cB	1 aA
CP2. Material retained in the upper sieve SBS	15 abB	7 aA	19 abA	44 aB	9 abcA	9 abA
CP3. Material retained in the lower sieve SBS	6 aA	17 aB	16 aA	45 aB	13 bcB	5 aA
CP4. Intermediate discharge FGS	14 abA	8 aA	19 abA	35 aB	11 abcA	7 abA
CP5. Intermediate discharge + Dyeing	7 abA	8 aA	33 abA	22 aA	1 aA	13 abB
CP6. Upper discharge SGS	15 abA	9 aA	14 aA	47 aB	6 abA	23 bB
CP7. Intermediate discharge SGS	23 bB	9 aA	17 aA	41 aB	10 abcA	18 bB
CP8. Lower discharge. SGS	9 abA	8 aA	43 bA	38 aA	16 bcA	10 abA
F Disinfestation (D)	7.26^{**}		42.92^{**}		0.02^{ns}	
F Conditioning process (CP)	1.35^{ns}		1.20^{ns}		2.16^{*}	
F (DxCP)	2.46^{*}		4.31^{**}		7.74^{**}	
C. V. %	72.82		37.39		72.78	

**,* and ^{ns} Significant at 1%, at 5% and not significant by the test F. Means followed by the same lowercase letter in the column and of the same capital letter in the row, do not differ among themselves by the Tukey test at 5% probability. SBS- Seed blower and sieves, FGS- First gravity separator, SGS- Second gravity separator.

In disinfested seeds, the reduction of these fungi seems to have favored the development of other fungi due to less competition for seed colonization. Disinfestation significantly increased the incidence of *Cladosporium* sp. in the seeds from the lower sieves machine (CP3). Disinfestation also significantly increased the incidence of *Fusarium* sp. in seeds obtained from almost all the stages of the conditioning process, except for those coming from the seed treatment equipment (CP5) and the lower discharge of the second gravitational table (CP8). In this last stage of the conditioning process, the non-disinfested seeds presented the highest percentages of this fungus compared to the sample that had not been subjected to the conditioning process (CP1).

The seeds from the lower discharge of the second gravitational table (CP8) are generally considered waste by the company. However, this material can be marketed and mixed with other seed lots to satisfy some less-demanding forage seed markets (Hessel et al., 2012; Mallmann et al., 2013). Therefore, these seeds may increase risk of fungi dissemination to pasture areas.

The lower discharge of the gravitational table usually separates *P. maximum* seeds (Melo et al., 2016a, 2016b) and *Nicotiana tabacum* seeds (Gadotti et al., 2012) that have a smaller dry mass, lower germination rate, and less vigor. For several species, it was found that seeds with these characteristics are more susceptible to attack by *Fusarium* sp. (Pereira et al., 2012; Mertz et al., 2007). In addition, the high number of contaminated seeds in the lower discharge, which generally consists of low-density seeds, may have occurred because the pathogens consume the dry matter of the seeds (Pereira et al., 2012).

Regarding the incidence of *Alternaria* sp. in the seeds without surface disinfestation, lower percentages of this pathogen were found in the seeds from the seed treatment equipment (CP5) and the upper discharge of the second gravitational table (CP6) in comparison to the control. The seeds attacked by this pathogen may have been removed due to their lower weight after being contaminated, as lower density seeds are separated at this stage of processing. Furthermore, the dye used in the seeds of the fourth stage could have created a barrier that prevented proliferation of the microorganism. Thus, the effectiveness of these steps of the conditioning process in the removal of seeds contaminated by this pathogen was verified.

In the seeds that were not subjected to the conditioning process (CP1) it was found that disinfestation drastically decreased contamination by *Alternaria* sp., with a decrease in incidence of 19%. In seeds collected from stages of the conditioning process, however, the beneficial effect of disinfestation was diminished, as disinfestation had little or no influence on the incidence of this fungus in seeds obtained in the upper and lower sieves of the seed blower and sieves machine (CP2 and CP3) or intermediate discharge of the first gravitational table (CP4). Furthermore, for the seeds obtained after treatment by the seed treatment equipment (CP5), upper and intermediate discharge of the gravitational table (CP6 and CP7), disinfestation favored the development of *Alternaria* sp..

During the conditioning process, seeds experience a series of physical collisions within the machine, causing injuries and cracks. This damage accumulates as the seeds move along the conditioning process, allowing microorganisms to access the interior of the seeds (Carvalho & Nakagawa, 2012) In the disinfested seeds, the reduction of superficial fungi may have allowed for the development of other fungi within the seed due to less competition for colonization. Therefore, it can be inferred that disinfestation performed before the sanitary test was efficient only in controlling *Alternaria* sp. in undesirable or less damaged seeds, since the microorganisms were housed on the surface of the seed, whereas in more damaged seeds these microorganisms are protected from disinfestation because they are lodged in the seed tissues.

For the occurrence of *Phoma* sp. and *Helminthosporium* sp. fungi in the Mombasa grass seeds, an interaction between the stages of the seed conditioning process and the disinfestation procedure was not found (Table 3). Nevertheless, disinfestation increased the incidence of both fungi (Table 2, Figure 2). These results resemble those found for *Fusarium* sp. in this study.

TABLE 2. Incidence of *Phoma* sp. and *Helminthosporium* sp. fungi in *Panicum maximum* 'Mombasa' seed with and without surface disinfestation with NaClO at different stages of the conditioning process.

Conditioning process	*Phoma* sp. (%)	*Heminthosporium* sp. (%)
CP1. Control (not conditioning process)	17 ab	6 ab
CP2. Material retained in the upper sieve SBS	20 ab	7 ab
CP3. Material retained in the lower sieve SBS	28 b	9 ab
CP4. Intermediate discharge FGS	20 ab	4 ab
CP5. Intermediate discharge + Dyeing	10 a	3 a
CP6. Upper discharge SGS	18 ab	11 b
CP7. Intermediate discharge SGS	16 ab	8 ab
CP8. Lower discharge. SGS	15 ab	9 ab
F Disinfestation (D)	11.82^{**}	10.30^{**}
F Conditioning process (CP)	3.42^{**}	3.00^{**}
F (DxCP)	1.52^{ns}	0.94^{ns}
C. V. %	50.02	100.89

** and ^{ns} Significant at 1% and not significant by the test F. Means followed by the same lowercase letter in the column and of the same capital letter in the row, do not differ among themselves by the Tukey test at 5% probability. SBS- Seed blower and sieves, FGS- First gravity separator, SGS- Second gravity separator.

The disinfestation of seeds and control of fungi such as *Cladosporium* sp., *Cercospora* sp. and *Epicoccum* sp. (Table 1, Figure 1) may have favored the development of other fungi such as *Fusarium* sp. by reducing competition for survival and seed colonization. This was also found for other types of fungi in research with forage grass seeds (Santos et al., 2014) and maize (Antonello et al., 2009).

During the stages of the conditioning process, it was found that some machines were able to select seeds with higher or lower incidence of *Phoma* sp. and *Helminthosporium* sp., but differences in percentages of infestation compared with control seeds were not statistically significant. Thus, even if these stages of conditioning can improve other attributes of the seeds for commercialization, they are not effective in terms of sanitary quality.

CONCLUSIONS

The conditioning process increases the incidence of *Fusarium* sp. in the Mombasa grass seeds.

Cladosporium sp. and *Alternaria* sp. are housed internally and externally in the seeds and can be disseminated by the seed conditioning machines.

The percentage of seeds with *Alternaria* sp. decreases after dyeing and the upper discharge of the second gravitational table.

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