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Evaluation of the sanitary and physiological quality of corn seeds (*Zea mays*) submitted to treatment with bioextracts and their binary combinations

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Abstract. The objective of the present study was to evaluate the effects of the application of bioextracts on seeds of creole corn, regarding their physiological and sanitary quality. The bioextracts of garlic (*Allium sativum L*), citronella (*Cymbopogon nardus*), cloves (*Caryophilus aromaticus*), ginger (*Zingiber officinale* roscoe), and their binary combinations were evaluated. Regarding the physiological quality of the seeds, the following parameters were evaluated: first germination (G1), germination (G), dry mass and seedling length (aerial and root). Regarding sanitary control, in vitro tests were carried out for the pathology of the seeds for *Fusarium* sp. and *Aspergillus* sp. The results of physiological quality showed a germination rate between 90.8-76.4% of the creole corn seeds treated with bioextracts and their combinations did not differ significantly from the control (84.9%), except when clove alone (3.0% of germination) was used. The results of dry mass, length of shoots and root plants followed a similar trend to that observed for germination analysis. Regarding sanity, the best result was obtained with clove bioextract, which completely inhibited the occurrence of *Penicillium* sp. Therefore, these studies allow to affirm that bioextracts can be efficient in controlling pathogens and maintaining the physiological quality of creole corn seeds.
Keywords: Germination, Seed pathology, Organic agriculture.

Introduction

The culture of maize (*Zea mays*) is one of the most important in the world, being the serial used for human consumption and for animal food. The maize trade is third in the ranking of agribusiness foreign sales in the cereal sector, sold mainly in the form of grain, flour and preparations (Santos et al., 2017; Dias et al., 2018).

In this sense, to produce the corn grain, regardless of the production scale, the producer needs good quality seeds for sowing. The physiological quality of corn seeds can be influenced by the genetic characteristics inherited from their progenitors, germination and vigor. These last two factors can be affected by environmental conditions, methods of harvesting, drying, processing, treatment, storage, and packaging. For the seed to maintain its physiological quality, most of the time treatments are carried out with synthetic substances, here called pesticides and/or synthetic pesticides. These pesticides are the basis of support for agribusiness, but their use in farming can directly impact ecosystems and biodiversity. The use of pesticides in a safe way usually presents greater difficulty on the part of the family farmer, mainly due to the lack of information. Concern about pesticide contamination of farm workers, communities close to the farming and the final consumer of these foods has been recurrent (Antonello et al., 2009; Nascimento et al., 2019).

Generally, the effects that pesticides cause on health, can be divided into two types: the first comprises the acute effects that are those that result from exposure to concentrations of one or more toxic agents, capable of causing apparent effective damage in a period of 24 h. The second is chronic effects, those that result from continued exposure to relatively low doses of one or more pesticides. The risks inherent in the use of pesticides can be substantially reduced, if it is possible to substitute for less toxic products that have similar efficiency (Recena et al., 2006; Peres et al., 2013; Queiroz et al., 2020).

In this sense, studies with vegetable bioextracts have shown efficacy in the control of several agriculture pathogens. The use of these natural products can be a more sustainable alternative, in the control of diseases and pests, without having the same harmful effect as pesticides. So that these products have shown promising results with the potential to produce foods with organic classification (Pascuali et al., 2018; Silva et al., 2019; Cortés-Rivera et al., 2019; Queiroz et al., 2020).

The use of vegetable bioextracts aims to offer an alternative in the control of the pathogen in agriculture and that allows the production of food with less environmental impact than conventional ones (Monteiro et al., 2020; Fonseca et al., 2014; 2018; Silva et al., 2019; Palfi, Konjevoda & Vrandečić, 2019; Queiroz et al., 2020).

The studies reported in the present work are important, as there are few studies on the effect of treatments with vegetable bioextracts on the physiological and sanitary quality of corn seeds, especially the creole variety. Therefore, these studies represent an important step towards the use of vegetable bioextracts in family farming to replace pesticides that have greater toxicity and more harmful environmental impact (Affonso et al., 2012; Rickli et al., 2011; Scheren, Ribeiro; Nobrega, 2014; Araújo et al., 2019; Pascuali et al., 2018).

Creole corn has great genetic variability, has been resistant to climate change and in Brazil the farmer himself is able to produce the seed. This corn breed is currently seen as a potential genetic bank for the improvement of cultivars in this series (Sandri; Tofanelli, 2008; Pipolo et al., 2010; Santo et al., 2017).In this sense, the objective of the present study was to evaluate the effects of the application of bioextracts, and their binary combinations, in creole corn seeds, regarding their physiological and sanitary quality for *Fusarium* sp. and *Aspergillus* sp.

Material and Methods

Obtaining and preparing of vegetable bioextracts

The experiments were carried out at the Agronomy and Chemistry Laboratories, of the State University of Mato Grosso, Campus Deputado Estadual Renê Barbour, in the city of Barra do Bugres, located at 15º04’21” south latitude and 57º10’52” west longitude, in the state of Mato Grosso, Brazil. To evaluate the effects of bioextracts on the physiological and sanitary quality on the creole corn seeds, experiments were conducted among the months of july to october 2019.

The seeds, free of synthetic pesticides, were harvested at the rainbow farm, Barra do Bugres, Mato Grosso state, cleaned and threshed manually, sieved, and stored at 25 ºC. The citronella was harvested from the university's own herbarium. The garlic bulb, ginger rhizome and clove flower buds were purchased in the commerce of the city of Barra do Bugres. The vegetable bioextracts alone and their combinations were evaluated as described in Table 1.

Table 1. Bioextracts and the binary combinations used in the tests of physiological and sanity quality of creole corn seeds.

Vegetable bioextracts alone	Binary combinations
Garlic (*Allium sativum* L.)	Citronella and Garlic
Citronella (*Cymbopogon nardus*)	Garlic and Clove
Clove (*Caryophilusaromaticus*)	Garlic and Ginger
Ginger (*Zingiber officinale* Roscoe)	Citronella and Clove
	Citronelaand Ginger
	Clove and Ginger

To obtain each vegetable bioextract, a mixture of 150 mL of ethyl alcohol, 20 mL of distilled water and 40 g of the vegetable was prepared. This mixture was kept under agitation for a period of five days in polystyrene packages (250 mL) at a temperature of 25± 2°C. Subsequently, the vegetable bioextracts were filtered with filter paper and placed in glass cups. Evaporation of ethanol was carried out at 45 °C in a water bath, until reaching a final volume of approximately 20 mL. Then, the containers containing the bioextracts were sealed with film paper and kept at 25 ± 2 °C until the moment of use.

For the binary combination tests (garlic-citronella; garlic-clove; garlic-ginger; citronella clove; citronella-ginger; clove-ginger), a ratio of 1:1 of the concentrate of each bioextract was used. Seed treatment was carried out with the application of 2 mL of bioextract for each 100 g of creole corn seeds. And the parameters analyzed for physiological quality were the first germination count (G1), germination (G), dry mass (m_d), seedling length (cm) of the aerial and root parts and in sanity, *Fusarium* sp. and *Aspergillus* sp.

Germination test

The germination test was carried out according to the Rules for Seed Analysis (RSA) protocol (Brazil, 2009). The method consists in the application of 2 mL of bioextract on 200 seeds, with eight repetitions, sown on substrate paper "Germitest", previously sterilized and moistened with distilled water. The rolls with 25 seeds (each) were placed in a "Mangelsdorf" germinator, at a constant temperature of 25±2 °C. The first count of germinated seeds (G1) was performed on the fourth day, after being placed in the germinator, and the second count (G) occurred after seven days (Brazil, 2009; Pascuali et al., 2018).

Root and shoot dry weight and length test

The length of the aerial and root part of the corn seedlings was performed with the assistance of a ruler graduated in centimeters, with the results expressed in cm/seedling (Brazil, 2009; Pascuali et al., 2018). The dry mass of the seedlings was measured at four days with the normal seedlings of the germination test. The seedlings were removed and separated into aerial and root parts. Subsequently, they were packed in Kraft paper bags and dried in an oven at 80°C, for a period of 48 h. The dried seedlings were weighed on semi analytical balance (Shimadzu, Japan with accuracy of 0.00001 g), thus determining the dry mass (m_d) of the seedlings, according to Equation 1 (Nakagawa, 1999).

$$m_d = \frac{m_t}{s_t}$$

Where, m_d is the seedling dry mass, m_t is the total mass and s_t is the total seedling quantity per repetition.

Pathology test

The pathology test was performed according to the methodology adopted by Pascuali et al. (2018) and in consonance of Brazil (2009). Where 200 seeds were used, in eight repetitions with 25 seeds, distributed in plastic gerbox boxes, with two sheets of blotting paper moistened with distilled water. The seeds were placed on the paper, keeping a distance from each other. Subsequently, they were incubated at a temperature of 20 ± 2 ºC, with a 12 h light regime for a period of seven days. After this period, the seeds were analyzed individually with the assistance of a stereomicroscope at a resolution of 30-80x, to visualize the incidence of fungi (IF) (Brazil, 2009). The analyzed fungi were *Penicillium* sp. and *Fusarium* sp., and the incidence of each fungus (IF) was expressed as a percentage, according to Equation 2 (Nascimento et al., 2019).

$$IF\ (\%) = infected\ seeds / total\ number\ of\ tested\ seeds\ x\ 100$$

Statistical analysis

The experimental design of the present study was completely randomized, with eight replicates per treatment. Comparisons of means were performed using the WinSat 1.0 software, at 5% probability, using the Duncan test (Machado; Conceição, 2005).

Results and discussion

Physiological quality of creole corn seeds treated with bioextracts

The results of the physiological and sanitary quality of creole corn seeds treated with vegetable bioextracts alone and the binary combinations are shown in Table 2. The first germination count (G1), performed at four days, shows better results for treatments with bioextracts of garlic (65.1%), ginger (55.6%) and for the garlic-ginger combination (69.5%) with germination rates higher than the control sample (23.8%) which received only distilled water. While clove bioextract (0.0%) inhibited the germination of creole corn seeds. In other treatments using bioextracts alone and binary combinations would not differ between themselves and the control sample (Table 2). In the germination (G) at seven days, the seeds treated with bioextracts alone and with the combinations did not differ from the control sample, with the exception of the treatment containing only the clove bioextract which presented 3.0% germination (Table 2).

However, there are studies that report that several bioextracts do not interfere on the germination of maize seeds, such as those reported by Faria et al. (2009) that show that the germination percentage of corn (BR 106) was not influenced by the treatment with concentrated bioextracts of millet (*Pennisetum americanum* (L.) Leeke) and pine (*Pinus* sp.). Rickli et al. (2011) show that bioextracts of neem leaves (*Azadirachta indica* A. Juss) concentration 100% did not inhibit the germination of corn seeds. Scheren, Ribeiro & Nobrega (2014) show that the rhizome and bulb bioextract of *Cyperus rotundus* L. at 30% concentration did not interfere in the germination of corn seeds. Studies by Araújo et al. (2019) report that the bioextracts of garlic (*Allium sativum*) and lemon balm (*Lippia alba*) with concentrations in the range of 25 to 100% of bioextract did not influence germination in *Chorisiaglaziovii* seeds.

On the other hand, studies with bioextract from the aerial part of *Cyperus rotundus* L. show a linear reduction in the germination of corn seeds with the concentration of the bioextract, reaching a 19% decrease in the germination rate of the seeds treated with the bioextract at 30% (76%), as compared to the control sample (95%) (Scheren, Ribeiro & Nobrega 2014). Sonego et al. (2012) observed a decrease in the germination of corn seeds with the increase in the concentration of Tanzania grass bioextract (*Panicum maximum* cv. Tanzania). Alves et al. (2015) studied the effect of treatment on soybean seeds with 10 and 20 mL of concentrated bioextract of black pepper (*Piper nigrum*), obtaining a reduction in the germination of the seeds treated with the bioextract and the death of all seeds when 20 mL of the bioextract is applied.

In addition, the results of the germination of creole corn seeds in the present study (Table 2) behave similarly to the studies reported by Silva et al. (2019), for rice seeds. In rice studies, bioextracts of citronella, ginger, cloves, garlic and the binary combination of these were used. These studies showed that treatments with garlic and clove bioextracts showed the best germination rates (89%) of rice seeds, similar to the control (86%). The combination of garlic-citronella bioextracts showed a germination rate of 92%, higher than that obtained by the control sample. In addition, the germination of the seeds treated with the garlic-clove, garlic-ginger, clove-ginger and ginger-citronella combinations showed no statistical difference as compared to the control. However, the combination of the clove-citronella bioextracts decreased, significantly, the germination of rice seeds (Silva et al., 2019).

Table 2. Effect of vegetable bioextracts on the physiological quality of creole corn seeds.

		G1 (%)	G (%)	Drymass (mg/pl)	Aeriallength(cm)	Rootlength(cm)
Individual bioextracts	Control	23,8^{c}	84,9^{abc}	4,6^{ef}	1,3^{d}	4,4^{d}
	Garlic	65,1^{a}	76,4^{c}	29,5^{a}	3,9^{a}	9,3^{a}
	Citronella	42,9^{bc}	83,3^{abc}	24,6^{abc}	2,6^{b}	8,3^{ab}
	Clove	0,0^{d}	3,0^{d}	0,0^{f}	0,0^{e}	0,0^{e}
	Ginger	55,6^{ab}	87,9^{a}	25,0abc	2,8^{b}	8,6^{ab}
Combinations of bioextracts	Garlic and Citronella	44,3^{bc}	84,7^{abc}	25,7^{ab}	2,7^{bc}	8,3^{ab}
	Garlic and Clove	38,0^{bc}	77,6^{bc}	16,8^{cd}	2,3^{b}	6,7^{bc}
	Garlic and Ginger	69,5^{a}	86,4^{ab}	20,1^{bcd}	2,9^{b}	8,4^{ab}
	Citronella and Clove	43,5^{bc}	83,1^{abc}	7,3^{ef}	2,2^{bc}	4,7^{cd}
	Citronella and Ginger	45,1^{bc}	90,8^{a}	12,8^{de}	2,4^{bc}	5,6^{cd}
	Clove and Ginger	34,5^{bc}	86,1^{abc}	6,2^{ef}	1,7^{cd}	5,0^{cd}

* The averages followed by the same letter in the column do not differ by the Duncan test at 5% probability.

The dry mass data presented in Table 2 showed that the best results were obtained with the bioextracts of garlic (29.5 mg / pl), citronella (24.6 mg / pl), ginger (25.0 mg/pl) and for the garlic-citronella combinations (25.7 mg/pl) and garlic-ginger (20.1 mg/pl), with higher values than the control sample (4.6 mg/pl). However, treatment with clove bioextract creole corn seedlings did not develop (Table 2). The dry mass results for the other treatments did not differ from the control sample. As for the seedlings aerial length, the treatment with garlic (3.9cm), citronella (2.6cm), ginger (2.8cm), for garlic-citronella (2.7cm), clove-garlic (2.3cm), garlic-ginger (2.9cm), citronella-clove (2.2cm) and citronella-ginger (2.4cm) bioextracts showed better results as compared to the control (1.3cm) (Table 2), while the treatment with the combination of bioextracts of clove-ginger differed to the control sample. Treatment with clove bioextract alone inhibited the development of creole corn seedlings (Table 2). For root length, the best results were obtained with garlic (9.3 cm), citronella (8.3 cm) and ginger (8.6 cm) bioextracts and with the garlic-citronella (8.3 cm) and garlic-ginger (8.4 cm) that are much higher than the control (4.4 cm). The treatments with the other combinations of bioextracts do not differ from the control. Creole corn seeds treated with clove bioextract did not develop radicle (Table 2).

In this sense, Scheren, Ribeiro & Nobrega (2014) observed that the bulb and rhizome bioextracts of *Cyperus rotundus* L. concentration 30% reduced the growth in 27% of the aerial part, however they did not interfere in the root growth of corn seedlings. However, the bioextract of the aerial part of *Cyperus rotundus* L concentration 30% reduced the growth in 77% of the aerial part and in 37% of the root part of the corn seedlings. Sonego et al. (2012) observed a linear decrease in the root compliance of corn seedling with the increase in the concentration of Tanzania grass bioextract (*Panicum maximum* cv. Tanzânia). In addition, studies by Pies et al. (2017) with bioextract from crotalaria (*Crotalaria juncea*) show an inversely proportional relationship, that is, the higher the concentration of bioextracts, the shorter the length of the barley roots (cultivar BRS Elis). Cruz-Silva et al. (2015), when studying the development of corn seeds, also reported an inverse relationship between concentration and root length, with a decrease from 4.8 cm (control) to 3.8 cm in the length of the roots of seeds treated with bioextracts, observed at 30% concentration.

Sanitary quality of creole corn seeds treated with bioextracts

The sanity results for *Fusarium* sp. and *Penicillium* sp. in creole corn seeds treated with bioextracts showed better results for clove bioextract, where it was inhibited completely the occurrence of *Penicillium* sp., with a considerably decreases on the percentage of occurrence of *Fusarium* sp. as compared to control and other treatments (Figure 1). In addition, it is highlighted that the treatments with garlic (13.9%), ginger (18.1%), citronella (14.8%) and the garlic-citronella and garlic-ginger combinations bioextracts favored the increase in the occurrence *Fusarium sp*., as compared to the control (7.2%). The treatments with clove-garlic, citronela-clove and ginger-clove showed a percentage of occurrence of the fungus similar to that obtained for the control sample (Figure 1A). In addition, treatments with citronella and of garlic-clove, citronella-clove, citronella-ginger and clove-ginger of bioextracts combinations reduced significantly the occurrence of *Penicillium* sp. (Figure 1B). However, the garlic and the garlic-ginger bioextracts combination favored the occurrence of this fungus. The other treatments performed showed results similar to the control sample (Figure 1B).

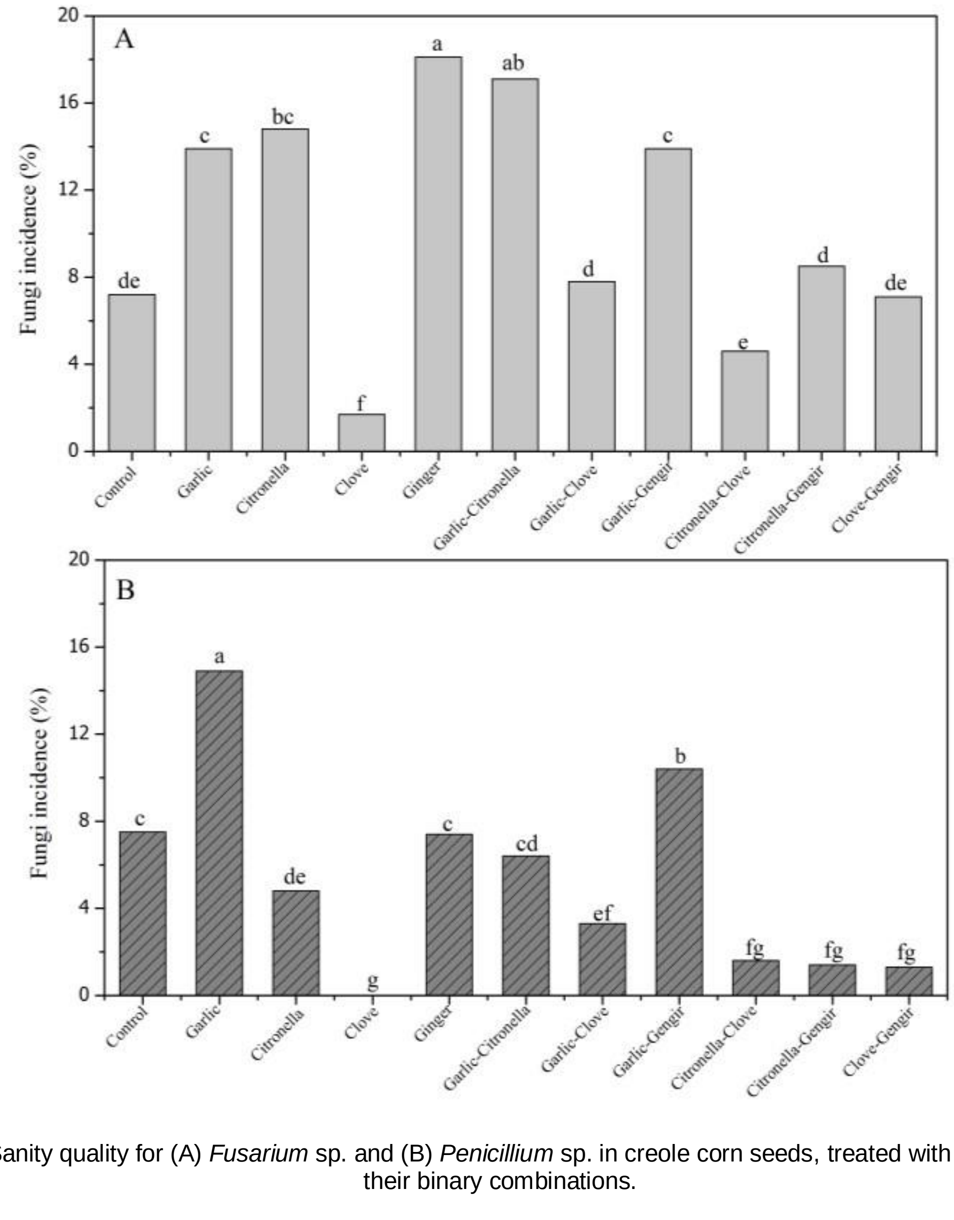

Figure 1. Sanity quality for (A) *Fusarium* sp. and (B) *Penicillium* sp. in creole corn seeds, treated with bioextracts and their binary combinations.

Sanity studies carried out by Araújo et al. (2013), with nine varieties of creole corn seeds treated with bioextracts from the peel of coumaru (*Dipteryx odorata*), orange (*Citrus vulgaris*) and chili peppers (*Capsicum frutescens*) showed that the bioextracts from orange peel and coumaru peel reduced the occurrence of *Penicillium* sp. However, Chili pepper bioextract favored the occurrence of the fungus (27%), as compared to the control sample (19%). Such results corroborate with the data found in the present study with creole corn (Figure1), since the bioextracts showed different efficacy for

Fusarium sp. and *Penicillium* sp. In addition, in vitro studies by Queiroz et al. (2020), with the fungi *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* isolated from soy (*Glycine max* L.) using the combination of citronela-lemon balm (*Cymbopogon citratus*), clove-lemon balm and clove-melissa (*Melissa officinalis*), in the concentration of 50,000 ppm, showed 100% inhibition of mycelial growth of fungi. Studies reported by Cortés-Rivera et al. (2019) showed that bioextracts of coconut mesocarp and exocarp (*Cocos nucifera* L.) significantly inhibit the mycelial growth of the fungus *P. italicum* in vitro.These differences in effects on the sanity quality of creole corn seeds reported in the present study can be attributed to the chemical composition of the bioextracts. So it is important to mention compounds that can be found in bioextracts, such as clove bioextract, that presents in its composition: eugenol, β-caryophyllene, α-humulene, eugenyl acetate and caryophyllene oxide (Affonso et al.,2014). Garlic (*Allium sativun* L.) has allicin, phytoterols and phenolic compounds (Silva, Moretti and Mattos, 2010).

Citronella contains the citronelal monoterpenes, geraniol, β-mircene, limonene, bergamal, linalool, β-citronelol (Veloso et al., 2012), citronella is found citral, linalool, geraniol, nerol and β-mircene (Silva et al., 2006). In melissa there is citral, citronelal, citronelol, limonene, linalool, geraniol and in ginger the geranial, neral, 1,8 cineol, geraniol, geranyl acetate and camphene (Andrade et al., 2012).

Conclusion

The bioextracts of garlic, ginger and citronella favored the germination, dry mass, aerial and root length and an improvement in the sanity of the creole corn seeds. Meanwhile, clove bioextract inhibited seed development. Treatments with combinations of bioextracts also improved germination, dry matter, air length, root length and the sanity of corn seeds, except for combinations of garlic with citronella and garlic with ginger, which favored the proliferation of fungi. Therefore, bioextracts can be efficient maintaining physiological quality or even improve and control fungi in creole corn seeds.

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