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RESEARCH ARTICLE

PROSO MILLET SEED PHYSIOLOGICAL AND SANITARY QUALITY WITH PIG SLURRY COMPOSTING DOSES

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ABSTRACT

The pig slurry composting has been recommended as an alternative to reduce the volume and pollution potential of liquid pig manure.The pig slurry composting application can influence the physiological and sanitary quality of proso millet(*Panicum miliaceum L.)* seeds and their effects vary with environmental conditions. The objective was to evaluate the effect of pig slurry composting in physiological and sanitary quality of proso millet crop seeds produced under field conditions.The research was conducted in southern Brazil under a no-tillage system in a Rhodic Hapludox. A randomized block experimental design was used with four replications and five treatmentsconsisted of: a) control without fertilization; and doses of pig slurry compost: b) 4.0 Mg ha^{-1} c) 8.0 Mg ha^{-1}; d) 12.0 Mg ha^{-1}.The application of PSC doses positively affects the vigor of proso millet seeds expressed by accelerated aging, seedling emergence in the field and germination. The PSC doses provided greater weight of thousand seeds, the doses being 8 and 12 Mg ha^{-1} the greatest results. The PSC doses did not influence the electrical conductivity, first count, cold test in soil. Sanity of proso millet seed is not influenced by the PSC doses.

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INTRODUCTION

Proso millet (*Panicum miliaceum L.)* is one of the oldest grasses domesticated by man (Kalinova and Moudry, 2006); most of the operation is designed to feed captive birds (Abrantes *et al.,* 2010). It is also used as a substrate for malting beers (Zarnkow *et al.,* 2010), and a potential alternative to its use is the production of ethanol fuel (Rose and Santra, 2013). Currently proso millet is quite cultivated in Eastern Europe, Russia, China, India and North America, for food and feed (Karam *et al.,* 2004). In Brazil proso millet is still little cultivated and reduced economic expression when compared to traditional crops. However, in recent years has aroused interest in some regions.

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Proso millet has been used as kind of soil cover in crop rotation under no-tillage due of its advantages to the system (Silva *et al.,* 2013). Advantages like low cost, abundant formation speed of straw and grain production, short cycle (Lu *et al.,* 2009), can be grown in almost all year, provided there is enough moisture in the soil and there is no risk of frost (Zancanella *et al.,* 2006). With increasing proso millet economic interest, so does the demand for quality seeds. Seed quality is characterized by genetic, physical, sanitary and physiological is of fundamental importance in the production process of any plant species, to influence the development and crop quality. There are several factors that affect the quality of proso millet seeds, among them, the fertilization is considered a key factor in terms of quality seeds (Imolesi *et al.,* 2001), since proper nutrition of plants influences the formation of the embryo and the reserve tissue (endosperm), with a significant effect on the vigor (Sá, 1994). Therefore, the application of nutrients can affect to plant growth, productivity and the quality of the grain (Didonet, 1994). With respect to the proso millet crop, few studies have been developed with organic nutrition, especially when it comes to seed production. Although pig slurry is one of organic waste with the highest potential as fertilizer, especially in southern Brazil, little is known concerning the quantities to be used, which allow obtaining satisfactory yields in production and improvement in the quality of seeds (Toledo *et al.,* 2009). The creation system used by most pig producers is the total confinement of animals, causing a large concentration of waste in liquid form in small areas. Successive applications of pig slurry in the same area leading to environmental problems, especially the gaseous emissions of ammonia (NH^3), nitrous oxide (N_2O) (Gonzatto *et al.,* 2013; Aita *et al.,* 2014) and groundwater contamination by nitrate (NO^{3-}) (Zaman and Blennerhassett, 2010). Pig slurry composting (PSC) has been recommended as an alternative to reduce the pollution potential of the pig slurry. In this process, the pig slurry is mixed substrates with high C / N ratio, such as wood shavings and sawdust (Fukumoto *et al.,* 2011). Based on the above, this study aimed to evaluate the effect of pig slurry composting doses in physiological and sanitary quality of millet seeds cv. AL Tibagi produced under field conditions.

MATERIAL AND METHODS

The study was conducted in the experimental area of the Universidade Federal de Santa Maria, in Frederico Westphalen (27º23'S, 53º25'W and altitude of 566 m), Rio Grande do Sul State, Brazil, in 2014. The local climate is humid subtropical (CFA), according to the Köppen classification (Moreno, 1961). Meteorological data for the maximum and minimum air temperatures and daily precipitation were collected from an automatic weather station located 500 m from the experiment site, being electronically recorded in one minute intervals, with values integrated for daily data (Figure 1). The soil is classified as Rhodic Hapludox, or the soil is classified as Latossolo vermelhodistróficotípico in the Brazilian System of Soil Classification (Embrapa 2013). During the implementation of the experiment, soil samples were collected from the 0-20 cm depth layer, at the beginning of the experiment. pH em H_2O (1:1) 5.0; SMP index 5.4; clay 700 g kg^{-1}; organic matter 3.0 g kg^{-1}; P-mehlich 6.9 mg dm^{-3}; potassium 219 $mg.dm^{-3}$; calcium 3.1 $cmolcdm^{-3}$; magnesium 2.4 $cmolcdm^{-3}$; H + Al 6.1 cmolcdm-3; Al 0.5 $cmolcdm^{-3}$; CTC 12.2 $cmolcdm^{-3}$; and a percentage of CTC with bases and Al of 49.8 and 7.6%, respectively. Six months before the experiment, lime was applied to the soil in order to raise the pH to 6.0 (CQFS-RS/SC 2004). A randomized blocks design, with four replications and five treatments, where each plot had an area of 4.0 m x 3.6 m (14.4 m²), was used. The treatments consisted of a control without fertilization and PSC doses (4.0 Mg ha^{-1}, 8.0 Mg ha^{-1} and 12.0 Mg ha^{-1}). The experiment was conducted under a no-tillage system. A culture succession of proso millet/soybean (*Glycine max*) was implemented. After the soybean harvest, the area remained in a fallow period of three months. The area was subjected to one application of glyphosate (3.0 L ha^{-1}) for experimental setup. The proso millet seeding process was held manually August 22 (2014), using the AL Tibagi cultivar, spaced 0.45 m between rows and with a density of 120 seeds m^{-1}. The emergence of proso millet occurred about 10 days after seeding, for both years. The cycles of the experiments were 83 days. A compost sample was collected and sent to a laboratory for analysis (Tedesco *et al*., 1995). The chemical composition and amount of nutrients added to each administered dose is shown in Table 1.

The PSC doses were manually applied to the crop in each year, immediately after the proso millet seeding. For the mineral fertilizer, a manual process was also used (CQFS-RS/SC 2004). The N was applied as urea (100 kg ha^{-1} of N), P_2O_5 in the form of single superphosphate (60 kg ha^{-1} of P_2O^5) and K_2O in the form of potassium chloride (40 kg ha^{-1}). The following characteristics were evaluated in the proso millet culture: a) Hundred seed weight (HSW): held from the weighing of eight repetitions of 100 seeds taken from the main sample (Brasil, 2009); b) Germination (GT)- eight replicates of 50 seeds per treatment were arranged in rolls of paper, moistened with distilled water corresponding to 2.5 times the weight of the dry paper. The rolls were stored in sealed plastic bags and kept in a B.O.D. incubator at 25 °C for 9 days. The evaluation was conducted at five and nine days after sowing; the percentage of normal seedlings was measured;c) First count (FC) - performed together with the germination test; the number of normal seedlings was assessed on the 5th day after the start of the test. The results were expressed as percentage of normal seedlings; d) Accelerated aging (AA) - four replicates of 50 seeds of each treatment were laid out in a single layer on a screen inside gerboxes, avoiding contact with 40 mL of distilled water placed at the bottom. The gerboxes were sealed and kept inside an oven at 42 °C for 72 hours. Immediately after the end of the aging period, each replicate was placed in paper towel rolls, and exposed to the same conditions described for the germination test, but the percentage of normal seedlings was assessed at five days after sowing (Brasil, 2009).e) Electrical conductivity (EC) - conducted through the center-of-mass system with four replicates of 50 seeds per treatment. The seeds were accurately weighed to two decimal places after the decimal point, and then placed in 200 mL plastic cups filled with 75 mL distilled water, and kept in an incubator at a constant temperature of 25 °C. After 24 hours of soaking, electrical conductivity was measured in the soaking solution, using a digital conductivity meter whose results were expressed in $\mu S.\ cm^{-1} g^{-1}$ (Krzyzanowski *et al.,* 1999);f) Cold test without soil: It used the method described by Krzyzanowski *et al*., (1999) adapted for millet culture.

They used four replicates of 100 seeds for each treatment was divided into germitest paper, moistened with a quantity of water equivalent to 2.5 times the paper weight. After seeding the rolls were placed inside plastic bags that once sealed with adhesive tape were kept in chamber at 5 ° C for seven days. Elapsed this period, the rollers inside the plastic bags were transferred to B.O.D type chamber at 20 ° C, where they remained for seven days. The interpretation was done by computing the percentage of normal seedlings;g) Seedling emergence in field: It was conducted jointly with the emergency speed index test. The evaluation was performed 21 days after sowing, determining the seedling emergence percentages (Nakagawa., 1994); h) Sanity: The sanitary quality of millet seeds was determined by the method of "Blottertest" without aseptic. For this, four replications of 25 seeds were placed in gerboxes containing three sheets of paper germitest previously dampened with deionized water and incubated in a growth chamber for seven days at a temperature of 25 ° C (± 2) under 12 hours photoperiod. After this incubation period the seeds were examined individually under stereoscopic microscope and / or optical microscope, by computing the fungi incidence percentage. The identification was based on its morphological characteristics and with the aid of specific literature. The results were submitted to analysis of variance and when significant for the variables, the averages were compared by Tukey test 5.0% probability. For these analyzes, we used the statistical program Assistat 7.7 beta.

RESULTS AND DISCUSSION

The results in Table 2 show that the pig slurry composting doses significantly influenced the variables: germination (GT), accelerated aging (AA), hundred seed weight (HSW), seedling emergence in field (SEF). However, the variables first count germination (FC), electrical conductivity (EC) and cold test without soil (CTS) were not significant (Table 2). In the crop cycle, 680 mm of precipitation were recorded during the experiment (Figure 1). During the cultivation period, the proso millet crop suffered two drought intervals, with a 12-days period in full bloom that occurred 42 days after emergence (Figure 1). Thus, water availability during the second year of cultivation may have been a limiting factor to the crop development (Moreno *et al*. 2014). The values of percentage of germination (GT), expressed by the percentage of normal seedlings showed significant differences depending on the pig slurry composting doses (Table 2).

Silva *et al.,* (2004), working with different levels of nitrogen in the quality of two cultivars of millet seeds also did not observe significant differences in germination. The no influence of nitrogen fertilization on germination was also observed in other crops such as oats (Nakagawa *et al*., 2000; Kolchinski and Schuch, 2004). Also in grass ramirez, Mecelis *et al*. (1991) found no relationship between the nutritional aspect and the physiological seed quality (germination). As for the first count was no significant response. The vigor tests have been using instruments increasingly routine for the seed industry to determine their physiological quality due to its high sensitivity to give answers, however, in this study showed no significant difference, perhaps the drought may have interfered in answer.

Crusciol *et al*. (2003) work with nitrogen fertilization on bean crop and Schuch *et al*. (1999) in oat, which to assess the effect of nitrogen on seed vigor found that the percentage of seedlings in the first count was lower when performed nitrogen fertilization. Already Silva *et al*. (2001) evaluated doses and nitrogen application times and Nakagawa *et al*. (2000) evaluated N rates in coverage, both with oat seeds, consisted no effect on the vigor. According to Nakagawa (1994) samples that have higher percentage of normal seedlings in the first count are the most vigorous, although it was significant for this work. These results were contrary to work Farinelli *et al*. (2006) in bean seeds, which found that the nitrogen fertilization promoted increases in seed vigor quadratically. The Brazilian Ministry of Agriculture to establish the grass seed and forage legumes standards through Ordinance No. 381/98, the standard germination is 40% for millet (*Panicum miliaceum L.)* (Brasil, 1998).

Table 1. Chemical characteristics and (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and N inorganic contents existing in each PSC dose

Dose PCS	C	N	P	K	Ca	Mg	N inorganic[1]	pH	C: N
	------------------------------- $g\ kg^{-1}$ -------------------------------								
$Mg\ ha^{-1}$	199	34.8	14.7	34.6	36.8	13.5	15.7	6.8	5.72
	------------------------------ $kg\ ha^{-1}$ added------------------------								
4.0	796	139.2	58.8	138.4	147.2	5.4	62.8	-	-
8	1592	278.4	117.6	276.8	294.4	10.8	125.6	-	-
12	2388	417.6	176.4	415.2	441.6	16.2	188.4	-	-

[1] N inorcanic, $N\text{-}NH_4^+$and NO_3

The application of doses of 4, 8 and 12 $Mg\ ha^{-1}$ PSC did not differ, however, increased germination percentage values compared to the control. These results show that the organic matter (pig slurry composting) by itself was able to provide increase in germination of seeds of proso millet. The large mineral nitrogen present content in the PSC can also significantly influence germination. These data support Passarin *et al.* (2016) studying the application of pig slurry in soybean had significant response in the percentage of soybean seed germination, seed germination was equal to 86% at the dose of 100 $m^3\ ha^{-1}$. Oliveira *et al.,* (2000) and Diniz (1995) studied the seed germination percentage in the bean crop had higher percentage of germination when the plants were fertilized with liquid cattle manure. Already Alves (1999) found no effect of manure on germination of bean seeds. Abrantes *et al.* (2010) studied nitrogen in top-dressing in proso millet cultivation no significant differences depending on nitrogen application in the percentage of millet germination.

For proso millet the lowest value found was 64.1% with the application of PSC (Table 2), and this value shows that germination is above the standard set for other species of the same genus. The PSC doses significantly interfere with the weight of a thousand seeds. The doses 8 and 12 $Mg\ ha^{-1}$ had the highest thousand seed weight. The weight of thousand higher seeds with the PSC doses may be the increased availability of nutrients and organic matter in the surface layer, as compared to control (Derpsch *et al.,* 1991; Santos and Tomm, 2003). Result contrary to that found by Abrantes *et al.* (2010) did not affect the mass thousand millet seeds to the study of nitrogen.

According to Table 2, it is observed that the physiological quality of millet seeds analyzed by the accelerated aging test showed a significant difference. A dose of 4 $Mg\ ha^{-1}$ presented the highest percentage of accelerated aging, followed by doses 12 $Mg\ ha^{-1}$, and the lowest values are observer to the doses of

8 Mg ha $^{-1}$ and 0 Mg ha^{-1}. and Vieira *et al.* (1995) evaluating the physiological quality of wheat seeds found that the dose of 120 kg ha^{-1} of nitrogen in a single application in wheat crop provided the highest germination in the accelerated aging of seeds obtained.

emergence speed, favoring the development of pathogenic microorganisms, therefore, the seeds resistant to these unfavorable conditions are more vigorous (Cicero and Vieira, 1994). Doses of compost pig slurry influenced seedling emergence in the field.

Table 2. Thousand seed weight (TSW), First count (FC), Germination (GT), Cold test without soil, Electrical conductivity (EC), Accelerated aging (AA), Seedling emergence in field (SEF), on the application of pig slurry composting doses

Treatments	PMS (g)	G (%)	PCG (%)	EA (%)	CE (uS cm g^{-1})	TF (%)	EC (%)
Control	4.14 b	59.7 ab	71 a	68 b	54 a	80 a	55 b
4 Mg ha^{-1}	4.41 ab	68.3 a	72 a	92 a	52 a	82 a	65 a
8 Mg ha^{-1}	4.57 a	65.4 a	68 a	63 b	58 a	76 a	61 a
12 Mg ha^{-1}	4.62 a	64.1 a	67 a	72 ab	55 a	77 a	62 a
Cv (%)	15.3	12.2	11.2	10.8	15	8	4.5

*Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

Table 3. Percentages of proso millet seeds contaminated with fungi by applying pig slurry composting doses

Treatments	*Alternaria*	*Aspergilus*	*Cladosporium*	*Bipolaris*
Control	14.50 ab	0.75 a	6.25 a	0.50 a
4 Mg ha^{-1}	13.50 ab	2.50 a	4.75 a	0.25 a
8 Mg ha^{-1}	21.50 a	1.00 a	4.75 a	0.75 a
12 Mg ha^{-1}	15.00 ab	0.50 a	8.00 a	0.25 a
Cv (%)	12.2	11.2	10.8	15
Treatments	*Epicocum*	*Fusarium*	*Penicilium*	*Phoma*
Control	0.75 a	1.75 a	6.50 a	0.50 a
4 Mg ha^{-1}	2.25 a	1.50 a	12.75 a	0.25 a
8 Mg ha^{-1}	2.50 a	0.50 a	16.50 a	1.50 a
12 Mg ha^{-1}	1.50 a	4.00 a	12.75 a	0.75 a
Cv (%)	17.2	15.1	8.9	11.4

* Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

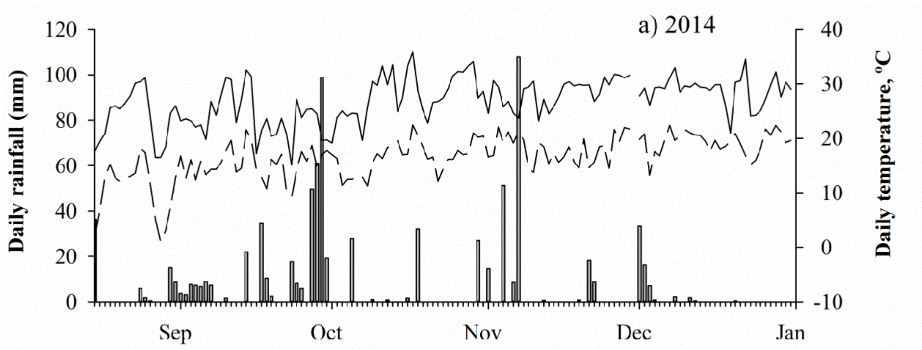

Figure 1. Average daily temperature and daily rainfall which occurred during the experiment. The rainfall data were obtained from the Meteorological Station in Frederico Westphalen – RS

The electrical conductivity was not influenced by pig slurry composting doses. The three doses did not differ from the control. A possible explanation for the lack of response may be low temperatures occurred during the experiment. Dias *et al.,* 1996 found that the electrical conductivity test is not effective for evaluating the deterioration of soybean seeds stored under low temperature conditions (10 ° C). Moreover, although the electrical conductivity test to provide reproducible results between laboratories, some factors may affect the results, such as genotype, seed development stage at harvest, changes in the structure and seed composition during development, size seed, temperature and soaking period and initial moisture content (Vieira *et al.,* 2002). The result of the cold test without soilindicated no statistical difference between treatments (Table 2). The combination of low temperatures and excess water in the soil can cause direct effects seed, reducing the

In this sense, probably the benefits provided by the addition of organic matter (PCS), allowed proso millet culture express greater seedling emergence in the field. In assessing the sanitary quality of the seeds, it was found the presence of various pathogens. Regarding the sanitary quality of millet seeds, quantification of the incidence of pathogenic fungi was detected: *Alternariaspp, Aspergillusspp, Cladosporiumspp, Bipolarisspp, Epicocumspp, Fusariumspp, Penicilliumspp, Phomaspp* (Table 3). Among the fungi only detected the fungus *Alternariaspp* presents statistical differences for the different treatments. Introducing higher incidence in treatment at a dose of 8 Mg ha^{-1}.The PSC has a large amount of nitrogen, the more recent the largest composting mineral N content in the ammonium form. According to Marschner (1995), high concentrations of nitrogen reduce the production of phenolic compounds, which have a fungistatic and lignification of the leaves, reducing the resistance to mandatory pathogens, and showing no action on those that are optional. The infection by fungal diseases is favored by nitrogen, as this increases the concentration of amino acids and amides in the apoplast and the cells of the mesophyll, substances that have greater influence than the sugars in the germination and development of conidia. The nutritional aspects of plant have an indirect effect on the incidence of pathogens in seeds, since one of the ways of contamination of the seeds is in the field. Thus plants growing in soils with nutritional stress are more susceptible to pathogens than plants growing in soil with balanced fertility. The use of plant pathogens of free seeds for crop establishment is of utmost importance, and, cultural practices, including the management of fertilization, can influence the contamination / infection of seeds obtained (Marschner, 1995).

Conclusion

The application of PSC doses positively affects the vigor of proso millet crops seeds expressed by accelerated aging, seedling emergence in the field and germination. The PSC doses provided greater weight of thousand seeds, the doses being 8 and 12 Mg ha-1 the greatest results. The PSC doses did not influence the electrical conductivity, first count, cold test in soil. Sanity of proso millet seed is not influenced by the PSC doses.

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