

Effects of *Eugenia dysenterica* leaf extracts on the growth of sesame and radish

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ABSTRACT

The sesame and radish were germinated and grown in different concentrations of *E. dysenterica* leaf extracts using filter paper and Cerrado soil as substrates. This study also evaluated the effects of fresh- and dry-leaf extracts and the extract pH (4.7 - 7.0) on target species. The extract did not influence the germination but drastically reduced the seedling growth. The roots were more affected by the extracts than shoots. Dry-leaf extracts were more inhibitory to sesame and radish seedlings than fresh-leaves extracts. The extract pH did not influence the allelopathic response of target species. The extracts resulted in the abnormal growth of seedlings viz., shorter roots, root tissue darkening, early lateral root development, less number of root hairs and lateral roots and altered gravitropic response. The inhibitory effects of leaves extracts on seedling growth were more intense, when soil was substrate. Thus *E. dysenterica* leaves have an allelopathic potential that could influence the germination and growth of plants under natural conditions.

Key Words: Allelopathy, Cerrado, *Eugenia dysenterica*, extract pH, root morphology, *Raphanus sativus*, *Sesamum indicum*.

INTRODUCTION

Allelopathy is the positive or negative interference of a plant upon other organisms through metabolic compounds released into the environment (9,15). These compounds, termed as allelochemicals, may be released through lixiviation of aerial parts, root exudates, volatilization and decomposition of plant material and are present in plant biomass, flowers, fruits, and seeds (9,15). The Cerrado is the second largest south-american biome comprising of 2.0 million Km² area in Central Brazil. Its vegetation types ranges from gallery forests to savannas and grasslands, however, savannas predominate. The dry season is from May to September and the rain season from mid-September to mid-May (total annual precipitation: 1600 mm). The Cerrado has very high biodiversity and degree of endemism (22). Several Cerrado species are allelopathic (4,10,18,19,20), including the tree *Eugenia dysenterica* D.C. (Myrtaceae), a deciduous tree. Its leaves accumulates in large quantity under the canopy mainly during the dry season. We noticed that in some areas below the trees, the recovery of neighboring plants is delayed during the rain season. This has made us to believe that lixiviates from the leaves could be involved

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in this process. Its aqueous leaf extracts reduced the initial growth of *Zea mays*, weeds (*Digitaria horizontalis*, *Bidens pilosa*) and the native grass *Melinis minutifolia* (1). In this study, we determined the allelopathic potential of its aqueous leaf extracts on the germination and initial growth of *Sesamum indicum* L. (sesame) and *Raphanus sativus* L. (radish). Besides, the effects of extract pH and preparation procedure (fresh or dry leaves) were also studied on the growth of these target species. These test species were selected because these are frequently used in allelopathic studies due to (i) fast germination of seeds and (ii) uniform growth of seedlings.

MATERIAL AND METHODS

Fully developed leaves of *E. dysenterica* were collected from four trees in the Brasilia University Campus (latitude 15° 46' 11" S and longitude 47° 52' 6" W at 1038 m altitude). For the fresh leaf-extracts, 50 g leaves were grinded and blended in 1000 mL of distilled water, resulting in 5% (w/v) solution. After 24 h at 6 °C (12), it was filtered through Whatman filter paper n° 1 and diluted to 3% and 1% for bioassays. For the dry-leaf extracts, the fresh leaves were dried for 24 h at 50 °C before grinding. The leaf water content was 53.77% (n=50, fresh weight basis). *Sesamum indicum* var. "Black" and *Raphanus sativus* var. Vip Crimson (Feltrin) were used as target-species to determine the effect of leaf extracts of *E. dysenterica* on their germination and seedling growth.

LABROTARY BIOASSAYS

The germination and growth experiments were conducted in BOD chambers (Marconi MA 403) under a photoperiod of 12h (400 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$) and temperatures of 25 and 30 °C for sesame and radish, respectively. The experimental treatments consisted of 3 factors: (i). Leaves types : 2 (Dry, fresh), (ii). Aqueous extracts concentration : 3 (0, 1, 3%) and (iii). Test Crops : 2 (Sesame, radish). The treatments were replicated three times in completely randomized design. Two types of experiments were conducted for (a) Germination and (b) Growth studies.

Germination experiments: Thirty seeds of each target-species were placed in three Petri dishes (10 seeds per plate) containing Qualy® filter paper. The number of germinated seeds were counted every 24 h and germinated seeds were removed. The seeds were considered germinated after the emergence of 1 cm long radicle (13). The germination was recorded for 2-days in sesame and for 4-days in radish. The experiment was replicated three times. The data was used to calculate the Germination Percentage (G %) and the mean Germination time (t) as under (13):

$$G\% = [\sum (n_i.N^{-1}).100]$$

Where, n_i : Number of seeds germinated and N: Number of seeds sown.

$$t = [\sum (n_i.t_i).\sum n_i^{-1}]$$

Where, n_i : Number of germinated seeds in a particular time interval t_i .

Leaf extract pH : The effect of extracts pH (original 4.7, adjusted 7.0) on the allelopathic response was investigated. The pH was adjusted to 7.0 with 200 mM NaOH. Aqueous extracts of both pH were assayed on seed germination and seedling growth of sesame and radish.

Growth experiments : To evaluate the effects of leaf extracts of *E. dysenterica* on seedling growth of sesame and radish seedlings, seeds of both target-species were first germinated in water, as per germination experiments. The germinated seeds were then selected by uniformity of radicle length and transferred to Petri dishes containing the aqueous extracts (0, 1, 3%). The growth parameters (size of shoots and roots, number of lateral roots, occurrence of root hairs, tissue darkening and abnormalities in the gravitropic response) were recorded 5-days after incubation.

POT CULTURE

A growth experiment was done using soil (Red Latosol) with sesame and radish. Red Latosol, the most common soil type in the Cerrado (23), was used as substrate. The collected soil was sieved to remove debris and coarser particles. This experiment had 3 treatments: Control (150 g soil), 1% (1.5 g leaves + 148.5 g soil) and 3% (4.5 g leaves + 145.5 g soil). Fresh leaves of *E. dysenterica* were slightly grinded and mixed with the soil as per treatments. Each treatment had 12 pots, each was sown with 3 germinated seeds of sesame or radish. The pots were irrigated consistently to keep the soil moist.

Statistical analysis: The data was analyzed by ANOVA and the differences among the means were tested by the Tukey test ($p < 0.05$) for the parametric data, and Kruskal-wallis ($p < 0.05$) for the non-parametric data. The results were expressed as the average of each analyzed parameter \pm Standard deviation (SD).

RESULTS AND DISCUSSION

PETRI DISH BIOASSAYS

Germination

The leaf extracts (1 and 3%) of *E. dysenterica* did not affect the germination of sesame and radish, whether prepared from fresh or dry leaves. In sesame, the germination was 86.7 to 96.7% and the mean germination times was 60.0 to 68.7 h. While in radish, the germination was 93.3 to 100% and the mean germination times was 24.8 to 30h. Indeed, the germination of both species was not affected by the leaf extracts pH (4.7 or 7.0). Several reports have shown that allelochemicals has little influence on germination. Fruit extracts (5% w/v) of *Solanum lycocarpum* did not effect the germination of sesame seeds (18). Similarly, the leaf extracts of *S. palinacanthum* had no effect on the seed germination of sesame (19). When germination is highly dependent upon the seeds reserves, it is less sensitive to external factors such as allelochemicals (12,16). However, leaf extracts of *Mimosa caesalpiniaefolia* influence the germination of *Tabebuia alba* (21), indicating that the germination process may sometimes be affected by chemicals present in the solution.

Seedlings growth

Fresh-leaf extracts did not influence the shoots growth in sesame, however, the 3% fresh-leaf extracts reduced the roots growth (Fig. 1). Conversely, dry-leaf extracts significantly reduced the growth of both shoots and roots (Fig. 1). For radish, fresh-leaf extracts had a significant smaller allelopathic impact on growth than the dry-leaf extracts (Fig. 1). Moreover, the shoots were less affected by type of extract assayed (from fresh/dry leaves) than roots. The dry-leaf extracts were slightly more inhibitory to seedling growth and the roots were more sensitive than shoots.

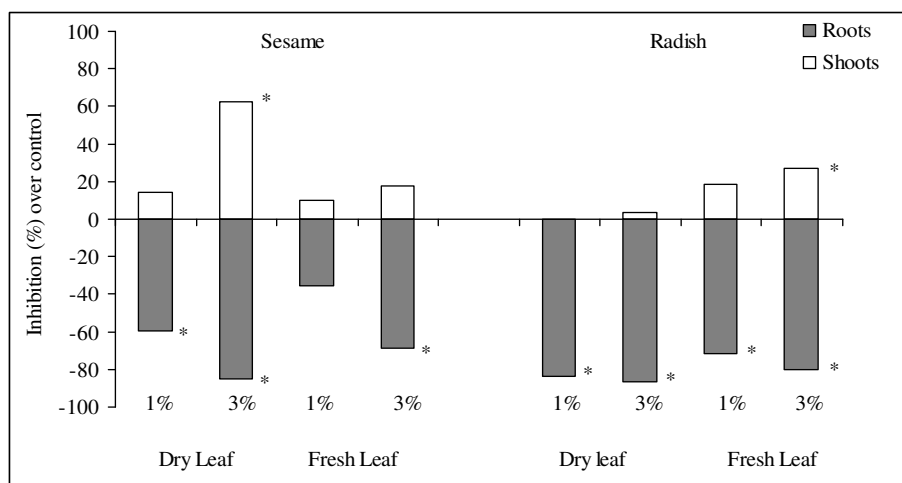


Figure 1. Inhibitory effects of fresh and dry leaves extracts of *Eugenia dysenterica* on root and shoot growth of sesame and radish seedling in Lab bioassay. Columns followed by an Asterik are significantly different than control as per Tukey test for shoots and Kruskal-Wallis test for roots ($p < 0.05$)

The extracts of dry plant parts, such as leaves, induces higher growth inhibition in target-species (6,11). Several factors may be associated with a higher effectiveness of extracts made of dry plant parts, being the difference in the amount of water in the material an important factor to be considered. The extracts of fresh plant material may induce similar allelopathic responses or even more intense than dry-organ extracts e.g. the soybean germination was equally affected by extracts of fresh or dry leaves of oats (7). Fresh-leaf extracts of *Mimosa caesalpinaefolia* inhibits and delays the germination of *Tabebuia alba* (21). Our results showed that drying the leaves of *E. dysenterica* at moderate temperatures do not affect its inhibitory effects on seedling growth, suggesting that under natural conditions the allelopathic potential of this species would not be affected by the dry season in the Cerrado biome.

Extract pH: The inhibitory effects of *E. dysenterica* leaf extracts on the growth of sesame and radish were very similar, irrespective the extract pH. In both species the roots were

severely affected by the extracts, although the shoots of sesame were also sensitive to the extracts (Fig. 2). Thus the pH of extracts was not a crucial factor in the allelopathic response of sesame and radish seedlings.

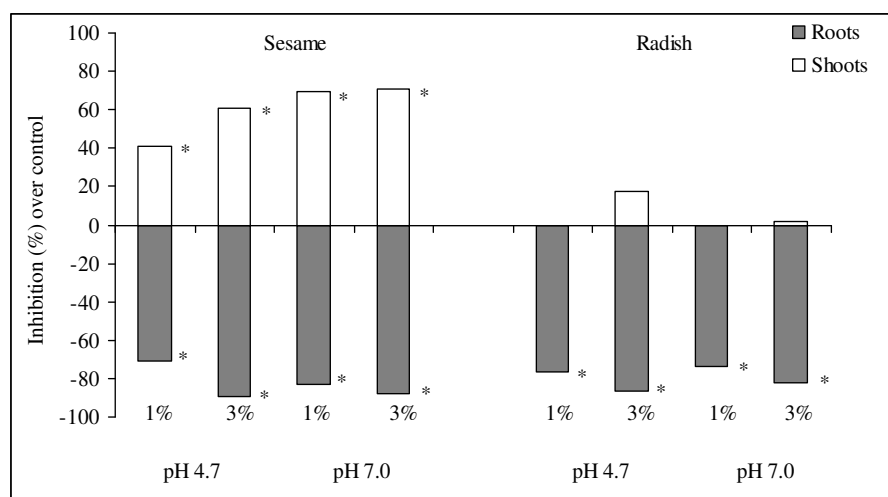


Figure 2. Inhibitory effects of pH levels of fresh leaves extracts from *E. dysenterica* on seedling growth of sesame and radish in Lab bioassay. Columns followed by an Asterisk are significantly different than control as per Tukey test for shoots and Kruskal-Wallis test for roots ($p < 0.05$)

Besides the pH, other physical-chemical factors such as the osmolarity have been considered when investigating the allelopathy of plant extracts (18, 20). In *E. dysenterica*, previous studies have shown that osmolarity was not involved on the effects of aqueous leaf extracts on seedling growth of four target-species (1). The value observed for dry-leaf extracts at 5% (w/v) was 23 mmol.kg^{-1} (1). This value is below 100 mmol.kg^{-1} , the minimum to produce osmotic effects on allelopathic bioassays (14). As the extract concentrations used in the present study were low (1% and 3%), hence, the osmotic effects of plant extracts on seedling growth were insignificant.

Root morphology : The aqueous extracts severely altered the roots of both sesame and radish seedlings (Table 1). Compared with their respective controls, there was a reduction in the number of seedlings that differentiated root hairs and lateral roots, as well as in the average number of lateral roots per seedling (Table 1).

The extracts also induced tissue darkening and affected gravitropic responses of the roots (Fig. 3). The regions of the root in contact with the extract became brown, which was more intense in higher concentration extracts. Additionally, the roots of seedlings subjected to the leaf extracts usually grew upwards, indicating a negative gravitropism.

Table 1. Effects of aqueous leaf extract of *E. dysenterica* on the roots development of sesame (*Sesamum indicum*) and radish (*Raphanus sativus*) at 5-days

Extract Concentration (%)	Seedlings with root hairs (%)		Seedlings with lateral roots (%)		Mean number of lateral roots/seedling	
	Sesame	Radish	Sesame	Radish	Sesame	Radish
Control (DW)	100.0 a	70.0 a	90.0 a	93.3 a	3.6 ± 2.01 a	1.7 ± 1.50 a
1%	86.7 a	53.3 a	83.3 a	33.3 b	3.6 ± 2.46 a	1.1 ± 1.66 b
3%	26.7 b	10.0 b	60.0 b	23.3 b	1.8 ± 1.78 b	0.3 ± 0.52 b

DW: Distilled water, Data are given as means ± Standard Deviation (SD), n=30. Means followed by the same letter within the same column are not significantly different by the Tukey test for the percentage of seedlings with lateral roots and the average number of lateral roots per seedling. Kruskal-wallis test was used for the percentage of seedlings with root hairs ($p < 0.05$).

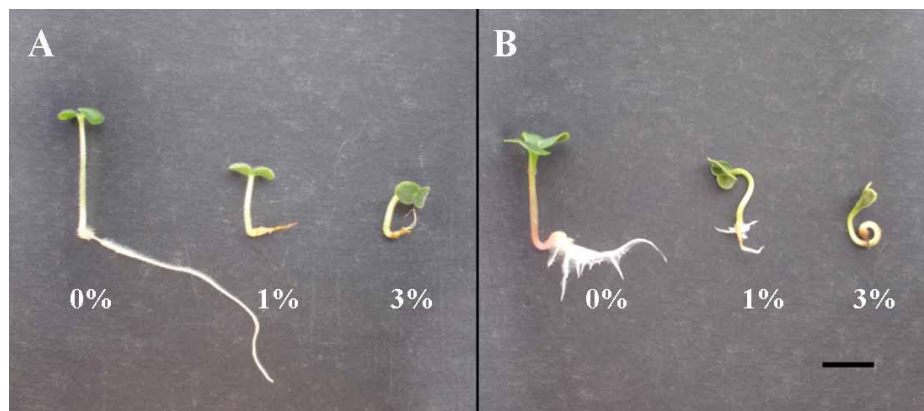


Figure 3. Effects of leaf extracts of *E. dysenterica* on the morphological parameters of 5-days-old sesame (*Sesamum indicum*) and radish (*Raphanus sativus*) seedlings. Scale bar = 1 cm.

Maize seedlings treated with *Ilex paraguariensis* fruit extracts showed a reduction in root hairs and early development of lateral roots (16). Moreover, when strawberry seedlings were treated with organic residues extracted from other strawberry plants, the roots turned red and no root hairs or laterals were formed (17). Morphological changes (number of root hairs, the number, length, and development of lateral roots) are common features in the allelopathic process. The reasons for these changes are still unknown, but these morphological effects may be associated with disruption of hormonal balance (5). The darkening of root tissue also appears to be highly associated with the allelopathic response. Roots of lettuce and radish growing with aqueous extract of *Andira humilis* turned dark (20). The darkening of tissue has been associated with the activation of genes related to the programmed cell death mechanism, as well as to genes of the oxidative stress and of the phenylpropanoid and terpenoid pathways, which may be triggered by allelochemicals (3). Alkaloids, phenols and terpenes are known to be associated with the allelopathic process (8).

Another reaction to the presence of *E. dysenterica* leaf extracts was the upward growth of sesame and radish roots. Such allelopathic response has also been observed in sesame seedlings treated with extracts of *Solanum lycocarpum* leaves (2). Additionally, hypocotyls of alfalfa seedlings bent upward when exposed to their own plant extracts (5). The cause of this phenomenon is likely to be a disturbance of the gravitropic response of the seedlings; however, anatomical studies are required to clarify such effects on root growth.

POT CULTURE

The leaf material mixed in Cerrado soil drastically inhibited the growth of sesame and radish seedlings (Fig. 4). The root growth inhibition was > 90% and the shoot growth inhibition was around 50% for both target species. This also shows that the allelopathic activity of *E. dysenterica* persists, when the leaves are subjected to lixiviation and decomposition under soil conditions, which opens the perspective for field experiments with *E. dysenterica*.

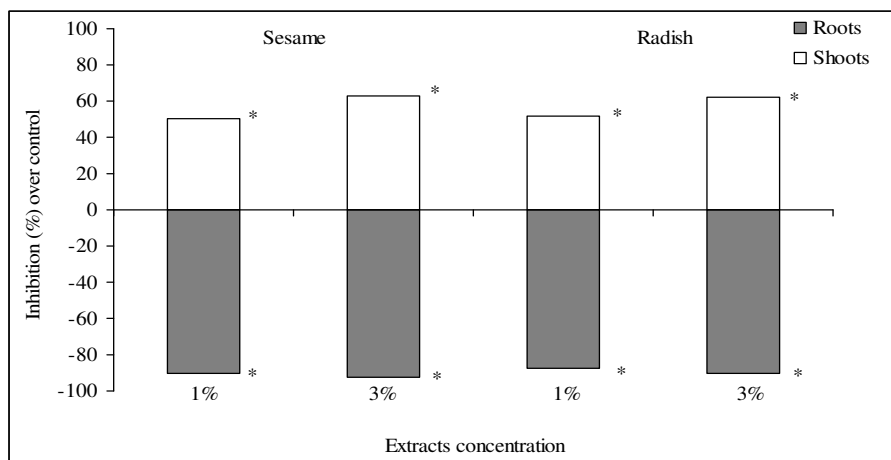


Figure 4. Inhibitory effects of powdered mature fresh leaves of *E. dysenterica* on seedlings growth of sesame and radish at 7 days in cerrado soil in pot culture. Columns followed by an Asterik are significantly different than control as per Tukey test for shoots and Kruskal-Wallis test for roots ($p < 0.05$)

CONCLUSIONS

The aqueous leaf extracts of *E. dysenterica* inhibited the initial growth, root hair and lateral root differentiation and impaired gravitropic responses of sesame and radish seedlings at concentration as low as 1%. The allelopathic potential was not affected neither by drying the leaves, nor by altering the pH of extracts. The roots were more sensitive than the shoots. Experiments in soil suggest that the observed responses may operate in natural conditions. Nevertheless, more research is needed to isolate and identify the allelochemicals involved, as well as how biotic and abiotic factors influence the effect of leaves of *E. dysenterica* on representative receptor plants in natural conditions.

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