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Seeds of *Calotropis procera* Treated With Essential Oils of *Copaifera langsdorffii* Desf. and *Syzygium aromaticum* L.

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Abstract

The Silk Flower (*Calotropis procera*) is widely used by farmers in the Northeast region, due to the adaptation of the climatic and soil conditions of the semi-arid region, but the incidence of pathogens has reduced the physiological and sanitary quality standards acceptable for sexual propagation of these plants in the field. Thus, the objective of this research was to verify the effects of the Copaíba (*C. langsdorffii*) and Cravo (*S. aromaticum*) oils on the health and physiological quality of silk flower seeds (*C. procera*) harvested in the city of Tacima, PB. The treatments were constituted by Copaíba (*C. langsdorffii*) and Cravo (*S. aromaticum*) essential oils at concentrations of 0.5; 1; 1.5; 2% and the fungicide Captan® (240 g, i.a. 100 kg^{-1} seed). The control 0 (zero) corresponded only to the immersion of the seeds in distilled and sterilized water (ADE). In the evaluation of sanity, the method of incubation on filter paper (Blotter test) was performed, using twenty replicates of 10 seeds for each treatment. The physiological quality was evaluated by the germination test (G%), first germination count (FGC), germination speed index (GSI) and seedling dry mass (SDM). A microflora composed mainly of *Alternaria* sp. (52%), *Fusarium* sp. (70%), *Helminthosporium* sp. (40%), *Cladosporium* sp. (50%), *Curvularia* sp. (20%) and *Nigrospora* sp. (5%). The essential oils considerably reduced the percentage of fungi associated with silk flower seeds, but there was moderate phytotoxic effect under the germination and vigor of *C. procera* seeds.

Keywords: alternative control, germination, vigor

1. Introduction

The Caatinga Biome has great economic potential still unexplored with a diverse native and exotic vegetation. Knowledge about the potentialities of caatinga species is necessary for the preservation of plant genetic resources, and the sustainability of the population that seeks food and income sources in this region (Oliveira et al., 2011). Among these species, the silk flower (*Calotropis procera* (Aiton) R. Br.), A shrub, belonging to the Apocynaceae family, although native to Asia, has adapted well to the Brazilian tropical environment. Has green fruits that turn yellow at the end of maturation, with numerous brown seeds, trapped by silky and long hairs, forming winged structures that allow their propagation through the air (Mariod et al., 2017).

The Silk Flower has several economical uses, such as forage production due to the there is no leaf fall even during the dry season of drought and crude protein content around 19.4% (Torres et al., 2010). An important characteristic of *C. procera* is its regrowth vigorous after cutting, giving this larger plant ease of handling and obtaining (Silva et al., 2017). It stands out as a raw material in the manufacture of fabrics, ornamentation, wood extraction for firewood and home made medicinal applications; its seeds are raw material for biodiesel production (Rangel & Nascimento, 2011, Oliveira-Bento et al., 2013).

Due to these characteristics, the study of the physiological quality of the seeds becomes important, since the seed is the basic input in the production, and vigorous plants originate from good quality seeds (Vechiat & Parisi, 2013). According to Henning (2005), the most efficient means of disseminating pathogens is by seed, which facilitates the introduction of diseases into new areas and reduces production due to seedling death. Thus, the use of alternative treatments appears as a preventive measure for problems related to the presence of pathogens in seeds, in addition to eliminating the fungal microflora, it is necessary that the treatment does not have negative effect on the physiological quality.

Alternative treatments have been gaining ground due to their efficiency in eliminating pathogens and reducing costs, in addition to the beneficial action to the environment, they do not leave residues, and avoid the resistance of microorganisms by the continuous use of chemical products (Santos et al., 2008, Lazarotto et al., 2009), and can replace fungicides, herbicides, insecticides and nematicides.

Among the alternative treatments, plant extracts and essential oils have effective fungal action; the results obtained are promising in the control of phytopathogens (Mondego et al., 2014). Santana (2015) working with Nim essential oils (*Azadirachta indica* A. Juss.); Thyme (*Thymus vulgaris* L.); Cinnamon (*Cinnamomum zeylanicum* J. Presl.); Camphor (*Cinnamomum camphora* L.) and Melalueca (*Melalueca alternifolia*). They observed a significant effect on both inhibition of mycelial growth (PIC) and Conidiapores.

Boukaew et al. (2017) tested essential oils of Clove (*Syzygium aromaticum* (L.) Merr. & LM Perry) and Vatica (*Vatica diospyroides*) on the antifungal activity of *Aspergillus flavus*, and found that both contained Eugenou and Benzyl Acetate, antifungal compounds, in their compositions; the essential oil of *V. diospyroides*, showed 100% inhibition of *A. flavus* conidia, whereas *S. aromaticum* oil inhibited 84.7% of the conidia in Zea mays seeds. The use of copaíba essential oil considerably reduced the incidence of fungi associated with bean (*Phaseolus lunatus*) seeds without reducing the physiological quality of the seeds (Guedes et al., 2016). The antimicrobial activity of an essential oil is linked to its functional groups and their composition, such as alcohols, phenols, terpenes and ketones (Sartorelli et al., 2007).

Because of the importance of *C. procera* and action provided by the use of essential oils with antifungal activity in seed treatment, aimed to verify the effects of Copaíba oil (*Copaifera langsdorffii*) and Cravo (*Syzygium aromaticum*) on the health and physiological quality of (*Calotropis procera*) seeds.

2. Material and Methods

The work was developed in the Laboratories of Seed Pathology and Seed Analysis, Federal University of Paraíba (UFPB), Campus II Areia, PB. Seeds of *C. procera* were obtained from the municipality of Tacima, PB 6°29′8″ South 35°37′51″ West. The seeds were collected randomly, directly from 4 matrices and in open fruits, at an advanced stage of maturation, and were then packaged in paper bags and benefited in the Seed Analysis Laboratory.

The treatments were constituted by Copaíba (*Copaifera langsdorffii*) and Cravo (*Caryophyllus aromaticus* L.) essential oils at concentrations of 0.5; 1.0; 1.5; 2.0% and the fungicide Captan® (240 g, i.a. 100 kg^{-1} seed). The control 0 (zero) corresponds to the immersion of the seeds in distilled and sterilized water (ADE). The seeds were immersed in the treatments for a period of five minutes, after which the sanity and physiological quality of the seeds were analyzed. This time was based on pre-liminary tests.

The sanitary quality of the seeds was evaluated by the method of incubation on filter paper (*Blotter test*), using 200 seeds (twenty replicates of 10 seeds) for each treatment. The seeds were distributed in Petri dishes, containing two sheets of filter paper (80 g/m^2) previously sterilized and moistened with sterile distilled water (ADE), incubated at 20±2 °C with photoperiod of 12 hours. The quantitative and qualitative evaluations of the fungi associated to the seeds were carried out after seven days of incubation, and the seeds were examined individually under the stereoscopic microscope.

The seed quality was determined by germination test (MAPA, 2009), using 200 seeds (four replicates of 50 seeds) for each treatment, using as substrate germitest®-type paper moistened with distilled water in the amount equivalent to three times the weight of dry paper (MAPA, 2009). Later they were placed to germinate in a germination chamber with a constant temperature of 30 °C, with photoperiod of 8 h light and 16 h dark (Oliveira-Bento et al., 2013). The evaluations for the first and last germination counts were performed at 5 and 10 days, respectively, after sowing (Oliveira-Bento et al., 2013). The results were expressed as percentage of normal seedlings (MAPA, 2009).

As for the vigor tests, the first germination count (FGC) was performed, which corresponds to the number of normal seedlings computed on the 5th day after the test installation and the germination speed index (GSI), both performed simultaneously with the test of germination, with evaluation of the seedlings daily, at the same time, from the first day after sowing. The evaluations were carried out until the last count (tenth day), using the formula proposed by Maguire (1962).

To evaluate the dry mass of the seedlings, 20 seedlings of each treatment were randomly chosen. The seedlings were conditioned in paper bags and placed in an oven at 65 °C until reaching a constant mass (48 hours), being weighed in an analytical balance (0.001 g). The mean dry matter mass of the seedlings was obtained by the quotient between the total mass recorded and the number of normal seedlings used, with results expressed in g. seedling^{-1} (Nakagawa, 2012).

The experimental design was the completely randomized in a factorial scheme (2 × 5), two oils versus five concentrations, with 4 replicates/treatment. The data were submitted to regression analysis using the means obtained in the ANAVA for the different oil concentrations. The SISVAR 4.5 software was used in the analysis of variance and regression (Ferreira, 2014).

3. Results and Discussion

The incidence of fungi associated with silk flower seeds (*Calotropis procera*) was represented by *Alternaria* spp. (52%), *Fusarium* spp. (70%), *Helminthosporium* spp. (40%), *Cladosporium* spp. (50%), *Curvularia* spp. (20%) and *Nigrospora* spp. (5%) (Figure 1).

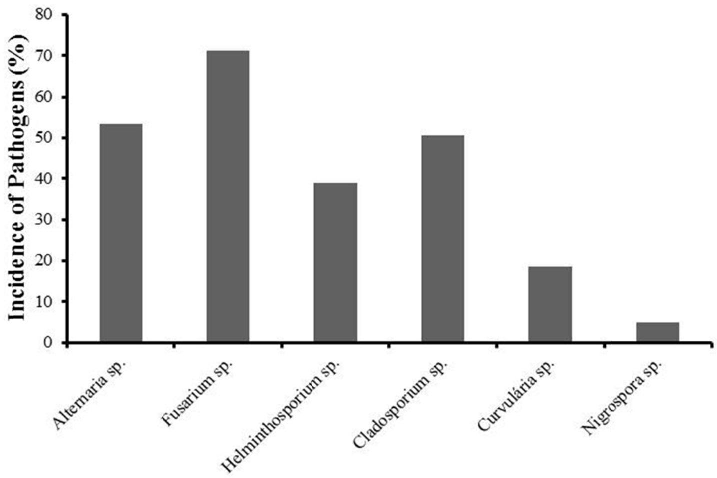

Figure 1. Incidence of fungi on seeds of *Calotropis procera* from the municipality of Tacima, PB

There was a significant effect for the treatment at a 1% probability level for all variables studied. Concentrations of the essential oils were also significant at 1% except for dry mass of the seedlings. The separated tested oils were significant at 5% probability germination Speed Index (Table 1).

Table 1. Summary of the analysis of variance for the characteristics percentage germination (G%), first germination count (FGC), germination speed index (GSI) and Dry mass of seedlings (DMS) in seeds of *Calotropis procera* submitted to treatment with *Copaifera langsdorffii* and *Syzygium aromaticum* oil

SV	DF	MS			
		%G	FGC	GSI	DMS
Treatment	9	453.377**	646.711**	23.944**	0.002**
Concentration	3	301.833**	307.458**	13.854**	0.000^{ns}
Linear	1	562.500**	342.225*	28.257**	0.0008*
Quadratic	1	40.500^{ns}	325.125*	1.739^{ns}	0.00004^{ns}
Oils	1	32.000^{ns}	10.125^{ns}	7.411*	0.00007^{ns}
C x O	3	49.333^{ns}	208.125*	5.097*	0.0001^{ns}
C/OC	3	84.916^{ns}	310.916**	7.436**	0.0004^{ns}
Linear	1	140.450^{ns}	174.050^{ns}	14.145**	0.001*
Quadratic	1	72.250^{ns}	756.250**	8.008*	0.000002^{ns}
D/OCR	3	266.250**	204.666*	11.515**	0.00006^{ns}
Linear	1	470.450**	168.200^{ns}	14.112**	0.00009^{ns}
Quadratic	1	0.250^{ns}	4.000^{ns}	0.931^{ns}	0.00006^{ns}
Fun vs 4 × 2 + 1	1	1254.400**	2310.400**	17.459**	0.008**
Water vs 4 × 2 + 1	1	1408.177**	1488.400**	121.661**	0.0089**
Error	30	35.133	51.266	1.099	0.0001
CV %		7.99	10.65	9.91	1.24
Average		69.87	67.2	10.58	0.95

Note. ** Significant at 1%, * Significant at 5% and ns: not significant. SV: Source of variation; d.f.: Degrees of freedom; MS: Mean squares; CV: Coefficient of variation. Fun (Fungicide); Oils Copaíba (OC); Oils Cravo (OCR).

As the concentration of the Copaíba (*Copaifera langsdorffii*) and Cravo (*Syzygium aromaticum*) essential oil was increased, there was a reduction in the percentage of the fungus. Being the concentration of 2.0% reduced the percentage of *Alternaria*, not statistically different from the fungicide used (Captan ®), with a 50% reduced pathogen percentage, to 30% with the use of oils (Figure 2A).

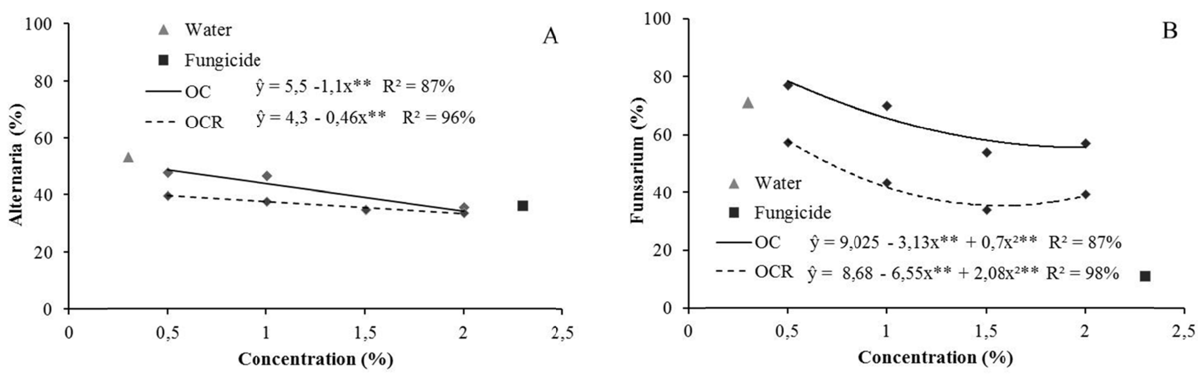

Figure 2. Incidence of *Alternaria* sp. (A) and *Fusarium* sp. (B) in seeds of *Calotropis procera* treated with essential oils of *Copaifera langsdorffii* and *Syzygium aromaticum* from the municipality of Tacima, PB

These results differ from Amaral and Bara (2005) in which they observed an antifungal action of clove oil in concentrations of 0.1 to 0.5% on phytopathogens present in rice, beans, soybean and corn (*Oriza sativa*, *Phaseolus vulgaris*, *Glycine Max* and *Zea mays*), respectively, reducing the pathogen by one hundred percent. The antifungal effect of copaiba oil is attributed to the substances present in its composition. Zimmermam-Franco et al. (2013) verified that the main substances found in the oil of this plant are β-caryophyllene, caurenoic acid compounds and γ-murolene, which are known to have antibacterial and antifungal properties, causing a synergistic effect resulting from the action of their functional groups (Boukaew et al., 2017).

The incidence of *Fusarium* spp. in the seeds of *C. procera*, the existence of the fungus in this area may be due to the fact that the fruits open at the end of maturation, some of which may have been infected (Figure 2B). The infected seeds are important source of primary inoculum for the fungi, causing deterioration, impairing the germination, causing root rot in the seedlings. There is a significant effect on the waste oils, with the percentage of the pathogen increasing from 60% in the control to less than 40% when using clove oil at a concentration of 2%. Similar results were observed by Costa et al. (2011) evaluated the action of the essential oil of *Syzygium aromaticum* on the hyphae of some phytopathogenic fungi (*Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum* and *Macrophomina phaseolina*), and concluded that the oil caused cell damage to fungi. This action of the oil can be justified by the presence of Eugenol, an antiseptic phenolic compound of known action (Ranasinghe et al., 2002; Daferera et al., 2003; Amaral & Bara, 2005; Gayoso et al., 2005).

Antifungal action of the oils on *Helminthosporium* infestation was observed (Figure 3A), while the concentrations of the oils increased, with a decrease in the percentage of the pathogen, however, clove oil at the concentration of 2% had a greater effect, reducing from 40% (distilled water) to 10%, but the fungicide (captan®) eliminated the pathogens from the seeds. The action of fungicides in the control of seed pathogens is an old practice performed by the producers. Goulart (2001) observed significant reductions in the incidence of pathogens in soybean seeds (*Glycine max*) when treated with tolylfluanid + thiophanate methyl, thiabendazole + thiram and thiophanate methyl + thiram. Similarly, Pereira et al. (2009) obtained an 82% reduction in the occurrence of *Colletotrichum truncatum* in soybean seeds (*Glycine max*). Similarly, Pereira et al. (2009) obtained an 82% reduction in the occurrence of *Colletotrichum truncatum* in soybean seeds (*Glycine max*).

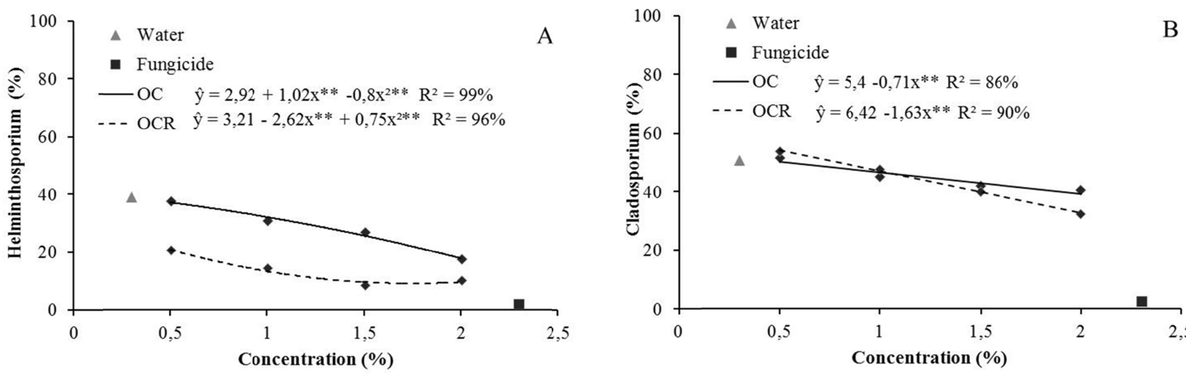

Figure 3. Incidence of *Helminthosporium* sp. (A) and *Cladosporium* sp. (B) in seeds of *Calotropis procera* treated with essential oils of *Copaifera langsdorffii* and *Syzygium aromaticum* from the municipality of Tacima, PB

Regarding the incidence of *Clasdosporium,* (Figure 3B) the oils tested were not as efficient in the control; a 50% reduction of the control to 30% was verified when the seeds were treated with 2% clove oil. In this case, the essential oils probably have pathogen specificity. Thus, it is important to know the pathogens occurring in the seeds; the correct diagnosis will provide subsidies for later adoption of measures of fungus management in the seeds and increase of productivity (Vida et al., 2004). Seeds contaminated with *Clasdosporium* may result in low germination and vigor, especially in untreated seeds (Carvalho et al., 2011).

The concentration of clove oil at 2% differed significantly from the control (19%), and did not differ from the fungicide (Captan®) (Figure 4A), showing control of the incidence of *Curvularia* sp. The oils studied at the concentration of 0.5% did not differ from the control (19%). Mondego et al. (2014), investigating methods of alternative microflora control in seeds of *Pseudobombax marginatum* with copaiba essential oil, for the incidence of *Curvularia* sp. observed that all doses of the oil differed significantly from the control (7%) and did not differ from the fungicide (Captan®). This fungus can be found frequently in different plant substrates such as saprophytic, phytopathogenic and endophytic, which can cause foliar stains in several plant families (Ferreira, 2010).

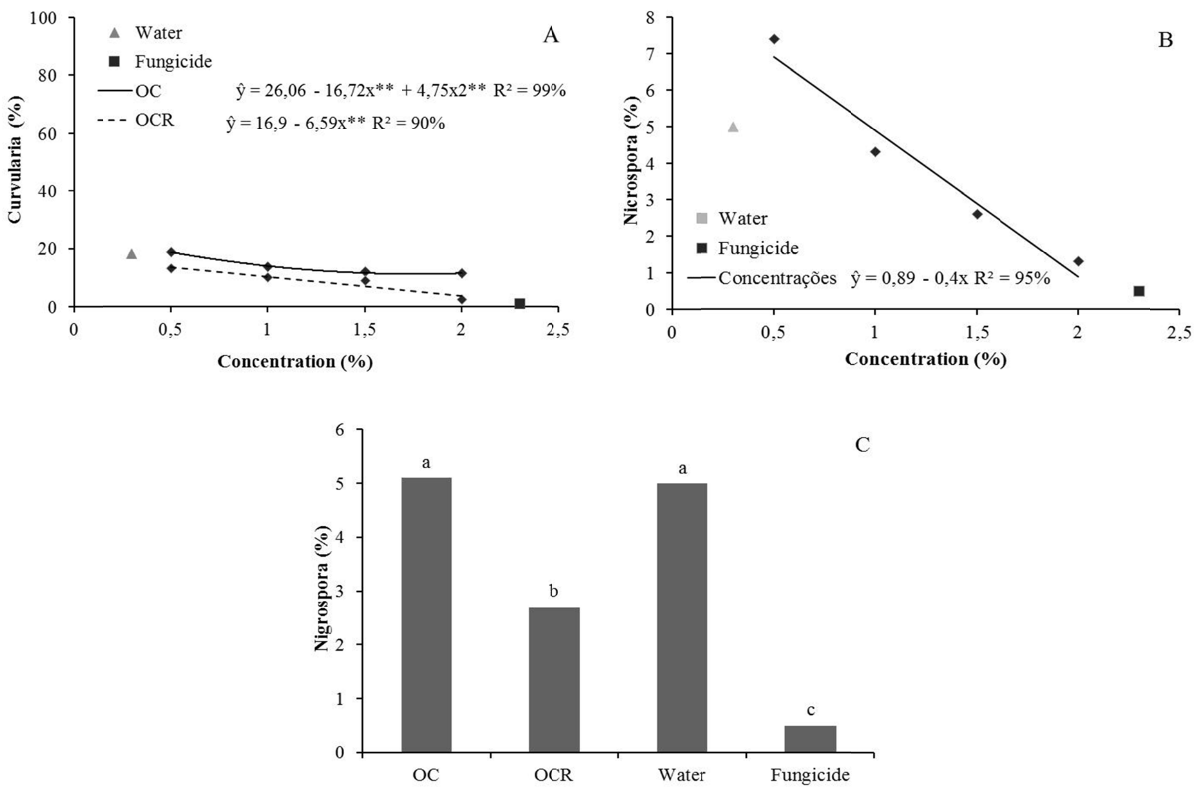

Figure 4. Incidence of *Curvularia* sp. (A) and *Nigrospora* sp. (B) in seeds of *Calotropis procera* treated with essential oils of *Copaifera langsdorffii* and *Syzygium aromaticum* from the municipality of Tacima, PB

Nigrospora sp. is a filamentous fungus widely distributed in soil, decaying plants and seeds. It is reported to be harmful to seed germination and seedling development (Sales, 1994). Even with low incidence on the seeds of *C. procera* is necessary its correct control. Clove essential oil showed a significant difference in relation to copaiba oil and water control, reducing the percentage of this fungus from 5% to 2.5% (Figure 4C). Among the concentrations used, 2% showed the best control, differing statistically from the other concentrations and not differing from the fungicide, resulting in almost complete elimination of the pathogen. Several authors have reported the use of essential oils in the control of this fungus. Mata et al. (2009) observed that the growth of *Nigrospora* sp., *Cladosporium* sp., *Curvularia* sp. is controlled with the essential oil of fennel (*Pimpinella anisum*) in all concentrations used, but the essential oil of citronella (*Cymbopogon winterianus*) only controls *Nigrospora* sp. and *Cladosporium* sp. these results show that there are specific alternative controls for various genus of fungi.

In the evaluation of the physiological quality of the seeds, there was a decrease of 15% in the percentage of seed germination as a function of the concentration of essential oils (Figure 5A). The negative influence observed can be attributed to the fact that essential oils have substances such as Eugenol, a phenyl propene (phenol). Eugenol is little soluble in water, which is present in the essential oil of *Syzygium aromaticum*, which binds strongly to the tannin by means of hydrogen bonds and consequently, inhibits seed germination (Oliveira et al., 2011; Boukaew et al., 2017).

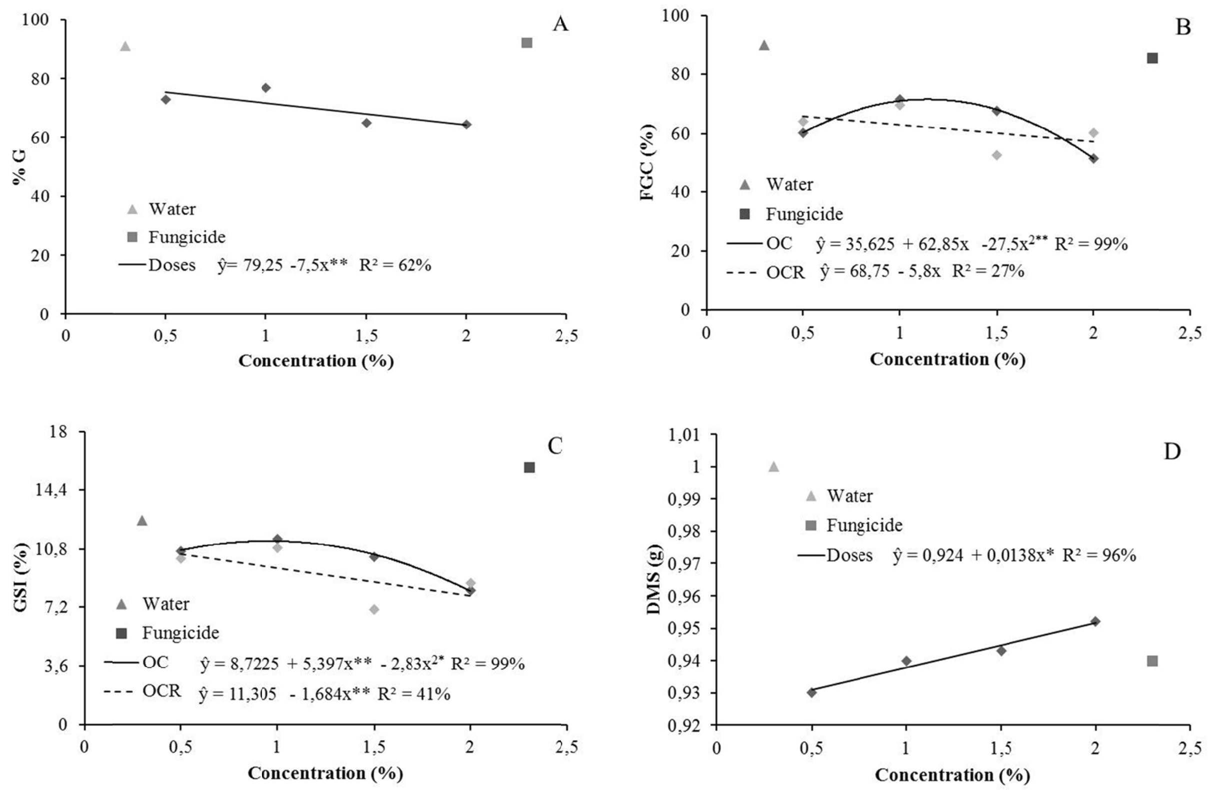

Figure 5. Germination (G); First Germination Count (FGC); Germination Speed Index (GSI); Dry mass of seedlings (DMS) in seeds of *Calotropis procera* submitted to treatment with *Copaifera langsdorffii* and *Syzygium aromaticum* oil

Other compounds are also responsible for inhibiting germination in different species such as Monotherpenes and oxygenated monoterpenes present in the oil of *Piper hispidinervium* C. DC. and sesquiterpenes, aliphatic and arylpropanoids found in the oil of *Pogostemon heyneanus* Benth (Souza Filho et al., 2009). In addition to the composition, the concentration of essential oils may influence the effect caused on germination as verified by Corlett et al. (2015), where concentrations of *Cymbopogon winterianus* oil above 0.05 mL caused inhibition of germination, with significant presence of abnormal and dead seedlings.

The first germination count (FGC) obtained maximum values of 69% and 66% in the concentration of 1.14% of essential oil of *Copaifera langsdorffii* and 0.5% of *Syzygium aromaticum*, respectively (Figure 5B), these doses favor the process germination because higher results at the first count result in higher percentages of normal seedlings. FGC is an important test of vigor, based on the principle that, samples with higher percentages of normal seedlings in the first count are the most vigorous, therefore, indispensable in evaluating the physiological quality of seeds (Nakagawa, 2012).

The germination speed index (GSI) presented a higher percentage (11.30%) in the concentration of 0.95% of the essential oil of *Copaifera langsdorffii*, the percentage values related to the essential oil concentrations of *Syzygium aromaticum* showed a negative linear behavior as concentrations increased, decreasing from 10.46% to 7.93% (Figure 5C). In general, the IVG values were influenced by the application of the essential oils, possibly due to the allopathic effects caused by the application of the essential oils in the seeds of *Calotropis procera*. This inhibits the emission of the radicles since they hinder the cell division process, besides other functions such as enzyme activation and membrane permeability (Pinã-Rodrigues & Lopes, 2001). This behavior may vary according to the species used, since in a research carried out by Mondego et al. (2014) using *Copaifera langsdorffii* essential oil as an alternative control of *Pseudobombax marginatum* seed microflora observed that the treatments did not affect germination or seed vigor.

For the dry matter of seedling (MS) there was a growth as a function of the concentrations of essential oils from 0.93 g to 0.95 g (Figure 5D). These results differ from those reported by Farias et al. (2016) when testing the three concentrations of oils (0, 0.5%, 1.0% and 2%) of Andiroba (*Carapa guianensis* Aubl.) And Copaíba (*Copaifera langsdorffi* Desf) on the health and physiology of seeds of two varieties (*Vigna unguiculata* L. Walp), BRS Gurgueira and Marataoã, in both varieties no differences were observed when comparing the control in the treatment with Copaiba oil, with a slight increase of the dry mass in the concentration of 2% of the oil of Andiroba. However, Mondego et al. (2016) recorded higher mean MS using *Copaifera langsdorffii* essential oil in this way proving beneficial effects and viability in their use.

4. Conclusions

The essential oils of Clove (*Syzygium aromaticum*) and Copaíba (*Copaifera langsdorffii*) considerably reduced the incidence of pathogens in silk flower seeds (*Calotropis procera*);

The concentration of 2.0% of the oil of *S. aromaticum* provided a reduction in the percentage of the fungal microflora of the genus *Altenaria* sp. and *Curvularia* sp., not differing from the fungicide;

The increasing concentrations of the oils *S. aromaticum* and *C. langsdorffii* present a phytotoxic effect, moderately reducing the germination and vigor of *C. procera* seeds.

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