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Research Article

Allelopathic Activity of the Hydrolate and Water Decoction of *Brachiaria humidicola* (Rendle) Plant Parts on the Germination of Four Tropical Leguminous Species

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Knowledge of allelopathic interactions between grasses and legumes can contribute for the successful establishment of mixed tropical pastures in Brazil. The purpose of this work was to evaluate the allelopathic effect of the hydrolate and water decoction of *Brachiaria humidicola* (Rendle) plant parts (root, shoot, and seeds) on four tropical forage legumes *Stylosanthes* spp. cv. Campo Grande, *Macrotyloma axillare, Calopogonium mucunoides, Desmodium ovalifolium*, and on lettuce (*Lactuca sativa* L.), this last species used as a sensitivity standard in allelopathic bioassays. The results obtained for roots and shoots showed, in the case of hydrolate, the highest inhibitory effect on germination rates of the receiving species, while seed hydrolates had a stimulating effect depending on the legume species. In contrast, water decoction extracts had the highest inhibitory effects on root and seed fraction, and the lowest on the shoot fraction. Regarding the receiving species, germination percentages of *M. axillare* showed higher tolerance to inhibitory effects of the aqueous extracts of *B. humidicola*, while *D. ovalifolium* showed the highest sensitivity.

1. Introduction

In Brazil, the predominant beef and dairy cattle production systems are based mostly on grazing and rely on native and cultivated pastures, which are the main source of animal feed [1] due to its lower costs of production [2]. In the tropical region of Brazil, sown pastures are dominated by African grasses of the genus *Brachiaria* [3]. Among them, *B. decumbens* and *B. brizantha* occupy millions of hectares, especially in Central Brazil [3]. *Brachiaria humidicola* (Rendle) Schweick, commonly known as "quicuio- da-Amazonia", has been further expanded to the humid tropics of South America [4]. This species is tolerant to low-fertility soils and other environmental stresses such as drought and cold conditions, and possess the ability to release chemical compounds from roots that suppress soil nitrification [5].

In spite of the good adaptability shown by these pastures, there has been, over the years, a decline in productivity. Pasture degradation is the name given for this process, the main cause of low productivity in the Brazilian livestock sector [1]. It is estimated that at least half of the cultivated pastures are degraded or in process of degradation [6]. Studies indicate that the two main drivers of degradative process are low-soil fertility (especially low soil N) and overgrazing [7].

The introduction of forage legumes in pastures in monoculture of grass can be an alternative to improve the fertility of tropical soils [8], to enrich the diet of the

animals, and, consequently, to increase productivity of pastures [9]. However, the use of legumes in the form of mixed pasture swards is still insignificant [10]. Some reasons may justify this fact, including failed attempts in the past. Although the use of mixed pastures of grasses and legumes is attractive, the rare use of this technology is due mainly because of its slow establishment and persistence in the tropical pasture of the *Leguminosae* [10]. This generally occurs due to competition factors, since the relationship between grass (C_4) and legumes (C_3) involves two species with distinct morphophysiological characteristics [11], with differentiated rates of growth. Grasses are more efficient than legumes in relation to photosynthesis, which make them more competitive within mixed pastures. However, there are other factors which may also affect the establishment and persistence of neighboring plants, such as the phenomenon known as allelopathy [12, 13]. Allelochemicals are produced by plants and released into the environment mainly through processes of leaching, exudation, and volatilization [12, 14–16].

In this context, knowledge of allelopathic interactions between plant species may contribute to the understanding of grass-legume relationships in mixed pastures [17]. There is an increasing demand for studies concerning secondary metabolites produced by plants, mainly those natural compounds that could act as archetypes of synthetic herbicides [14].

Although research confirms that *B. humidicola*, as well as other *Brachiaria* species, possess allopathic activity [17] there are no reports that consider the possible allelopathic activity of different plant parts including roots and seeds, using the technique of hydrodistillation. Therefore, the purpose of this work was to evaluate the laboratory germination responses of the tropical forage legumes *Stylosanthes* spp. cv. Campo Grande, *Macrotyloma axillare*, *Calopogonium mucunoides, and Desmodium ovalifolium* to the effects of the hydrolate and water decoction of *Brachiaria humidicola* plant parts (root, shoot, and seeds).

2. Material and Methods

The experiment was carried out in the laboratories of the Institute of Agronomy, Universidade Federal Rural do Rio de Janeiro (UFRRJ), at Seropédica, Rio de Janeiro State, Brazil (22°46′59″ S; 43°40′45″ W and 33 m.a.s.l.). *Brachiaria humidicola* was collected from an established monoculture (25 years old) on lowland Planossoil, submitted to a lenient grazing regime by goats and occasionally daily heifers [18]. Shoots were cut 0.05 m above soil surface and roots were obtained through direct excavation, which was favored by the sandy texture of the soil surface layer. A previous study on *B. humidicola* root distribution in this profile indicated that at 0.2 m soil depth were concentrated about 70% coarse root and 80% fine root dry mass in relation to total root dry mass at 0.7 m [19]. Root samples were washed free of soil as described by Roberts et al. [20]. These sampling procedures were made in the morning (07 : 00 h, local time) and plant material was transported to the lab in isothermic box. Seeds were collected in January 2010, during the period of grass flowering, and set to dry in the shade at room temperature.

Table 1: Dormancy-breaking methods, incubation temperatures, and duration of photoperiod for allelopathic bioassays of lettuce (*L. sativa*) and four tropical legume species.

Species	Dormancy-breaking method(1)	Incubation temperature(2) (°C)	Photoperiod duration(3) (h)
Lactuca sativa	—	20	16
Macrotyloma axillare	Cuticule remotion	25	8
Calopogonio mucunoides	—	25	0
Stylosanthes spp.	Water 80°C	35	0
Desmodium ovalifolium	Escarification	25	8

(1), (2), (3) According to Brasil [26].

Roots and shoots were cut for analytical processing in fragments of approximately 5 cm. Thereafter, 0.5 kg of each fraction was placed in a 5 liter round-bottom flask with two thirds of distillated and deionized water. The flask was coupled to a Clevenger-type system to perform steam distillation. The same procedure was followed for the seeds except for their previous grinding in a blender. Plant materials remained in contact with boiling water for 4 h in order to extract the essential oil of *B. humidicola*. However, no oil was obtained, probably due to its low concentration. The product of evaporation and subsequent condensation was considered as the hydrolate of the respective fractions, while the water that remained in the flask constituted the water decoction. To obtain sufficient quantities of both fractions, it was necessary to perform four successive cycles of hydrodistillation.

Alellopathic susceptibility of the receiving species was evaluated through germination tests [21–24]. Seeds of *Stylosanthes* ssp. cv. Campo Grande, *Calopogonium mucunoides*, *Macrotyloma axillare* cv. Java and *Desmodium ovalifolium* cv. Itabela were obtained from the germplasm bank of Embrapa Agrobiologia, at Seropédica, Rio de Janeiro State, Brazil. Lettuce (*Lactuca sativa* L.) seeds came from commercial source. This species was chosen because it is considered a standard for sensitivity in bioassays of allelopathic activity [21, 25]. When necessary, seed dormancy-breaking of the leguminous species was performed following the specifications of the rules for seed analysis [26] as indicated in Table 1.

Aliquots of 5 ml of each hydrodistilled extract (hydrolate) and residual water decoction were applied on the surface of three sheets of autoclaved (120°C) Germitest paper in gear box type containers, and then 25 seeds of each receiving species were set uniformly on the moistened paper. In control treatment, Germitest paper was moistened with deionized water. The gear box containers were incubated in BOD chambers under variable conditions of light and temperature, according to the species (Table 1).

Calculations of seed germination rates followed Brazilian specifications. Accordingly, germination of seeds corresponded to the proportion of seeds that produced seedlings classified as normal, that is, those who had the primary root and shoot well developed [26]. The germination data were expressed in percentage (%) and transformed in arc sen $\sqrt{\%/100}$ to ensure residual normality [27].

Treatments consisted in combinations of five receiving species, with or without extract and three parts of the donor species with four repetitions in a completely randomized design. Differences between extraction methods were assessed only qualitatively. Data were analyzed using the statistical procedures of the Statistical Analysis System [SAS, [28]]. The PROC GLM was used for the analysis of variance (ANOVA), and mean data were compared by Tukey test (5%).

3. Results and Discussion

3.1. Allelopathic Activity of Shoot. Overall, the results of bioassays showed statistically significant differences ($P \leq 0.05$) between receiving species for both the hydrolate and water decoction of shoot pieces of *B. humidicola* when compared with their respective controls (Figures 1(a) and 1(b)). As we would expect, the shoot hydrolate completely inhibited the germination of lettuce [21], but also had equal suppressing effect on *D. ovalifolium*. Similarly, *Stylosanthes* spp. cv. Campo Grande was severely affected with an absolute reduction of 66% or 54% considering its germination percentage in deionized water. In contrast, the application of the hydrolate had no effect on absolute or relative germination rates of *C. mucunoides*, whereas for *M. axillare* the reduction was relatively small (16%), but statistically significant (Figure 1(a)). In relation to water decoction of shoot parts, its inhibitory effects were smaller in comparison with the hydrolate extracts, as evidenced by the fact that no full inhibition of germination in any of the studied species was observed (Figure 1(b)). In fact, there were no significant differences between *M. axillare* and *C. mucunoides* compared to their controls. On the other hand, germination rates of *Stylosanthes* and *Desmodium* in response to application of water decoction was lower than those for lettuce, a species that is reasonably immune, despite being considered a standard of sensitivity.

Collectively, these results indicate that the allelochemical agents are present in higher concentrations in the hydrolate.

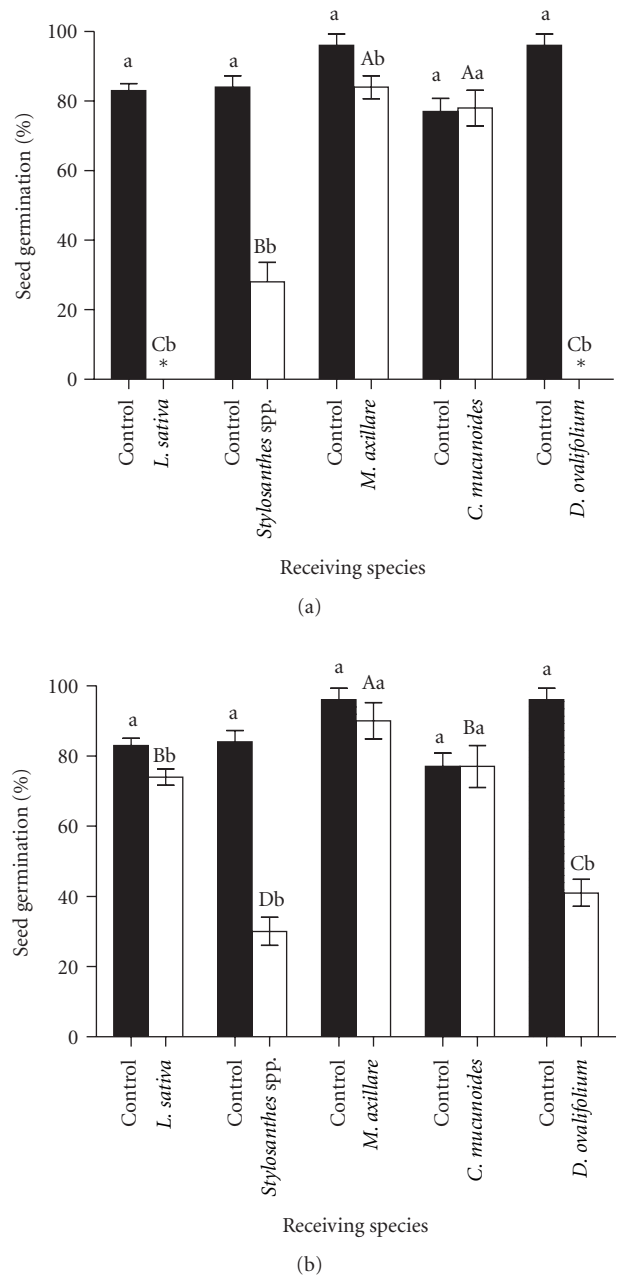

Figure 1: Germination percentages of five selected receiving species in response to application of hydrolate (a) or water decoction (b) extracts prepared from shoot of *B. humidicola* compared to the control treatment (only deionized water). Uppercase letters on top of bars denote statistical differences ($P \leq 0.05$) of the averages between species of receptors. Lowercase letters show the difference of each species and their respective control.

3.2. Allelopathic Activity of Roots. Considering the allelopathic effects attributable to hydrolate or water decoction, root extracts had a greater inhibitory capacity than shoot extracts (Figures 1 and 2). Full inhibition (lettuce and *Desmodium*) or severe reduction (*Stylosanthes*) of germination percentages were observed when seeds were treated with root hydrolates (Figure 2(a)). Even the most tolerant *M. axillare* and *C. mucunoides* species showed significant decrease in their germination rates in relation to control treatments (Figure 2(a)). Also, in opposition with the shoot behavior, water decoction of the roots showed a high-biological activity (Figures 1(b) and 2(b)). In fact, full inhibition of *D. ovalifolium* and *C. mucunoides* was observed, the latter being an unexpected result. Furthermore, seed germination percentages of *Stylosanthes* spp. and *L. sativa* did not differ significantly (Figure 2(b)).

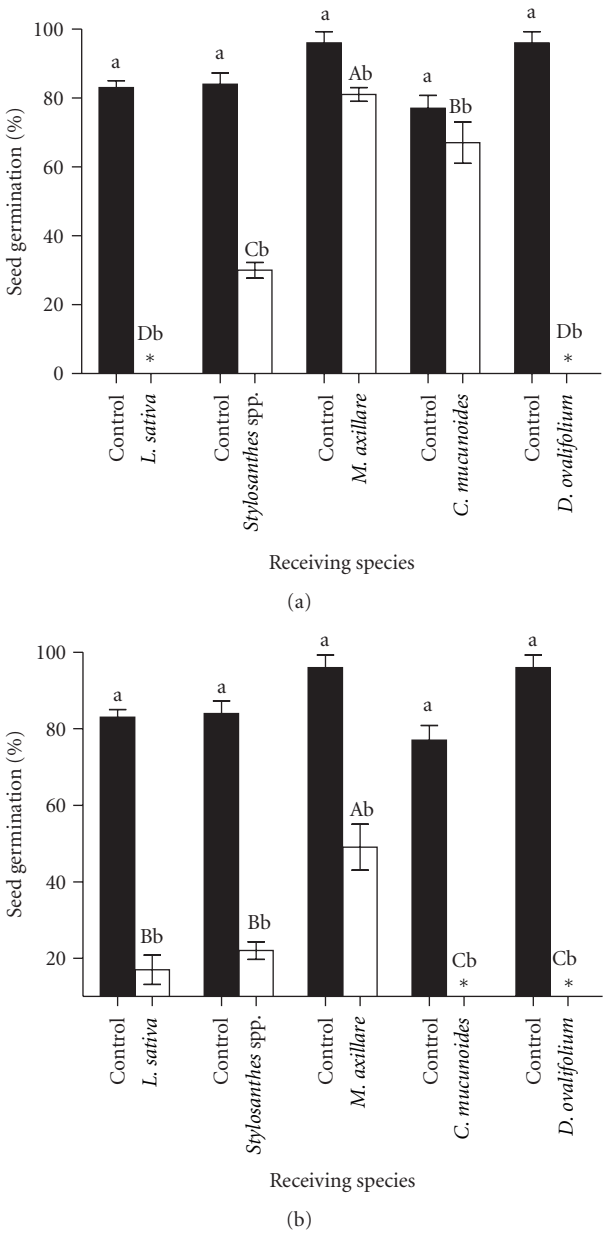

Figure 2: Germination percentages of five selected receiving species in response to application of hydrolate (a) or water decoction (b) extracts prepared from roots of *B. humidicola* compared to the control treatment (only deionized water). Uppercase letters on top of bars denote statistical differences ($P \leq 0.05$) of the averages between species of receptors. Lowercase letters show the difference of each species and their respective control.

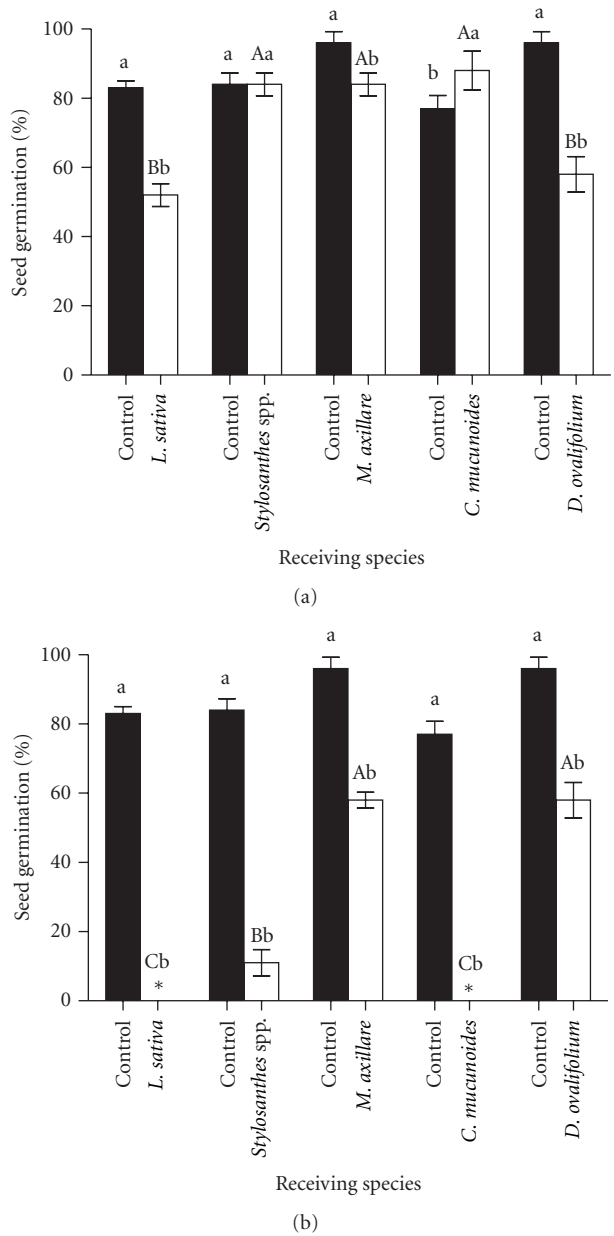

Figure 3: Germination percentages of five selected receiving species in response to application of hydrolate (a) or water decoction (b) extracts prepared from seeds of *B. humidicola* compared to the control treatment (only deionized water). Uppercase letters on top of bars denote statistical differences ($P \leq 0.05$) of the averages between species of receptors. Lowercase letters show the difference of each species and their respective control.

The better response in terms of resistance was expressed by *M. axillare*, with 96% of germination for the control treatment versus 49% for water decoction, a performance significantly higher ($P \leq 0.05$) than the other species studied. Thus, data suggest that for the roots of *Brachiaria humidicola*, allelopathic activity is associated with nonvolatile molecules that probably are concentrated in the water decoction fraction.

3.3. Allelopathic Activity of Seeds. Figures 3(a) and 3(b) show percentages of seed germination of the receiving species when treated with extracts obtained from hydrolates or water decoction seeds of *B. humidicola*. In regard to the hydrolate, inhibitory effects of allelopathic agents were lenient, as for *M. axillare,* or totally absent as in the case of *Stylosanthes* spp. or even expressed as a significant stimulatory effect ($P \leq 0.05$) in *C. mucunoides*. Again, the data confirmed

the higher sensitivity of *D. ovalifolium* in relation to the other species, since its inhibitory response equaled that of lettuce (Figure 3(a)). In contrast with the limited inhibitory power of seed hydrolate, water decoction showed an acute allelopathic activity involving complete inhibition of seed germination of *C. mucunoides* and *L. sativa*, a response very similar to that observed for extracts of water decoction of roots (Figures 2(b) and 3(b)). Likewise, *Stylosanthes* spp. response to seed water decoction parallels the effect for roots and the inhibitory behavior of *M. axillare* mirrored that seen for root water decoction (Figures 2(b) and 3(b)).

Taken together, these results indicate that root and shoot hydrolates had the highest inhibitory effect on germination rates of the forage legume species studied. Seed hydrolates had a lighter inhibitory effect, including an allelopathic stimulation on the seed germination rate of *C. mucunoides*. In contrast, water decoction extracts had the highest inhibitory effects on the root and seed fractions, and the lowest on the shoot fraction. Regarding the receiving species, germination percentages of *M. axillare* showed bigger tolerance to inhibitory effects of the aqueous extracts of *B. humidicola*, while *D. ovalifolium* showed the highest sensitivity.

4. Conclusion

In general, the hydrolate and decoction water of *B. humidicola* parts (shoot, root, and seed) showed inhibitory effect on receiving species, *L. sativa*, *Stylosanthes* spp., *M. axillare*, *C. mucunoides,* and *D. ovalifolium*.

M. axillare showed the highest rates of germination under the application of the extracts of *B. humidicola*, while *D. ovalifolium* showed the highest sensitivity.

Water decoction, mainly from the roots, resulted in a greater inhibitory effect. In contrast, the seed hydrolate showed less activity.

In relation to the parts of *B. humidicola*, the shoot extracts showed a more lenient result for the inhibition of germination of the receiving species.

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