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Sanitary and physiological quality of corn seeds (*Zea mays*) treated with essential oil emulsion

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Abstract. The aim of the present studies was to evaluate the effects of treatments with essential oil emulsion on the sanitary and physiological quality of creole corn seeds. In the studies were evaluated the essential oils of neem (*Azadirachta indica*), citronella (*Cymbopogon nardus*), lemon balm (*Cymbopogon citratus*), melissa (*Melissa officinalis* L.), and the combinations neem-citronella, neem-lemon balm, neem-melissa, citronella-lemon balm, citronella-melissa and lemon balm-melissa. The tests carried out were germination, length of seedlings (aerial part and root), dry mass and pathology for *Fusarium* sp. and *Aspergillus* sp. in seeds of creole corn produced without the use of synthetic pesticides. The studies were carried out in vitro and consisted of the preparation of water emulsion, 5,000 ppm of essential oil and 1% of triton X100 and direct application on the seeds of creole corn, following the protocol of Rules of Seed Analysis (RSA). The results showed a significant decrease in the occurrence of fungi *Fusarium* sp. and *Aspergillus* sp. with treatment with essential oils of citronella, lemon balm and melissa, while the oil of neither reduced the occurrence of *Fusarium* sp., but increased the occurrence of *Aspergillus* sp. in almost four times. All binary combinations of essential oils reduced the occurrence of both fungi. On the other hand, the physiological quality was strongly affected by the death of creole corn seeds, except for the treatment with lemon balm essential oil. Therefore, these studies allow to affirm that essential oils can be efficient in the control of pathogens, however they can kill the seeds of creole corn.

Keywords: Organic agriculture, Seed pathology, Physiological quality.

Introduction

Corn (*Zea mays* L.) is a diploid and allogamous species, which belongs to the *Poaceae* family (*Gramineae*), its origin occurs approximately from seven to ten thousand years ago in a region that covers Mexico and continues to Central America. It is the species that has the most detailed genetic characterization among the cultivated ones, being one of the oldest cultivated plants, and one of the most researched cereal (Galinat, 1995).

Corn cultivars can be subdivided into two main types: hybrids and varieties, with hybrids being single, triple or double. The simple hybrid is the result of the crossing between two pure lines and it is indicated for production systems that use high technology, as it has the greatest productive potential. The triple hybrid is obtained from the crossing between a pure line and a simple hybrid and is indicated for medium to high technology, while the double hybrid is the result of the cross between two

simple hybrids, being indicated for medium technology (Cruz, et al., 2012).

In addition to hybrid corn, there are creole populations, also known as local breeds, which are less productive as compared to commercial corn, but these varieties have better nutritional contents, as well as, genetic variability, resistant and adapted to the environment of origin (Sandri and Tofanelli, 2008).

However, in order for quality corn to be produced, it is necessary that the seed to maintain a good physiological quality and to maintain this quality, most of the time they are subjected to treatments with synthetic products, and these products are the basis of support for agribusiness. But these products have significant destructive potential for ecosystems and biodiversity (Rocha, 2010).

On the other hand, consumer concern with the quality and safety of food that reaches the table has grown, with those that have not been treated with

synthetic products being more economically valued and that production has less environmental impact (Bettiol, 2013; Queiroz et al., 2020).

In this sense, studies have shown that essential oils have the potential to be used in the control of diseases that attack crops (Bastos et al., 2004; Silva et al., 2019; Queiroz et al., 2020). The essential oils are products from plant parts (leaves usually) and they are repellent or attractive substances of plants and can have properties mainly of a therapeutic nature. Such substances are generally known as aromatic or called volatile oils (Andrade et al., 2012).

Although Brazil occupies a prominent position in the production of essential oils, but there are still few studies on the effect of essential oils on the sanitary and physiological quality of corn seeds. Therefore, the present study aimed to evaluate the effects of essential oil emulsions on the sanitary and physiological quality of creole corn seeds in vitro.

Methods

Obtaining essential oils, preparing emulsions, and carrying out sanitary and physiological quality studies.

The experiments were carried out at the State University of Mato Grosso (UNEMAT), Barra do Bugres-MT. To evaluate essential oils in the sanitary and physiological quality of creole corn seeds, five experiments were conducted between July and October of 2019.

Creole corn seeds, free of synthetic products, were harvested at the rainbow farm (geographic coordinates 15°08'21.47" south 56°59' 37.53.50), cleaned and threshed manually, sieved and kept at 25 °C until the time of the studies.

The citronella, lemon balm and melissa vegetables were harvested from the UNEMAT herbarium, Barra do Bugres. The extraction of essential oils was carried out with the technique of steam dragging in clevenger using water as a solvent. The essential oils obtained were stored in an amber glass bottle and kept at 25 °C. Neem essential oil was purchased at the Bauru LTDA distillery (Lot: DBBION-ONEMPR 170315).

For the preparation of emulsions, with water and essential oil, with each type of essential oil and its binary equal mixtures (neem-citronella, neem-lemon balm, neem-melissa, citronella-lemon balm, citronella-melissa and lemon balm-melissa) were used 5,000 ppm concentration, plus 1% (1,000 ppm) of the Tween 80 emulsifier, with analytical purity.

The parameters analyzed in physiological quality were the first count of germination, germination, dry mass, length of seedlings of the aerial and root parts as well as, sanitary quality to *Fusarium sp* and *Aspergillus sp*.

Seed treatment was carried out with the application of 2 mL of essential oil emulsions on the

100 grams of creole corn seeds, as used in the studies performed by Silva et al. (2019).

Germination studies

In the germination studies, 2 ml of the essential oil emulsion was applied on the 200 seeds, with four replications, sown on a paper substrate "Germitest", autoclaved and moistened with distilled water, having a proportion of 2.5 times the weight of the paper, following the protocol of Rules for Seed Analysis (RSA) (Brasil, 2009). The rolls with 25 seeds (each) were placed in a "Mangelsdorf" type germinator, at a constant temperature of 25 °C. The first count of germinated seeds was performed on the fourth day after they were placed in the germinator, and the second count on the seventh day (Brasil, 2009; Pascuali et al., 2018).

Seedling length and dry mass

The seedling length was obtained using a ruler and performed together with the germination test, being analyzed the seedling length of 200 seeds with four replications with 25 seeds (Nakagawa, 1999; Brasil, 2009; Pascuali et al., 2018).

The dry mass of the seedlings was measured at four days with the normal seedlings of the germination test, which were removed, separated in aerial and root parts, placed in Kraft paper bags and taken to the oven at 80 °C, for 48 hours. The dry seedlings were weighed on a scale with an accuracy of 0.0001 g, thus determining the dry mass weight of normal seedlings. Seedling (mass/number of seedlings) x100 (Nakagawa, 1999).

Pathology studies

Pathology studies were performed in gerboxes, with two sheets of blotting paper moistened with distilled water. In these studies, 200 seeds were used, being distributed in eight repetitions with 25 seeds each. The seeds were placed on the paper, later incubated at 20 ± 2 °C, with a 12-hour light regime for seven days. After this period, the seeds were analyzed individually with the aid of a stereomicroscope at a resolution of 30-80x, to check the incidence of fungi. The fungi analyzed in the present studies were *Penicillium sp.* and *Fusarium sp.*, and the result reported in percentage (Brasil, 2009; PASCUALI et al., 2018).

Statistical analysis

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

Results and discussion

The results of sanitary quality of seeds for creole corn treated with emulsion of essential oils are presented in Table 1. These data show that seeds treated with emulsions of essential oils of citronella, lemon balm and melissa showed a significant decrease in the percentage of *Fusarium sp.* and

Penicillium sp. (Table 1). In the treatment with neem essential oil the result for *Fusarium* sp. shows a lower occurrence of the fungus as compared to the control, while for the *Penicillium* sp. the occurrence of this fungus increased almost four times (Table 1). These results suggest that neem essential oil favored the appearance of *penicillium* sp. in the treated seeds.

On the other hand, in the treatment using essential oil combinations, all treatments led to a decrease in the percentage of *Fusarium* sp. and *Penicillium* sp., with better results to the combinations of neem-melissa, citronella-citron, citronella-melissa and lemon balm-melissa essential oils showing more significantly reduced the percentage of fungi on the seeds (Table 1).

In this sense, Domene et al. (2016) evaluated the treatment of corn seeds of the AL Bandeirante variety, with essential oils from the leaves of *C. citriodora* and *E. camaldulensis* at 3.4 ppm. These studies show a significant decrease of the *Penicillium* sp. and *Fusarium* sp. However, the essential oil of *E. camaldulensis* proved to be less effective in controlling *Penicillium* sp. than *C. citriodora*. However, the combination of the essential oils of *C. citriodora* and *E. camaldulensis* showed less efficiency in controlling *Fusarium* sp. and *Penicillium* sp. as compared to the essential oil individually (Domene et al., 2016).

Table 1. Effects of treatment with essential oils on the sanitary quality of creole corn seeds.

Essential oils and binary combinations	Sanity (%)	
	<i>Fusarium</i> sp.	<i>Penicillium</i> sp.
Control	3.40 ^a	4.70 ^b
Neem	1.90 ^b	18.20 ^a
Citronella	0.20 ^c	0.10 ^d
Lemon balm	0.30 ^c	0.10 ^d
Melissa	0.10 ^c	0.10 ^d
Neem and Citronella	2.60 ^b	1.30 ^c
Neem and Lemon balm	0.80 ^c	0.10 ^d
Neem and Melissa	3.60 ^a	0.20 ^d
Citronella and Lemon balm	0.00 ^c	0.10 ^d
Citronella and Melissa	0.10 ^c	0.10 ^d
Lemon balm and Melissa	0.10 ^c	0.00 ^c

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

In addition, studies carried out by Brum (2012) with essential oil of citronella (*Cymbopogon nardus*) at 25 ppm showed a reduction of 27% in the growth of the *Rhizoctonia solani* when the essential oil was incorporated into the culture medium. Studies using emulsion of essential oils of citronella (*Cymbopogon nardus*), clove (*Caryophyllus aromaticus*), lemon balm (*Cymbopogon citratus*), melissa (*Melissa officinalis* L.) and neem (*Azadirachta indica*) have shown potential to inhibit mycelial growth of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* isolated from seeds of either in BDA (potato dextrose agar) culture medium (Queiroz et al., 2020).

The results of germination, dry mass, length of aerial and root seedling are shown in the Table 2. In the first germination count (G1) and in germination (G), the treatment with lemon balm essential oil was the only one that did not inhibit germination with a similar result to the control sample. The other essential oils caused the death of the seeds, showing a phytotoxic effect (Table 2). The results of dry mass, as well as in the aerial length test of seedlings of seeds treated with lemon balm essential oil were similar to control. In the root length of seedlings, the seeds treated with lemon balm essential oil show results that do not differ from the control (Table 1). In addition, the binary combinations of citronella-lemon balm, citronella-melissa and lemon balm-melissa

essential oils also caused the death of creole corn seeds and seeds treated with combinations of neem-citronella, neem-lemon balm and neem-melissa showed smaller germination as compared to the control (Table 2). The dry mass, aerial part and root length of seedlings were also smaller.

The results obtained in the present study corroborate with studies carried out by Silva et al. (2019) using 5,000 ppm of neem, citronella, lemon balm and melissa of essential oils on the rice seeds of caiapó variety. These studies show that citronella and lemon balm essential oils practically killed the seeds and only 1% of the rice seeds germinated when treated with melissa oil (Silva et al., 2019).

However, the results with creole corn seeds reported in the present study differ from the studies carried out by Domene et al. (2016) with corn seeds of the variety AL Bandeirante, treated with essential oils extracted from leaves of *C. citriodora* and *E. camaldulensis*, at a concentration of 3.4 ppm, showed that they did not significantly interfere with germination or seedling length, since they did not differ statistically from the control. Studies performed by Silva et al. (2019) showed that the combinations of essential oils of citronella-melissa and citronella-melissa, at 5,000 ppm, inhibited the development of rice seeds, and the combinations neem-melissa and citronella- lemon balm reduced seed germination to

1 and 2%, respectively. These studies show that essential oils can interfere in the physiological quality of different seeds.

The effects on the sanitary and physiological quality of essential oils can be attributed to diverse bioactive molecules (Veloso et al., 2012). In this context, it is worth mentioning biomolecules present in the essential oils used in the present study. Characterization and quantification studies of the composition of bioactive molecules present in citronella essential oil (*Cymbopogon nardus*) report the presence of citronelal monoterpenes, geraniol, β -mircene, limonene, bergamal, linalool, β - citronelol among others, which have antioxidant and

antimicrobial properties (Veloso et al., 2012). In lemon balm essential oil was found citral, linalool, geraniol, nerol and β -mirceno (Silva et al., 2006). In the lemon balm essential oil was found citronellal, citronellol, limonene, linalool and geraniol (Andrade et al., 2012). Neem (*Azadirachta indica*) has azadiractin, azadiradione, nimbin and salannin (Oliveira, 2015). Therefore, antifungal activity in the treatment of creole corn seeds of essential oils can be attributed to these bioactive molecules (Silva et al., 2006; Andrade et al., 2012; Lins et al., 2015; Silva, Moretti and Mattos, 2010).

Table 2: Effects of treatment with essential oil emulsion on the physiological quality of Creole corn seeds.

	1 ^o Germination Count (G1) %	Germination (G) (%)	Dry Mass	Aerial Length	Root Length
			(mg/pl)	(cm)	(cm)
Control	23.00 ^a	37.00 ^a	24.00 ^a	4.50 ^a	6.90 ^a
Neem	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c
Citronella	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c
Lemon balm	25.00 ^a	33.00 ^a	29.00 ^a	5.00 ^a	7.30 ^a
Melissa	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c
Neem and Citronella	0.00 ^b	1.00 ^{bc}	0.00 ^c	0.00 ^c	0.00 ^c
Neem and Lemon balm	0.00 ^b	1.00 ^{bc}	0.00 ^c	0.00 ^c	0.00 ^c
Neem and Melissa	0.00 ^b	3.00 ^b	5.00 ^b	0.90 ^{bc}	1.90 ^b
Citronella and Lemon balm	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c
Citronella and Melissa	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c
Lemon balm and Melissa	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c

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

Conclusion

The studies carried out allow to affirm that except the neem essential oil the others showed activity against the occurrence of the *Penicillium* sp. and *Fusarium* sp. in the seeds of creole corn. In addition, all binary combinations of essential oils were effective in reducing the occurrence of fungi in the seeds. However, treatment with essential oils caused the death of the seeds, except treatment with lemon balm essential oil. Therefore, essential oils show potential to be used to control pathogens in creole corn seeds, but further studies are needed to reduce the damage to the physiological quality of seeds in the presence of essential oils.

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