Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v45i1.65301

Adaptive advantage of *Sesbania virgata* (Cav.) Pers. in the phytochemical production: the influence on fungi occurring in seeds

Vera Lygia El Id* **and Nelson Augusto dos Santos Junior**

Laboratório de Sementes, Núcleo de Conservação da Biodiversidade, Instituto de Pesquisas Ambientais, Avenida Miguel Stéfano, 3687, 04301-012, São Paulo, São Paulo, Brazil. *Author for correspondence. E-mail: verinhalygia@gmail.com

ABSTRACT. *Sesbania virgata* is a native Brazilian plant species. It exhibits rapid growth, a high soil cover potential, and efficient soil seed bank formation and is used in environmental restoration projects. The soil seed bank is susceptible to fungal infection and other biotic factors. However, only a few studies have reported on the fungi on the surface of *S. virgata* seeds. Moreover, little is known about how substances present in the seed integument affect fungal communities and their role in adapting to and thriving in new environments. Herein, *S. virgata* seeds were collected from populations that produce or do not produce the flavonoid catechin in the seed coat. These seeds were subjected to laboratory tests to identify and quantify the fungal populations in the integument. We selected and subjected three genera to irrigation and inoculation tests with *S. virgata* extracts and seeds from both populations. We observed that the aqueous seed coat extracts inhibited *Alternaria* sp. micellar and augmented *Phoma* sp. growth. *Phoma* sp. also caused post-germinated seed lethality. Our data indicate that the seed coat of *S. virgata* contains antifungal substances that endow this species with an adaptive advantage.

Keywords: antifungals; fungal incidence; germination; micellar growth.

Received on October 6 2022.
Accepted on August 16, 2023.

Introduction

Allelopathy is a natural phenomenon through which one organism produces biochemicals that affect the germination, growth, reproduction and/or survival of other living beings (De Conti & Franco, 2011; Pinto et al., 2016). It can also influence microorganism populations (Arruda et al., 2006; Ferreira, Medeiros, & Soares, 2008; Souza Filho, Vasconcelos, Zoghbi, & Cunha, 2009; De Conti & Franco, 2011; Gomes, Fortes, Silva, Bonamigo, & Pinto, 2013), such as phytopathogenic fungi (Hasse, May-de-Mio, & Lima Neto, 2007). It is directly related to maintaining the ecosystem's balance.

It has been shown that allelopathy is influenced by interactions between environmental conditions and secondary metabolite production (Iganci, Bobrowski, Heiden, Stein, & Rocha, 2006). Indeed, some allelopathic species exhibit defense mechanisms based on synthesizing specific metabolites, thus providing them with an adaptive advantage (Lara-Nuñez et al., 2006; Gonçalves, 2015). Ceballos, Hossaert-Mckey, Mckey and Andary (1998) verified the exudation of secondary metabolites, including catechins, proanthocyanidins and luteolin, by two *Sesbania* species. In *Sesbania drummondii* (Rydb) Cory (perennial species), the amount of the flavonoid (+)- catechin was greater than (-)- catechin. Additionally, there was a predominance of (-)- catechin in *Sesbania vesicaria* (Jacq.) Ell. (annual species). Notably, it was found that *S. vesicaria* was more susceptible to attack by the fungus *Alternaria* sp. and that *S. drummondii* could inhibit this microorganism's growth. Thus, it seems plausible that the (+)- catechin has antimicrobial activity.

Additionally, Simões et al. (2008) detected phytotoxin quercetin and the alkaloid sesbanimide A in *Sesbania virgata* (Cav.) Pers. seeds. The former is a toxic substance not exudated into the medium during germination, and the latter has antifungal properties. Sesbanimide A was detected in *S. virgata* seed exudate and could inhibit the growth of the fungus *Cladosporium sphaerospermum* Pemzig. Furthermore, Praxedes, Zerlin, Dias and Pessoni (2011) reported that proteins from *S. virgata* seeds could inhibit the growth of the filamentous fungi *Aspergillus niger*, *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides* and *Fusarium solani*.

When examining the effects of crude exudates from *S. virgata* seeds and roots on arbuscular mycorrhizal fungi, Coelho, Mignoni, Silva and Braga (2019) observed that these fungi were stimulated in the presence of seed exudates. In this sense, substances from *S. virgata* seeds have inhibitory and stimulatory effects, depending on the fungus in the environment in which it develops.

Sesbania virgata (Cav.) Pers. belongs to the Fabaceae family. It is native to South America (Araújo, Mendonça, Barbosa, Lamonica, & Silva, 2004). In Brazil, it mainly grows in the Cerrado and Atlantic Forest areas (Pott & Pott, 1994). It is commonly recommended and employed for restoring mining-degraded areas (Coutinho et al., 2005; Florentino & Moreira, 2009; Souza, Agra, Andrade, Oliveira, & Oliveira, 2010). *S. virgata* is a pioneer species that can form a soil seed bank with high longevity. Notably, with fast germination and development rates, this species efficiently covers the soil (Coutinho et al., 2005) and has been referred to as a super-dominant species (Matos & Pivello, 2009).

In addition to these characteristics, it has allelopathic properties against native (i.e., Brazil) and exotic plant species (Id, Braga, & Santos Junior, 2020; Mignoni, Simões, & Braga, 2017). Its allelopathic action is mainly due to the flavonoid (+)- catechin, which displays phytotoxic potential and is released by the seeds in significant quantities (Simões et al., 2008). In some soils and for some species, the interaction between the catechins and the soil microbial communities can inhibit plant growth (Inderjit, Wardle, Karban, & Callaway, 2011).

We hypothesize that ecological characteristics, including seed bank formation capability, rapid growth, and allelopathic and potentially antifungal substance (e.g., catechins) production, may justify the aggressiveness of *S. virgata* in the environment. This work's objective was to describe the *S. virgata* seed mycota and determine how these fungi react to *S. virgata* seed coat extracts and how the seeds respond to inoculation with specific fungal species.

Material and methods

Samples

In laboratory tests, fungi on the seed coat of *S. virgata* were identified and quantified according to their incidence and severity. We assessed the effect of applying *S. virgata* seed coat extract to fungal spores and inoculating *S. virgata* seeds with the mycelium of these fungi.

Samples were collected from three *S. virgata* populations with detectable levels of the allelochemical (+)-catechin in the plant material and ripe fruits. We also collected samples from three populations where the allelochemical was not present at detectable levels. All populations are located in the Lavras (MG) municipality and were characterized according to preliminary studies (Id et al., 2015).

Sesbania virgata integument extract production

Seed integument extraction involved homogenizing 800 seeds from catechin-producing and non-producing *S. virgata* populations in batch. Each seed was manually scarified with P60 sandpaper and placed in black plastic boxes (11×11×3.5 cm). The seeds were soaked in 70% ethyl alcohol (diluted with autoclaved water) for seven hours in an air-conditioned chamber at 25°C for disinfection. This soaking step also facilitates integument extraction. Next, we manually extracted the seed integuments with a sterile scalpel blade and transferred them to aluminum foil envelopes. The samples were then frozen, lyophilized (48 hours) and macerated in a ball mill.

The material was weighed and dissolved in autoclaved water. This mixture was then filtered and diluted to the following weight/volume recommendations: 0.1, 0.5 and 1.0%. These values as based on previous experiments using seed extracts from the catechin-producing and non-producing populations (Simões et al., 2008; Id et al., 2015). Aqueous extracts of commercially available (+)- catechin standard (Sigma-Aldrich) were used as the positive control at a concentration of 1 mg mL^{-1}.

Identification and severity of fungi

We utilized the filter paper method (Brasil, 2009) to identify and isolate fungi in the *S. virgata* seeds. This method involves depositing *S. virgata* seeds into sterile Petri dishes (90×15 mm) containing two sterilized and moistened (with autoclaved water) sheets of filter paper. We used 200 seeds from the catechin-producing and 200 from the non-producing populations.

Additionally, we assessed the effects of sterilization and scarification on the filter paper method results. Towards this goal, groups of seeds from the catechin-producing and the non-producing populations were formed to evaluate the following treatment conditions: nonsterilized and non-scarified (NE), nonsterilized and scarified (E), sterilized and non-scarified (S) or sterilized and scarified (SE).

Sterilization was achieved by immersing the seeds in 70% alcohol (1 min.), 2% sodium hypochlorite (1 min.) and finally distilled and sterilized water (1 min). Scarification was performed manually with a flame-sterilized scalpel blade, and seeds were incubated in an air-conditioned chamber for eight days at 20 ± 2ºC with a 12-hour photoperiod. Each treatment was repeated ten times, using five seeds per repetition.

Identifying the fungal colonies on the seed coat and evaluating their incidence and severity was carried out using a stereomicroscope. This approach allows for the visualization of morphological characteristics of the fungi, such as conidia (Barnett & Hunter, 1999).

The degree of fungi severity was based on the scale proposed by Françoso and Barbedo (2016). A score of 'zero' on this scale corresponds to seeds not being infected with the evaluated fungus, 'trace' corresponds to very low numbers of small colonies, 'weak' corresponds to colonies with weak/sparse growth occupying <40% of the seed, 'moderate' corresponds to colonies with slow superficial growth occupying 41–100% of the seed and 'intense' corresponds to dense and evenly distributed colonies, with intense growth occupying 41 to 100%.

Following the identification and incidence analyses, six main genera of fungi were identified, and after evaluating the degree of severity results for each fungus in the *S. virgata* seeds, we decided to isolate three fungi species (*Phoma* sp., *Cladosporium* sp. and *Alternaria* sp.).

For the extract application studies, the fungal colonies were formed by inoculating the central point of the Petri dishes containing potato-dextrose-agar (BDA) culture medium. The plates were incubated at 20 ± 2ºC with a 12-hour photoperiod (Brasil, 2009).

Application of extracts to fungal colonies

Mycelia of the three fungi isolates, previously selected and grown in BDA culture medium, were removed and placed, with the aid of a flamed stylet, in the center of sterile Petri dishes (90×15 mm) containing agar-agar culture medium. Following inoculation and subsequent fungal colony formation, the plates were treated with 5 mL of the aqueous *S. virgata* integument extracts (at previously defined percentages), distilled and autoclaved water (control) or an aqueous extract with commercial catechin (positive control). The plates were sealed with plastic material and incubated for 15 days in an air-conditioned chamber at 20 ± 2ºC with a 12-hour photoperiod.

The reaction of the fungi to the extract was assessed by measuring and calculating the average radial growth of the colonies. This was accomplished using a millimeter ruler and measuring two diameters, previously marked on the Petri dish's bottom. We evaluated each treatment's effect five times for each selected fungi, using four plates per repetition.

Germination of infected *Sesbania virgata* seeds

To assess the effect the selected fungi had on the *S. virgata* germinative process, seeds from the catechin-producing and non-producing populations were disinfected with 2% sodium hypochlorite (1 min.), 70% alcohol (1 min.) and distilled and sterilized water (1 min.). Following disinfestation, the seeds were manually scarified with a flame-sterilized scalpel blade.

The seeds were transferred to sterile Petri dishes containing two sterilized and moistened (with distilled and autoclaved water) sheets of filter paper. With a flamed stylus, mycelia of 1 mm^2 of each fungus were removed and used to inoculate the seed coat of *S. virgata* seeds. This procedure was performed for the three genera of fungus, thus representing three treatments applied to the seeds of *S. virgata*. For each fungus, that is, for each treatment, five repetitions were used with four plates per repetition (with five seeds per plate). The material was then incubated at 20 ± 2ºC with a 12-hour photoperiod.

The treatments lasted ten days. At the end of this period, germination percentage, the germination speed index (Maguire, 1962) and seed mortality were calculated.

For each experimental test with fungi, a completely randomized design was employed. Data were analyzed using analysis of variance (ANOVA), and significant differences between the means of treatments were identified by the Tukey test ($p<0.05$). Statistical analyses were conducted with the SISVAR 5.1 statistical program (Ferreira, 2010).

Results and discussion

Severity of fungi

We initially selected six fungal genera: *Phoma* sp., *Pestalotiopsis* sp., *Cladosporium* sp., *Fusarium* sp., *Rhizopus* sp. and *Alternaria* sp. (Figure 1). According to the graphs, it was observed that the most prevalent fungi in the *S. virgata* seed coat were *Phoma* sp., *Pestalotiopsis* sp., *Cladosporium* sp. and *Fusarium* sp genera. In contrast, fungi of the genera *Rhizopus* sp. and *Alternaria* sp. were less prominent. We also observed a strong influence on the results when considering the sample treatment method and seed origin. Interestingly, these factors influenced the presence or absence of these fungi. Based on these results, we also evaluated these treatments using severity tests.

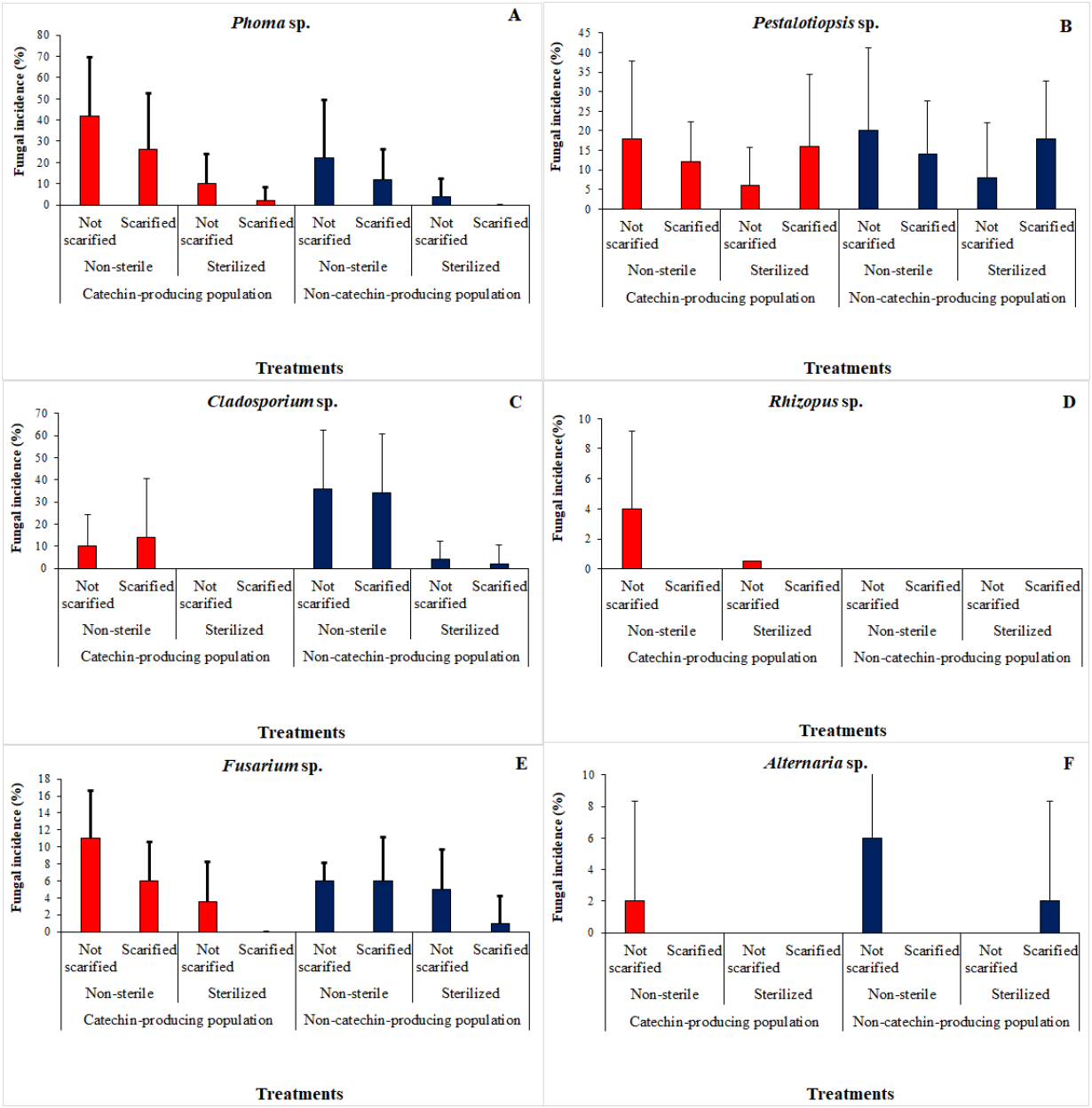

Figure 1. Mean values of incidence (%) for *Phoma* sp. (A), *Pestalotiopsis* sp. (B), *Cladosporium* sp. (C), *Rhizopus* sp. (D), *Fusarium* sp. (E) and *Alternaria* sp. (F) in *S. virgata* (Cav.) Pers. seeds originating from catechin-producing (red bars) and non-producing (blue bars) populations and after the following treatments: nonsterilized and non-scarified, nonsterilized and scarified, sterilized and non-scarified and sterilized and scarified.

According to the data obtained and presented in Figure 2, some fungi, such as *Phoma* sp., *Pestalotiopsis* sp., *Cladosporium* sp. and *Fusarium* sp., were more incident and had higher severity rates. Notably, the incidence and severity varied depending on the seed origin. For example, we observed that *Rhizopus* sp. and *Alternaria* sp., fungi with the lowest incidence and severity, exhibited origin-specific differences in the degree of severity.

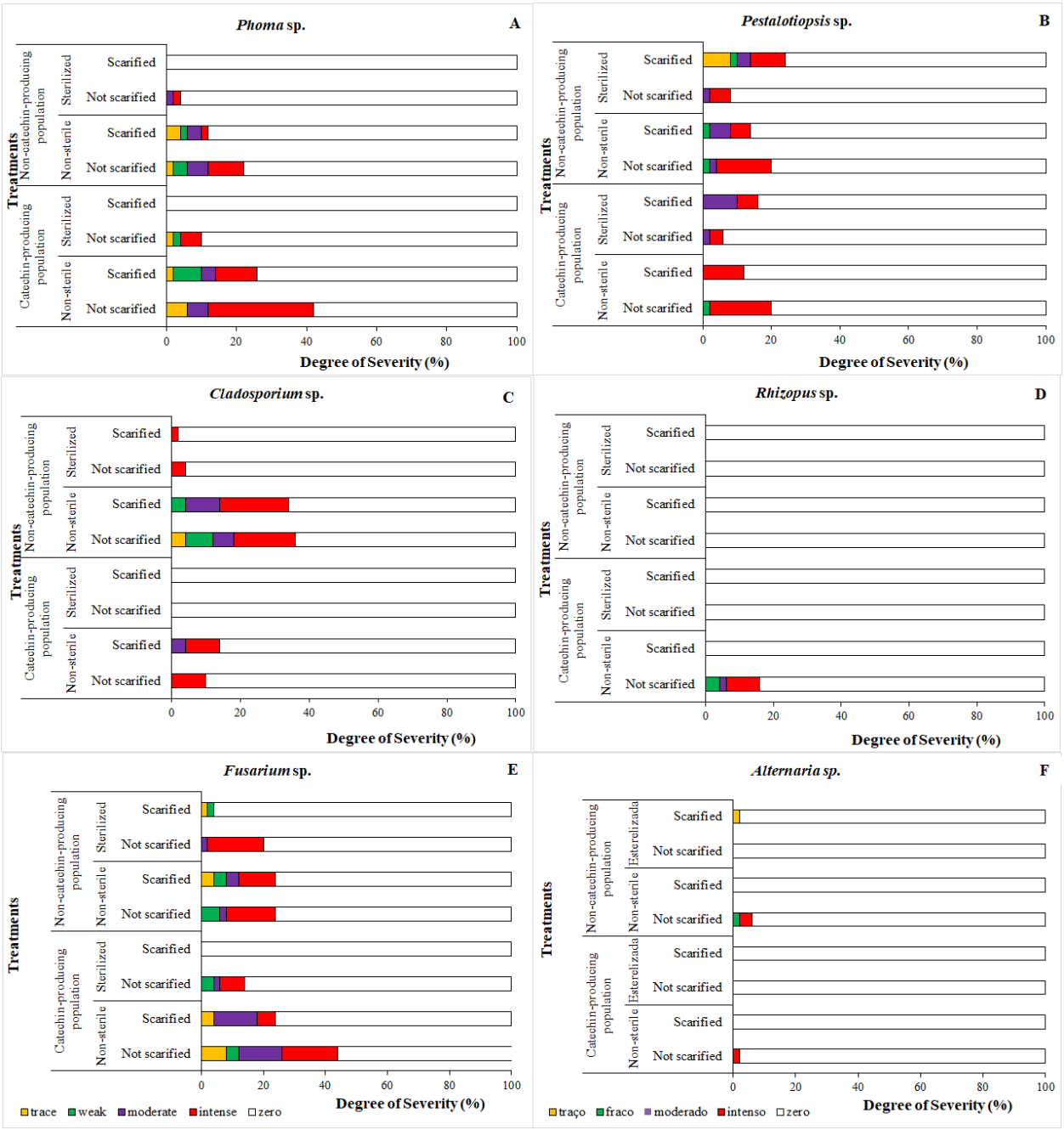

Figure 2. Mean values of the degree of severity (%) for *Phoma* sp. (A), *Pestalotiopsis* sp. (B), *Cladosporium* sp. (C), *Rhizopus* sp. (D), *Fusarium* sp. (E) and *Alternaria* sp. (F) in *S. virgata* (Cav.) Pers. seeds originating from catechin-producing and non-producing populations and after the following treatments: nonsterilized and non-scarified, nonsterilized and scarified, sterilized and non-scarified and sterilized and scarified. Degree of severity 'zero' (white bar), degree of severity 'trace' (yellow bar), degree of severity 'weak' (green bar), degree of severity 'moderate' (purple bar) and degree of severity 'intense' (red bar).

It is plausible that these observed variations in occurrence are related to evolutionary mechanisms of fungal tolerance, such as the ability to degrade allelopathic substances (Zhu, Zhang, & Ma, 2011). Indeed, fungal tolerance strategies include enzyme production, self-resistance or efflux mechanisms (Morrissey & Osbourn. 1999), which prevent intracellular antifungal compounds from accumulating to toxic levels.

When analyzing each fungus individually, it was noted that the *Phoma* sp. (Figure 2A) displayed 'intense' severity in *S. virgata* seeds from catechin-producing and non-producing populations. However, this fungus was more present in seeds producing catechin, regardless of sterilization and scarification treatment. A severity of 'zero' was observed in sterilized catechin-producing seeds, with and without scarification. Overall, *Phoma* sp. was a 'weak' to 'moderate' grade fungus in both seed populations, with greater severity observed under nonsterilized conditions. Previous work showed that soil fungi are attracted to seeds that secrete nutritive exudates, which are typically released by the seeds during imbibition in the germinative process

(Nelson, 2004). Changes in nutritional compound availability caused by the edaphic characteristics (Long, Steadman, Panetta, & Adkins, 2009) can influence microbial growth and change its persistence in the soil (Wagner & Mitschunas, 2008; Pakeman, Small, & Torvell, 2012). On the other hand, the micellar growth of mycorrhizal fungi species can also be stimulated when contacted by substances exuded by *S. virgata* seeds. Thus, some substances can be toxic or beneficial to particular plant species, depending on their role (Coelho et al., 2019), a behavior that seems to occur with this genus.

For *Pestalotiopsis* (Figure 2B), the severity was 'intense', regardless of the origin or sterilization and scarification treatment. Interestingly, this fungus only exhibited 'moderate' severity in sterilized catechin-producing seeds. In contrast, the severity level was more expressive for seeds from non-producing populations in scarified and nonsterilized seeds. 'Trace' severity of this fungus was only detected in seeds subjected to both treatments. The degree of severity variability of some of the genera, such as *Pestalotiopsis* sp., after sterilization, could be accounted for by fungi, initially inside the *S. virgata* seeds, being released during integument processing. *Pestalotiopsis* sp. is potentially pathogenic to seeds and seedlings (Maciel et al., 2012) and is responsible for seed rot (Santos, Medeiros, & Santana, 2001). These features are consistent with the degrees of severity observed in the present study, with *Pestalotiopsis* sp. having 'moderate' and 'intense' degrees of severity in all *S. virgata* seed groups, regardless of catechin-producing status or treatment.

In general, forest seeds have a high incidence of *Pestalotiopsis* sp. and *Cladosporium* sp. (Maciel et al., 2012). Shreelalitha and Sridhar (2015) identified endophytic fungi in *Sesbania bispinosa* seeds, including *Pestalotiopsis* sp. and *Cladosporium* sp. These observations suggest that the present study's scarification treatment could have contributed to the disappearance of some of the fungi on the seed surface. It has been stated that *S. bispinosa* seeds produced more fungal isolates when compared to other parts of the plant, but the richness and species diversity were low (Shreelalitha & Sridhar, 2015). Similar to the results obtained herein, Cherobini, Muniz and Blume (2007) reported the presence of *Alternaria* sp., *Penicillium* sp., *Nigrospora* sp. and *Cladosporium* sp. on the surface of *S. virgata* seeds.

The severity traces revealed 'intense' *Cladosporium* sp. severity when the seeds from both origins when not sterilized, and scarification did not affect this observation (Figure 2C). A 'zero' degree of severity for this fungus was observed in sterilized catechin-producing seeds. On the other hand, seeds from non-producing populations displayed 'intense' severity under the same treatment conditions. It has been reported that *Cladosporium* sp. presents saprophytic behavior and, when detected in high incidence, may reduce the germinative power of the seeds (Vechiato & Parisi, 2013).

Despite the low incidence of *Rhizopus* sp. (Figure 1D), it presented 'intense' severity in the nonsterilized/nonscarified seeds from the catechin-producing populations (Figure 2D). It should be noted that it also displayed 'moderate' and 'weak' degrees of severity in seeds of the same origin and treatment. The field fungus, *Rhizopus* sp., is established in the seed before harvest, during the growth and maturation periods (Vechiato, 2010). This genus is widely distributed in nature, surviving saprophytically in the soil and organic residues with the potential to invade plant tissues (Velázquez-del Valle, Bautista-Baños, Haernández-Lauzardo, Guerra-Sánchez, & Amora-Lazcano, 2008) in the flowering, seed formation, harvesting and processing stages (Jaccoud-Filho & Dabul, 2011). The lack of pre-harvest contamination supports the low incidence of *Rhizopus* sp. observed here. At the same time, the 'intense' degree of severity in the *S. virgata* seeds can be explained by the fungus' saprophytic nature.

Since the sterilization and scarification treatments altered the degree of severity in all genera, it appears that imbibition by the *S. virgata* seeds increases as the tegument breaks, consequently releasing some substances, like catechin, in large quantities.

Fusarium sp. presented traces of *Fusarium* sp. (Figure 2E). This fungus was found to have 'intense' severity in seeds from catechin-producing and non-catechin-producing populations. A 'zero' degree of severity was observed in sterilized and scarified catechin-producing seeds. Additionally, 'trace' and 'weak' severity degrees were found in non-producing populations subjected to the same treatments. Notably, the degree of severity is more evident in non-catechin-producing seeds. Previously, Costa, Chagas Junior, Ramos, Soares and Scheidt (2017) reported that flavonoids, the chemical class to which the catechin metabolite belongs and present in alcoholic extracts produced with garlic bulbs, inhibited the micellar growth of *Fusarium* sp. Furthermore, Gregolin, Bonaldo, Sinhorin, Banderá and Wobeto (2019) found that ethanol extracts of propolis, which contain flavonoids such as quercetin and kaempferol, exhibited antifungal action against *Fusarium* sp. Notably, quercetin has been detected in the coat of *S. virgata* (Simões et al., 2008). This

relationship between *Fusarium* sp. and secondary metabolites belonging to the flavonoid group may have influenced *Fusarium* sp. severity in *S. virgata* seeds. In this sense, many authors highlight the importance of studies on the effect of extracts on seed fungi as a possible phytosanitary control measure as well (Choudhury, Dobhal, Srivastava, Saha, S., & Kundu, S., 2018; Dourado et al., 2020; Almeida et al., 2021).

Lastly, under nonsterilized non-scarified treatment conditions, *Alternaria* sp. is present with 'intense' severity in seeds from the catechin-producing and non-producing populations (Figure 2F). It should be pointed out that a 'moderate' degree of severity was also detected in the seeds. Additionally, 'trace' severity was detected in sterilized and scarified non-producing seeds. Since *Alternaria* sp. is present in seeds and, later, in sesbania seedlings, it is likely that this fungus is transmitted to the plant in the field, resulting in high disease rates and diminished green mass and plant height. In the study by Cherobini et al. (2007), they also proposed that *S. virgata* seed origin influenced the genera of fungi occurring in its seeds. In this sense, our results provide further evidence in favor of this proposal since the genus *Alternaria* sp. did not display a high incidence in *S. virgata* seeds. Interestingly, even with a degree of 'intense' severity, the isolates in question suffered significant micellar growth reduction following treatment with aqueous extracts of *S. virgata* seed coat integuments. This demonstrates that the *S. virgata* seeds, originating from catechin-producing populations (Id et al., 2015), produce a phytotoxic substance that inhibits the micellar growth of the fungus in question.

Application of extracts in fungal colonies

Applying *S. virgata* seed coat extract to colonies of *Cladosporium* sp. did not elicit significant changes in the mycelium's radial growth (Figure 3). Based on the results with *Cladosporium* sp., it appears this genus has evolved mechanisms to tolerate the exudation of *S. virgata* allelochemicals. Moreover, this genus helps break down the integument and initiate germination (Delgado-Sanches, Ortega-Amaro, Jimenez-Bremont, & Flores, 2011; Berendsen, Pieterse, & Bakker, 2012), thus benefiting the soil seed bank.

In contrast, a gradual and significant reduction in the radial growth of *Alternaria* sp. mycelium was observed following extract treatment (Figure 3). At 0.1 and 0.5% extract concentrations, radial growth was reduced to 3.52 to 3.86 cm, respectively. Borges et al. (2017) previously demonstrated that the catechin flavonoid, produced by various plant species, has antifungal activity. This sensitivity of *Alternaria* sp. to the aqueous *S. virgata* seed coat extracts and the low frequency of this genus in the seed coat corroborate it.

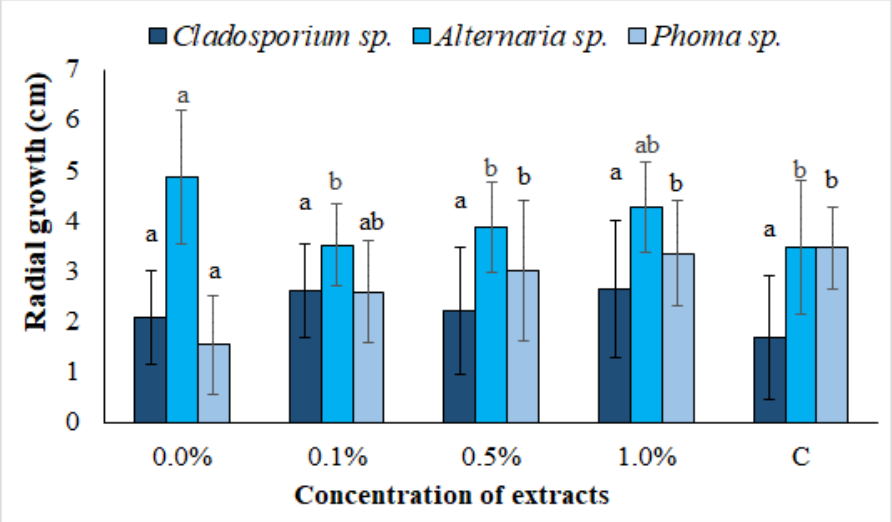

Figure 3. Mean radial growth values of *Cladosporium* sp., *Alternaria* sp., and *Phoma* sp. after the irrigation treatment with aqueous extracts of *S. virgata* (0.1, 0.5 and 1.0%), distilled and autoclaved water (0%), or commercial catechin (1 mg mL^{-1}) (C). Means followed by the same letter between treatments (0.0, 0.1, 0.5 and 1.0% and C) and within a given species are not significantly different, as determined by the Tukey test at a 5% probability level.

In contrast, *Phoma* sp. presented a gradual and significant increase in mycelium growth when treated with the extracts (Figure 3). The highest values of 3.01 cm and 3.36 cm were observed for colonies treated with 0.5 and 1.0% of the extract, respectively. Also, for this fungus, the extract produced with commercial catechin caused a significant increase in mycelium growth (3.46 cm) compared to the control group (1.55 cm).

Germination of infected *Sesbania virgata* seeds

As shown in Table 1, inoculating the seed coat with the three selected fungi did not cause any alterations in the germination rate or the germination speed index. However, seeds inoculated with *Phoma* sp. exhibited a four-fold increase in post-germination *S. virgata* seed mortality compared to those inoculated with *Cladosporium* sp. or *Alternaria* sp. On some seeds, this genus of fungi can form a barrier against the entry of other fungi that can release mycotoxins and damage the seed (Rezende, Couto, Borges, Silva, & Batista, 2013).

Table 1. Germination Rate (G%), Germination Speed Index (GSI) and Mortality Rate of post-germinated *S. virgata* (Cav.) Pers. seeds (M%), after inoculation with isolates from *Alternaria* sp., *Cladosporium* sp. and *Phoma* sp.

Fungus	Parameter		
	G%	GSI	M%
Alternaria sp.	99 a	3.66 a	4 a
Cladosporium sp.	98 a	3.86 a	4 a
Phoma sp.	94 a	3.99 a	16 b

Means followed by the same letter in the column are not significantly different by the Tukey test with a 5% probability.

Another point verified in the present study is that *S. virgata* seeds inoculated with *Phoma* sp. isolates displayed increased mortality of post-germinated seeds. It is worth pointing out that seed germination is one of the most vulnerable stages of plants. This phase is sensitive to variations in substrate, temperature, gases, humidity and light (Cosmo, Gogosz, Rego, Nogueira, & Kuniyoshi, 2017). Moreover, phytopathogen-infected seeds have been shown to have reduced germination and can spread diseases to seedling nursery areas (Carneiro, 1987; Santos, Parisi, & Menten, 2011; Martins, Santos Junior, & Barbedo, 2022). Consequently, the association between fungi and seeds of native species can simultaneously attenuate germination and the emergence of seedlings during sowing, reducing plants' establishment in the field and propagating the fungus (Fagan, Ramirez, Schwan-Estrada, Cruz, & Stangarlin, 2004). Identifying microorganisms in the seed does not guarantee that they will infect the plant of that seed because the amount of inoculum, soil microflora, climatic conditions and the pathogen's survival time in the seed and the seed itself can influence transmission (Cherobini et al., 2007). Therefore, the seed pathogen's association represents a potential for transmission and the possible disease establishment in the field.

In general, seeds can protect themselves from lethal infections through mechanisms such as physical barriers, making their seeds impervious to pathogens; endogenous chemical defenses and production; rapid seed germination, beneficial associations with other microorganisms and elimination of microorganisms with saprophytic potential (Dalling, Davis, Schutte, & Arnold, 2011). The latter may explain the ecological relationship between fungi and *S. virgata* seeds.

Conclusion

The origin of the seeds was directly related to the fungi present, with variations in the composition of the fungi depending on the origin. The extracts of the species inhibited the growth of some fungi and stimulated others. This result was probably linked to the fact that, evolutionarily, while *S. virgata* developed features and tools to inhibit specific pathogens, some of them, on the other hand, also evolved to tolerate this inhibition. In general, the seed-coating fungi seem to modulate the growth of other fungi that can be potentially lethal to the seed bank of the species in the soil. Importantly, this study demonstrated that the seeds of *S. virgata*, as a superdominant species, presented adaptive advantages in the environment.

Acknowledgments

This work is part of the doctoral thesis of Vera Lygia El Id from the Institute of Environment Research (IPA) and received financial support from *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq). Thanks to CNPq, to the Seed Laboratory of the IPA, where this work was developed and to Drª Cibelle Ferreira Françoso for the teachings in the initial identification of genera of fungi.

References

Almeida, A. R., Silva, I. C., Souza, C. A. F., Avelino, J. R. L., Santos, J. E. C. C., Medeiros, E. V., & Pinto, K. M. S. (2021). Plant extract as a strategy for the management of seed pathogens: a critical review. *Research, Society and Development, 10*(14), e174101421846. DOI: https://doi.org/10.33448/rsd-v10i14.21846

Araújo, E., Mendonça, A. V., Barbosa, D. G., Lamonica, K. R., & Silva, R. F. (2004). Caracterização morfológica de frutos, sementes e plântulas de *Sesbania virgata* (Cav.) Pers. *Revista Brasileira de Botânica*, *26*(1), 105-110. DOI: https://doi.org/10.1590/S0101-31222004000100016

Arruda, T., Antunes, R. M., Catão, R. M., Lima, E. O., Sousa, D. P., Nunes, X. P., ... Cunha, E. V. (2006) Preliminary study of the antimicrobial activity of *Mentha villosa* Hudson essential oil, rotundifolone and its analogues. *Revista Brasileira de Farmacognosia*, *16*(3), 307-311. DOI: https://doi.org/10.1590/S0102-695X2006000300005

Barnett, H. L., & Hunter, B. B. (1999). *Ilustrated genera of imperfect fungi*. Upper Saddle River, NJ: Prentice Hall.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in plant Science*, *17*(8), 478-486.

Borges, I. V., Cavalcanti, L. S, Neto, A. F., Almeida, J. R. G. S., Rolim, L. A., & Araújo, E. C. C. (2017). Identificação da fração antimicrobiana do extrato da *Mimosa tenuiflora*. *Comunicata Scientiae*, *8*(1), 155-164.

Brasil (2009). *Regras para análise de sementes*. Brasília, DF: Ministério da Agricultura, Pecuária e Abastecimento.

Carneiro, J. S. (1987). Testes de sanidade de sementes de essências florestais. In J. Soave, M. M. V. S. Wetzel (Eds.), *Patologia de sementes* (p. 363-393). Campinas, SP: Fundação Cargill.

Ceballos, L., Hossaert-Mckey, M., Mckey, D., & Andary, C. (1998). Rapid deployment of allelochemicals in exudates of germinating seeds of *Sesbania* (Fabaceae): roles of seed anatomy and histolocalization of polyphenolic compounds in anti-pathogen defense of seedlings. *Chemoecology*, *8*(1), 141-151. DOI: https://doi.org/10.1007/s000490050019

Cherobini, E. A. I., Muniz, M. F. B., & Blume, E. (2007). Avaliação da qualidade de sementes e mudas de *Sesbania virgata* Poir. FABACEAE. *Cerne*, *13*(1), 136-144.

Choudhury, D., Dobhal, P., Srivastava, S., Saha, S., & Kundu, S. (2018) Role of botanical plant extracts to control plant pathogens – a review. *Indian Journal of Agricultural Research, 52*(4), 341-346. DOI: https://doi.org/10.18805/IJARe.A-5005

Coelho, L., Mignoni, D. S., Silva, F. S., & Braga, M. R. (2019). Seed exudates of *Sesbania virgata* (Cav.) Pers. stimulate the asymbiotic phase of the arbuscular mycorrhizal fungus *Gigaspora albida* Becker & Hall. *Hoehnea*, *46*(1), e272018. DOI: https://doi.org/10.1590/2236-8906-27/2018

Cosmo, N. L., Gogosz, A. M., Rego, S. S., Nogueira, A.C., & Kuniyoshi, Y. S. (2017) Morfologia de fruto, semente e plântula, e germinação de sementes de *Myrceugenia euosma* (O. Berg) D. Legrand (Myrtaceae). *Floresta*, *47*(4), 479-488. DOI: https://doi.org/10.5380/rf.v47i4.46933

Costa, N. C., Chagas Junior, A. F., Ramos, A. C. C., Soares, L. P., & Scheidt, G. N. (2017). Atividade antimicrobiana e análise fitoquímica preliminar do extrato vegetal de alho no controle de fungos fitopatogênicos. *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, *12*(1), 161-166. DOI: https://doi.org/10.18378/rvads.v12i1.4406

Coutinho, M. P., Carneiro, J. G. A., Barroso, D. G., Rodrigues, L. A., Figueiredo, F. A. M. M. A., Mendonça, A. V. R., & Novaes, A. B. (2005). Crescimento de mudas de *Sesbania virgata* (Cav.) Pers. plantadas em uma área degradada por extração de argila. *Floresta*, *35*(2), 231-239. DOI: https://doi.org/10.5380/rf.v35i2.4608

Dalling, J. W., Davis, A. S., Schutte, B. J., & Arnold, A. E. (2011). Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology*, *99*(1), 89-95. DOI: https://doi.org/10.1111/j.1365-2745.2010.01739.x

De Conti, D. Y, & Franco, E. T. H. (2011) Efeito alelopático de extratos aquosos de *Casearia sylvestris* Sw. na germinação e no crescimento de *Lactuca sativa* L. *Revista Brasileira Agrociência*, *17*(2-4), 193-203.

Delgado-Sanchez, P., Ortega-Amaro, M. A., Jimenez-Bremont, J. F., & Flores, J. (2011). Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae). *Plant Biology*m, *13*(1), 154-159. DOI: https://doi.org/10.1111/j.1438-8677.2010.00333.x

Dourado, G. F., Santos e Silva, M. S. B., Oliveira, A. C. S., Cândido e Silva, E. K., Oliveira, L. J. M. G., & Rodrigues, A. A. C. (2020). Alternative seed treatment methods for plant pathogen control in sweet pepper crops. *Revista Brasileira de Ciências Agrárias, 15*(3), e8420. DOI: https://doi.org/10.5039/agraria.v15i3a8420

Fagan, C., Ramirez, C. A., Schwan-Estrada, K. R., Cruz, M. E. S., & Stangarlin, J. R. (2004). Efeito do extrato bruto de *Laurus nobilis* e *Zingiber officinale* no crescimento micelial de fungos fitopatogênicos. *Fitopatologia Brasileira*, *29*(2),128-134.

Ferreira, D. F. (2010). *SISVAR-Sistema de análise de variância*. Lavras, MG: Universidade Federal de Lavras.

Ferreira, N. R., Medeiros, R. B., & Soares, G. L. G. (2008) Potencial alelopático do capim-annoni-2 (Eragrostisplana Nees) na germinação de sementes de gramíneas perenes estivais. *Revista Brasileira de Sementes*, *30*(2), 43-59. DOI: https://doi.org/10.1590/S0101-31222008000200006

Florentino, L. A., & Moreira, F. M. S. (2009). Características simbióticas e fenotípicas de Azorhizobium doebereinerae, microissimbiote de *Sesbania virgata*. *Revista Árvore*, *33*(2), 215-226. DOI: https://doi.org/10.1590/S0100-67622009000200003

Françoso, C. F., & Barbedo, C. J. (2016). Osmotic and heat treatments on control of fungi associated with seeds of *Eugenia brasiliensis* and *E. pyriformis* (Myrtaceae). *Journal of Seed Science*, *38*(3), 195-203. DOI: https://doi.org/10.1590/2317-1545v38n3159481

Gomes, F. M., Fortes, A. M. T., Silva, J., Bonamigo, T., & Pinto, T. T. (2013). Efeito alelopático da fitomassa de *Lupinus angustifolius* (L.) sobre a germinação e desenvolvimento inicial de *Zea mays* (L.) e *Bidens pilosa* (L.). *Revista Brasileira de Agroecologia*, *8*(1), 48-56.

Gonçalves, T. S. (2015) Ecological and evolutive interactions between: plants, herbivory and natural enemies. *Agropecuária Científica no Semiárido*, *3*(3), 1-9.

Gregolin, F. S., Bonaldo, S. M., Sinhorin, A. P., Banderó, J. L., & Wobeto, C. (2019). The *in vitro* control of *Fusarium proliferatum* by propolis ethanolic extracts. *Revista de Ciências Agrárias*, *42*(2), 456-463. DOI: https://doi.org/10.19084/rca.17471

Hasse, I., May-de-Mio, L. L., & Lima Neto, V. C. (2007). Efeito do pré-plantio com plantas medicinais e aromáticas no controle de *Plasmodiophora brassicae*. *Summa Phytopathologica*, *33*(1), 74-79. DOI: https://doi.org/10.1590/S0100-54052007000100011

Id, V. L. E., Braga, M. R., & Santos Junior, N. A. (2020). Germination and initial development of forest species under the action of catechin, presents in seeds of *Sesbania virgata* (Cav.) Pers. (Fabaceae). *Hoehnea*, *47*(1), e472020.

Id, V. L. E., Costa, B. V., Mignoni, D. S. B., Veronesi, M. B., Simões, K., Braga, M. R., & Santos Junior, N. A. (2015). Phytotoxic effect of *Sesbania virgata* (Cav.) Pers. on seeds of agronomic and forestry species. *Journal of Forestry Research*, *26*(2), 339-346. DOI: DOI: https://doi.org/10.1007/s11676-015-0026-z

Iganci, J. R. V., Bobrowski, V. L., Heiden, G., Stein, V. C., & Rocha, B. H. G. (2006). Efeito do extrato aquoso de diferentes espécies de boldo sobre a germinação e índice mitótico de *Allium cepa* L. *Arquivos do Instituto Biológico*, *73*(1), 79-82. DOI: https://doi.org/10.1590/1808-1657v73p0792006

Inderjit, Wardle, D. A., Karban, R., & Callaway, R. M. (2011). The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology and Evolution*, *26*(12), 655-662. DOI: https://doi.org/10.1016/j.tree.2011.08.003

Jaccoud Filho, D. S, & Dabul, A. N. G. (2011). Novos métodos de detecção de fungos em sementes florestais. In A. F. Santos, J. J. D. Parisi, & J. O. M. Mentem (Eds.), *Patologia de sementes florestais* (p. 69-96). Colombo, PR: Embrapa Florestas.

Lara-Nuñez, A., Romero-Romero, T., Ventura, J. L., Blancas, V., Anaya, A. L., & Cruz-Ortega, R. (2006) Allelochemical stress causes inhibition of growth and oxidative damage in *Lycopersicon esculentum* Mill. *Plant, Cell & Environment*, *29*(11), 2009-2016. DOI: https://doi.org/10.1111/j.1365-3040.2006.01575.x

Long, R. L., Steadman, K. J., Panetta, F. D., & Adkins, S. W. (2009). Soil type does not affect seed ageing when soil water potential and temperature are controlled. *Plant and Soil*, *320*(1-2), 131-140. DOI: https://doi.org/10.1007/s11104-008-9878-8

Maciel, C. G., Ferrera, T. S., Bovolini, M. P., Gonzatto, R., Muniz, M. F. B., & Buriol, G. A. (2012). Seed transmission and pathogenicity of *Pestalotiopsis* sp. in seedlings of *Schinus terebinthifolius* Raddi. *Semina: Ciências Agrárias*, *33*(6), 2767-2774. DOI: https://doi.org/10.5433/1679-0359.2012v33n6Supl1p2767

Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, *2*(2), 176-177. DOI: https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Martins, R. S., Santos Junior, N. A., & Barbedo, C. J. (2022) Seed pathology of non-domesticated species of tropical ecosystems. *Journal of Seed Science*, *44*, e202244029. DOI: https://doi.org/10.1590/2317-1545v44262592

Matos, D. M. S., & Pivello, V. R. (2009). O impacto das plantas invasoras nos recursos naturais de ambientes terrestres- Alguns casos brasileiros. *Ciência e Cultura*, *61*(1), 27-30.

Mignoni, D. S. B., Simões, K., & Braga, M. R. (2017). Potential allelopathic effects of the tropical legume *Sesbania virgata* on the alien *Leucaena leucocephala* related to seed carbohydrate metabolism. *Biological invasions*, *20*(12), 165-180. DOI: DOI: https://doi.org/10.1007/s10530-017-1524-z

Morrissey, J. P., & Osbourn, A. E. (1999). Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiology and Molecular Biology Reviews*, *63*(3), 708-724. DOI: https://doi.org/10.1128/MMBR.63.3.708-724.1999

Nelson, E. B. (2004). Microbial dynamics and interactions in the spermosphere. *Annual Review of Phytopathology*, *42*(1), 271-309. DOI: https://doi.org/10.1146/annurev.phyto.42.121603.131041

Pakeman, R. J., Small, J. L., & Torvell, L. (2012). Edaphic factors influence the longevity of seeds in the soil. *Plant Ecology*, *213*(1), 57-65. DOI: https://doi.org/10.1007/s11258-011-0006-0

Pinto, E. N. F., Souto, J. S., Leonardo, F. A. P., Borges, C. H. A., Barroso, R. F., & Medeiros, A. C. (2016) Crescimento de plântulas de alface (*Lactuca sativa* L.) em solo oriundo de um povoamento de *Luetzelburgia auriculata* (Allemão) Ducke. *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, *11*(2), 33-38. DOI: https://doi.org/10.18378/rvads.v11i2.4260

Pott, A., & Pott, V. J. (1994). *Plantas do Pantanal*. Corumbá, MS: Emprapa/CPAP/SPI.

Praxedes, P. G., Zerlin, J. K., Dias, L. O., & Pessoni, R. A. B. (2011). A novel antifungal protein from seeds of *Sesbania virgata* (Cav.) Pers. (Leguminosae-Faboideae). *Brazilian Journal of Biology*, *71*(3), 687-692. DOI: https://doi.org/10.1590/S1519-69842011000400013

Rezende, E. D. F., Couto, F. A., Borges, J. G., Silva, D. M. D., & Batista, L. R. (2013). Potencial enzimático e toxigênico de fungos isolados de grãos de café. *Coffee Science*, *8*(1), 69-77.

Santos, A. D., Parisi, J. J. D., & Menten, J. O. M. (2011). *Patologia de sementes florestais*. Colombo, PR: Embrapa Florestas.

Santos, A. F., Medeiros, A. C. S., & Santana, D. L. Q. (2001). Fungos em sementes de espécies arbóreas da Mata Atlântica. *Boletim de Pesquisa Florestal, 1*(42), 51-60.

Shreelalitha, S. J., & Sridhar, K. R. (2015). Endophytic fungi of wild legume *Sesbania bispinosa* in coastal sand dunes and mangroves of the Southwest coast of India. *Journal of Forestry Research*, *26*(4), 1003-1011. DOI: https://doi.org/10.1007/s11676-015-0103-3

Simões, K., Du, J., Kretzschmar, F. S., Broeckling, C. D., Stermitz, F. R., Vivanco, J. M., & Braga, M. R. (2008) Phytotoxic catechin leached by seeds of the tropical weed *Sesbania virgata. Journal of Chemical Ecology*, *34*(5), 681-687. DOI: https://doi.org/10.1007/s10886-008-9443-1

Souza, V. C., Agra, P. F. M., Andrade, L. A., Oliveira, I. G., & Oliveira, L. S. (2010) Germinação de sementes da invasora *Sesbania virgata* (Cav.) Pers. sob efeito de luz, temperatura e superação de dormência. *Semina: Ciências Agrárias, 31*(4), 889-889. DOI: https://doi.org/10.5433/1679-0359.2010v31n4p889

Souza Filho, A. P. D. S., Vasconcelos, M. A. M. D., Zoghbi, M. D. G. B., & Cunha, R. L. (2009) Efeitos potencialmente alelopáticos dos óleos essenciais de *Piper hispidinervium* C. DC. e *Pogostemon heyneanus* Benth sobre plantas daninhas. *Acta Amazonica*, *39*(2), 389-396. DOI: https://doi.org/10.1590/S0044-59672009000200018

Vechiato, M. H. (2010). *Importância da qualidade sanitária de sementes florestais na produção de mudas*. Retrieved from http://www.infobibos.com/Artigos/2010_3/SementesFlorestais/index.html

Vechiato, M. H., & Parisi, J. J. D. (2013). Importância da qualidade sanitária de sementes de florestais na produção de mudas. *Biológico, 75*(1), 27-32.

Velázquez-del Valle, M. G., Bautista-Baños, S., Hernández-Lauzardo, A. N., Guerra-Sánchez, M. G., & Amora-Lazcano, E. (2008). Estrategias de control de *Rhizopus stolonifer* Ehrenb. (Ex Fr.) Lind, agente causal de pudriciones postcosecha en productos agrícolas. *Revista Mexicana de Fitopatologia*, *26*(1), 49-55.

Wagner, M., & Mitschunas, N. (2008) Fungal effects on seed bank persistence and potential applications in weed biocontrol: a review. *Basic and Applied Ecology*, *9*(3), 191-203. DOI: https://doi.org/10.1016/j.baae.2007.02.003

Zhu, X., Zhang, J., & Ma, K. (2011). Soil biota reduce allelopathic effects of the invasive *Eupatorium adenophorum*. *PLoS One*, *6*(9), e25393. DOI: https://doi.org/10.1371/journal.pone.0025393