Ecotoxicology
https://doi.org/10.1007/s10646-019-02133-y

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Secondary metabolites that could contribute to the monodominance of *Erythrina fusca* in the Brazilian Pantanal

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Accepted: 2 November 2019

Abstract

Erythrina fusca is a dominant species in the Brazilian Pantanal. We hypothesized that *E. fusca* possess allelopathic potential and we evaluated effects of extracts on germination and development of *Lactuca sativa*, a bioindicator species. We tested the effect of leaves, bark, roots, and seeds extracts of *E. fusca* on germination and speed index, using high, moderate and low concentration (0.2, 1 and 5 mg mL^{-1}). To evaluate effects on development, we subjected seedlings of *L. sativa* to the same treatments and measured root and aerial part length. High concentration of extracts reduced *L. sativa* germination; leaves extract caused the maximum reduction on germination of *L. sativa*, similar to 2,4-Dichlorophenoxyacetic acid (2,4-D); this extract has flavonoids and saponins as main compounds, classes that also occur in the bark and roots extracts in lower concentrations; bark and roots (5 mg mL^{-1}), leaves and roots (1 mg mL^{-1}) decreased these traits as well, but in lower magnitude. A significant reduction in root length was induced by highest concentration of all extracts (5 mg mL^{-1}); the results suggest that erythrinic alkaloids should interfere in the root length once the seeds accumulate almost exclusively this class of compounds. Our results showed that all parts of *E. fusca* had adverse effects on germination or development of *L. sativa*, showing that different class of compounds secondary metabolites is involved in this activity. Possibly, this phytotoxicity influences monodominance of *E. fusca* in Pantanal, but studies are essential to evaluate effects of it on other native species.

Keywords Allelopathy · Flavonoids · Erythrinic alkaloids · Tree dominance · Tropical wetland

Introduction

Erythrina fusca Lour. (Fabaceae) is a monodominant tree species found in the Brazilian Pantanal and one of the most widespread species of the genus (Russo and Baguinon 1997; Pott et al. 2011). *E. fusca* occurs in riparian forests, mainly in the Amazon region and Pantanal, and is monodominant only in the plains of the northern Paraguay River, in the Cáceres sub-region of the Pantanal (Lorenzi 1998; Pott et al. 2011).

Monodominant species can grow aggregate and may represent more than half of the total number of trees from a plant community (Connell and Lowman 1989; Hart et al. 1989). One mechanism that may influence the dominance of some plant species is allelopathy since this process can affect plant-plant and plant-environment interactions (Hart 1990; Macías et al. 2008).

According to Rice (1984), allelopathy refers to both inhibitory as well as stimulatory effect, direct or indirect, which one plant species performs on another may have

Supplementary information The online version of this article (https://doi.org/10.1007/s10646-019-02133-y) contains supplementary material, which is available to authorized users.

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Published online: 14 November 2019

inhibitory impacts, mediated by secondary metabolites such as flavonoids, saponins, and alkaloids. Thus, allelopathy is the capacity to accumulate metabolites that affect germination, development and/or reproduction of other organisms (Ooka and Owens 2018).

The family Fabaceae frequently showed allelopathic potential (Oliveira et al. 2008; Cândido et al. 2010; Aguilera et al. 2015; Id et al. 2015). Allelopathy is also common in the genus *Erythrina* (Soares et al. 2002; Centenaro et al. 2009; Oliveira et al. 2012, 2013); this genus is rich in unusual secondary metabolites, such as tetracyclic alkaloids (erythrinic alkaloids), terpenoids, flavonoids (especially pterocarpans and *C*-hexoside), coumarins, and saponins (Yenesew et al. 2003; Juma and Majinda 2004; Dao et al. 2009; Pérez et al. 2015). Studies of metabolites from *E. fusca* confirmed the presence of flavonoids, such as pterocarpans (Innok et al. 2009, 2010). Despite the monodominance and the presence of phytotoxicity metabolites, no study has covered the allelopathic effects of *E. fusca*, not even on a bioindicator species.

The use of the bioindicator *Lactuca sativa* L. (Asteraceae) is a usual method for testing allelopathic potential since it has a rapid life cycle and is highly sensitive to the action of allelochemicals (even in low concentrations) and also has as all stages of development well known (Ferreira and Aquila 2000). Meanwhile, the germination and development patterns are not known for many wild species, and it is challenging to infer about the potential allelopathic effects on these wild plant species. Therefore, the results of the experiment using *L. sativa* are quick and easy to understand, because if we regulate all external factors and compare percentages of germination and/or development of *L. sativa* with controls (negative and positive), any change in this trait could be attributed to the adverse potential of the tested extracts/compounds. Several recent studies use *L. sativa* as a plant model in allelopathy experiments (e.g., Wang et al. 2016, 2019; Fernandes et al. 2018; Carvalho et al. 2019; Scrivanti and Anton 2019; Silva et al. 2019). Furthermore, considering that *E. fusca* affects the development of *L. sativa* seeds, we can infer about its probable effect on wild eudicots.

Therefore, we believe that *E. fusca* has allelopathic potential and, we hypothesized that: (1) extracts from different parts of the plant retard or inhibit seed germination and development of the bioindicator species (*L. sativa*); (2) inhibitory activity increase with increasing concentration of each extract. Thus, to get an initial idea about this, we aimed, in this study, to evaluate the effects of *E. fusca* leaves, bark, root, and seed extracts on germination and development of the bioindicator *L. sativa* and identify the secondary metabolites of different tested parts.

Materials and methods

Plant material and study area

E. fusca is a deciduous species that blooms from May to September with fructification occurring in November (Lorenzi 1998). This species is monodominant in the Pantanal sub-region of Cáceres, more specifically in the region of Taiamã Ecological Station (Pott et al. 2011), where this vegetation type occupies 16% of the total area (Frota et al. 2017). This vegetation is characterized by low-density vegetation predominating with arboreal individuals of *E. fusca*, as well as few individuals of other arboreal species. A previous study showed that *E. fusca* represents 77% of the total of individuals in these stands (Gris et al. unpubl. res.). The herbaceous stratum is very homogeneous, dominated by grasses, with soil covered by a histosol layer with leaf litter, primarily composed by *E. fusca* leaves (Gris et al. unpubl. res.). We collected the material only from adult plants: mature leaves and seeds, without predator attack; branches of secondary roots and bark slices of the basal trunk region.

We performed the fieldwork during the dry season, in November 2013, in these monodominant stands at Taiamã Ecological Station, between the coordinates: 16°50′58.2″S 57°28′25.7″W and 16°52′57.4″S 57°30′22.2″W. The regional climate is Aw (with dry winter) according to the Köppen classification (Alvares et al. 2013), with two seasons: dry season from May to September and rainy season from October to April. The average precipitation is 1227 mm, and the average annual temperatures are around 26 °C, we calculated these values from the data obtained from National Institute of Meteorology (INMET 2019).

We prepared a fertile sample using herbarium techniques (Mori et al. 1989; Bridson and Forman 2004) and the voucher was deposited in the CGMS Herbarium under registration CGMS 40967.

Plant extraction

We dehydrated plant material in an air-dry oven at 50 °C for 24 h. We briefly ground and homogenized each plant part separately in a Willey-type mill. The seed extract was prepared through percolation, using ethanol:water (7:3), 20 drops per minute during three days of extraction. Other plant tissues were submitted to a pressurized fluid extractor (DIONEX®—ASE 150), using a mixture of ethanol:water (7:3), with temperature of 130 °C, static extraction time of 4 min, 150% volume wash, five cycles of extraction and 100 s of purge. The extracts were concentrated in a rotary evaporator, lyophilized and maintained at −20 °C until tests were performed.

Bioassay and analysis

For the germination experiment, we used Petri dishes (9 cm in diameter) containing two sheets of filter paper, previously autoclaved. We solubilized all extracts in MES buffer (2-morpholinoethanesulfonic acid) 10 mM, pH 6.0.

The experimental design was completely randomized with 14 treatments consisting of MES buffer solution (negative control); solution of 2,4-Dichlorophenoxyacetic acid (2,4-D) 1% diluted in distilled water (positive control) and three concentrations of each extract of leaves, bark, roots and seeds of *E. fusca* (0.2, 1 and 5 mg mL^{-1}). We used four replicas for each treatment, with 25 seeds of *L. sativa* (lettuce) and 2 mL of the extract solution for the respective treatment. We kept all dishes in an incubator of biochemical oxygen demand (BOD) at 25 °C and a photoperiod of 12 h. Every 12 h during seven days, we counted germinated seeds (seeds with at least 2 mm of radicular protrusion) and, on the last evaluation day, we counted the normal seedlings (well-developed, complete, proportionate, and healthy seedlings). We calculated the percentage of germination (G), germination speed index (GSI), the percentage of normal seedlings (NS) and the normal seedling speed index (NSSI). GSI and NSSI were calculated by adapting the formula according to Maguire (1962), for example, GSI = G1/N1 + G2/N2 + .. + Gn/Nn, where G1, G2, Gn = number of seeds germinated in each count day (from first to last day) and N1, N2, Nn = number of days from first to last count day (Maguire 1962; Labouriau and Valadares 1976).

We also considered growth as a parameter to measure allelopathic potential. To evaluate the effects of the same treatments described above on growth of seedlings of *L. sativa*, we first germinated the seeds in autoclaved Petri dishes, which contained two sheets of filter paper moistened with 2 mL of buffer solution MES. After 3 days, we selected the normal seedlings and transferred them to dishes containing the 14 treatments described above (four replicates of 25 seedlings each). After 4 days, we measured the root and aerial part length of seedlings.

We used R software (R Development Core Team 2019) to analyze the results with a two-way analysis of variance (with the factors: plant part and concentration of the extracts), which was followed by a Tukey test (5%).

Chemical analysis in UFLC-DAD-MS

All the extracts were solubilized in methanol and ultrapure water (7:3 v/v) at the concentration of 1 mg mL^{-1} The analysis were performed in a UFLC LC-20AD (Shimadzu, Kyoto, Japan) coupled to a diode array detector (DAD) and a high-resolution mass spectrometer ESI-qTOF (MicroTOF-Q III, Bruker Daltonics, Billerica, USA). The analyses were done in the negative and positive ionization mode, but we only depicted the chromatogram at positive mode (*m/z* 120–1200). The UV wavelength was monitoring between 240 and 800 nm. The capillary voltage applied was 4500 Kv, and Nitrogen was used as the nebulizer gas (4 Bar) and drying gas (9 L/min) as well. The mobile phase used was ultrapure water (solvent A) and acetonitrile (solvent B) both added with 0.1% of formic acid (v/v). The elution profile applied was 0–2 min 3% of B; 2–25 min 3–25% of B; 25–40 min 25–80% of B followed by washing and reconditioning of the column. The chromatographic column was a Kinetex® C-18 (2.6 µ, 150 × 2.2 mm, Phenomenex®) inside an oven set on a temperature of 50 °C and the flow rate was 0.3 ml per minute. These data were processed in Data Analysis 4.2 (Bruker Daltonics, Billerica, USA).

Results

Effects of plant parts on germination and seedling formation

We observed statistical differences in the effects of extracts on the germination of *L. sativa* in different concentrations and from different parts of *E. fusca*. These statistical results were significant on the germination of *L. sativa* ($P < 0.05$), germination speed index ($P < 0.05$), percentage of normal seedlings ($P < 0.05$), and normal seedling speed index ($P < 0.05$). Some extracts presented inhibitory activity on the percentage of germination and formation of normal lettuce seedlings (Fig. 1 and Fig. 2), as well as on the germination and seedling speed indexes (Fig. 1 and Fig. 2). For most parts of the plant, increasing the concentration of extracts led to reducing *L. sativa* germination. Seed extracts at concentrations of 1 and 0.2 mg mL^{-1}, as well as bark, leaves and root extracts at a concentration of 0.2 mg mL^{-1} did not have any negative impact on germination and normal seedling formation of *L. sativa*. Leaves and root extracts at 1 mg mL^{-1} concentration and bark and root extracts at 5 mg mL^{-1} concentration caused a reduction on percentage of germination, germination speed index, percentage of normal seedlings, and normal seedling speed index of lettuce, but in lower magnitude. Leaves extract at a concentration of 5 mg mL^{-1} caused the most significant reduction on all these traits, which were similar to the positive control (2,4-D).

Effects of plant parts on developing of root and aerial part

The analysis of variance indicates significant differences in root lengths ($P < 0.05$) (Fig. 3). Root length of lettuce

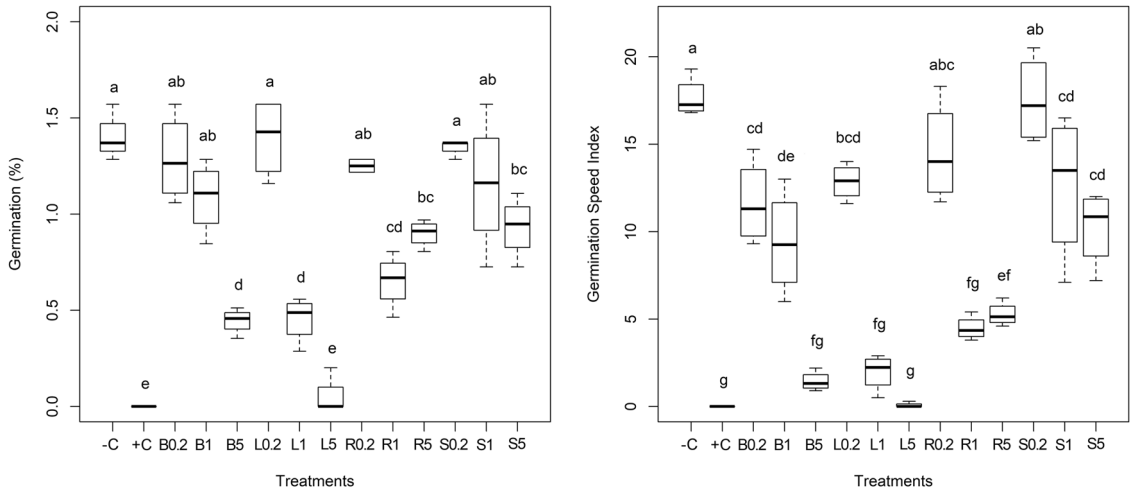

Fig. 1 Results of averages of percentage of germination **a** and germination speed index **b** with default error of *L. sativa* submitted to controls with buffer (−C) and 2,4-D (+C) and different concentrations of extracts from *E. fusca*: seeds 5 mg mL^{-1} (S5); seed 1 mg mL^{-1} (S1); seed 0.2 mg mL^{-1} (S0.2); leaves 5 mg mL^{-1} (L5); leaves 1 mg mL^{-1} (L1); leaves 0.2 mg mL^{-1} (L0.2); bark 5 mg mL^{-1} (B5); bark 1 mg mL^{-1} (B1); bark 0.2 mg mL^{-1} (B0.2); root 5 mg mL^{-1} (R5); root 1 mg mL^{-1} (R1); root 0.2 mg mL^{-1} (R0.2). Data is expressed as average percentage from experiments containing four replicates with 25 seeds each. Different letters in the columns express statistically different averages detected with Tukey test ($P < 0.05$)

exhibited a reduction in treatments with the highest concentration (5 mg mL^{-1}) of all extracts presenting similar averages to that observed for the 2,4-D control, but only roots lengths growing in treatment with 5 mg mL^{-1} of seeds extract differed significantly from the negative control.

Chemical profile of the extracts from each part of E.fusca

The chemical profile of the extracts from *E. fusca* (Fig. 4) showed that *C*-hexosyl flavones and saponins are the main metabolites of leaves. Some of these flavonoids are also present in the bark, root and seed extract, but in lower concentrations than in leaves; saponins are also present in the root extract. Two pterocarpans were detected in the bark and root extract, highly concentrated in bark; seed extract is characterized for the high concentration of alkaloids. Table 1 shows all identified compounds and relative intensity for each plant parts.

Discussion

We observed that different parts of *E. fusca* have allelopathic potential in variable degrees. According to Ooka and Owens (2018), subtropical and tropical species are successful in the development of allelochemicals, probably to assist competition with other species in such growth-friendly environments. The presence of allelopathy is frequent in species of Fabaceae (Oliveira et al. 2008; Cândido et al. 2010; Aguilera et al. 2015; Id et al. 2015). Studies with *Erythrina velutina* (Centenaro et al. 2009; Oliveira et al. 2012, 2013) and *Erythrina speciosa* (Soares et al. 2002) showed that different parts of the plants affect the germination and development of *L. sativa*. The differential toxicity between leaves, bark, roots, and seeds of *E. fusca* is due to a distinct pattern of allelopathic compounds, which vary in composition and concentration according to each plant part tested.

Is this way, *Erythrina* genus are excellent sources of secondary metabolites, such as tetracyclic alkaloids, flavonoids, coumarins, and saponins (Tanaka et al. 2002; Yenesew et al. 2003; Juma and Majinda 2004; Innok et al. 2009, 2010; Pérez et al. 2015). Secondary metabolites are essential for plant survival, propagation, interactions, defense against pathogens and herbivores, signaling and communication between plants and environment (Harborne 1996; Hebets and Papaj 2005; Stevenson et al. 2017; Brunetti et al. 2018), in addition, plant exudates can participate in soil ecology and biogeochemical cycles (Scavo et al. 2018) Also, these classes of metabolites found in this genus are known to present allelopathic activity (Rice 1984; Ferreira and Aquila 2000). Thus, the observed allelopathic potential of *E. fusca* may be related to such secondary metabolite compounds.

According to Ferreira and Aquila (2000), metabolites vary in different parts and tissues of the plant, in concentration, location, and composition and can be excreted into air, soil, or leached. In this study, we observed that among the four analyzed parts, leaves of *E. fusca* presented the highest allelopathic potential, and the major metabolites from this part are *C*-glycosyl flavones and saponins. According to the germination and seedling formation results it is possible to suggest that *C*-hexosyl flavones identified in leaves may be the main metabolites that affect these

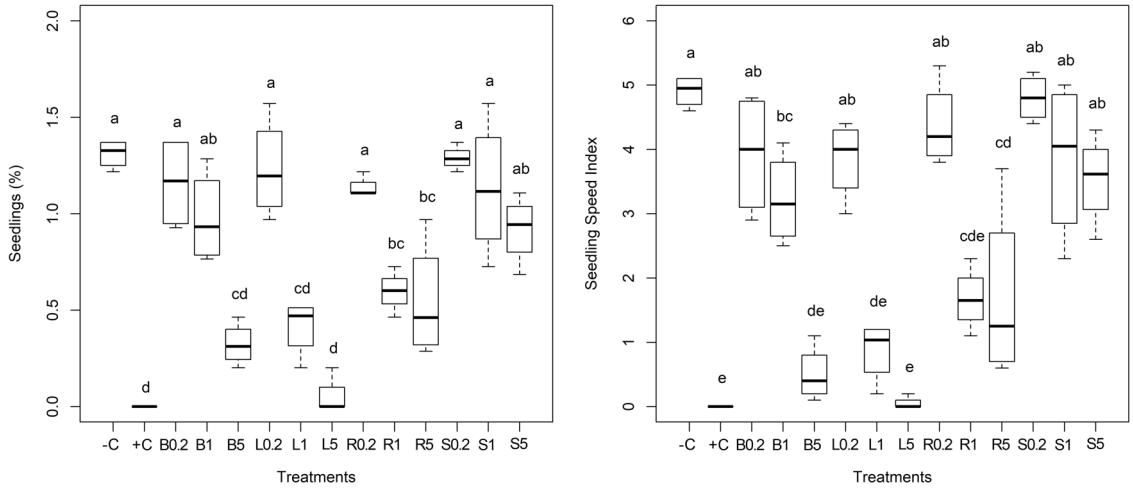

Fig. 2 Results of averages percentage of normal seedlings **c** and normal seedling speed index **d**, with default error of *L. sativa* submitted to controls with buffer (−C) and 2,4-D (+C) and different concentrations of extracts from *E. fusca*: seeds 5 mg mL^{-1} (S5); seed 1 mg mL^{-1} (S1); seed 0.2 mg mL^{-1} (S0.2); leaves 5 mg mL^{-1} (L5); leaves 1 mg mL^{-1} (L1); leaves 0.2 mg mL^{-1} (L0.2); bark 5 mg mL^{-1} (B5); bark 1 mg mL^{-1} (B1); bark 0.2 mg mL^{-1} (B0.2); root 5 mg mL^{-1} (R5); root 1 mg mL^{-1} (R1); root 0.2 mg mL^{-1} (R0.2). Data is expressed as average percentage from experiments containing four replicates with 25 seeds each. Different letters in the columns express statistically different averages detected with Tukey test ($P < 0.05$)

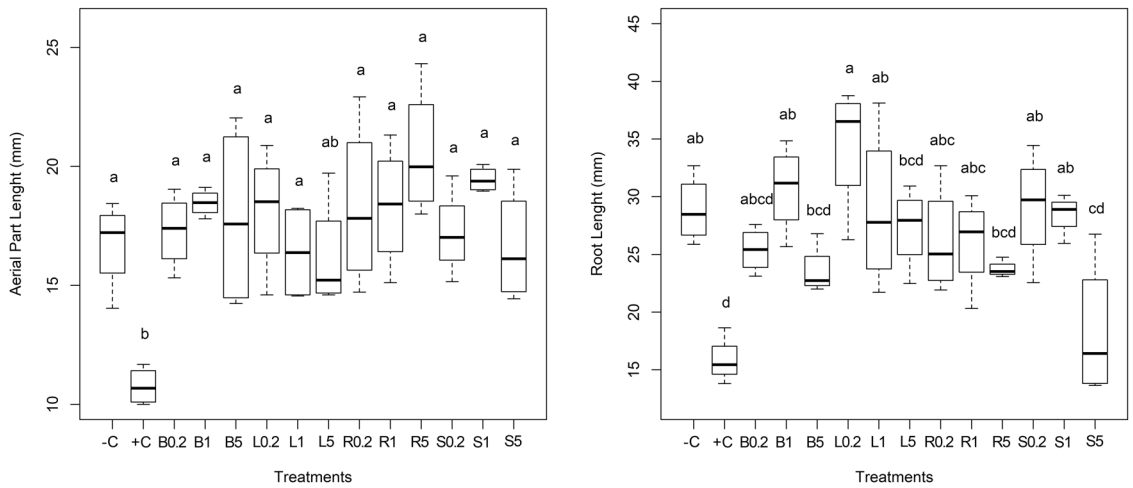

Fig. 3 Results of averages of aerial part length **a** and root length **b**, with default error of *L. sativa* submitted to controls with buffer (−C) and 2,4-D (+C) and to different concentrations of extracts from *E. fusca*: seeds 5 mg mL^{-1} (S5); seed 1 mg mL^{-1} (S1); seed 0.2 mg mL^{-1} (S0.2); leaves 5 mg mL^{-1} (L5); leaves 1 mg mL^{-1} (L1); leaves 0.2 mg mL^{-1} (L0.2); bark 5 mg mL^{-1} (B5); bark 1 mg mL^{-1} (B1); bark 0.2 mg mL^{-1} (B0.2); root 5 mg mL^{-1} (R5); root 1 mg mL^{-1} (R1); root 0.2 mg mL^{-1} (R0.2). Data is expressed as average percentage from experiments containing four replicates with 25 seeds each. Different letters in the columns express statistically different averages detected with Tukey test ($P < 0.05$)

parameters since they are intense in leaves and they are also present in the bark and root extracts, which demonstrated some influence in the germination and seedlings indexes as well. The presence of saponins in the leaves and roots can have some influence on the allelopathic potential, increasing the effect of flavonoids due to their amphipathic property that assists in the dissipation of flavonoids in a liquid medium (this interaction could help the distribution of these compounds in the Pantanal flood period). Saponins were not detected in the extracts of the seeds and this extract exhibited the lowest allelopathic potential for all parameters of seed germination and seedling formation. On the other hand, seed extract exhibited the most substantial effect on the root length, which can reduce the plant establishment after the germination, this extract presented the highest effect in the plant development, especially in the growth of the roots, it is probable that this effect can be caused by the alkaloids, which were detected in high intensities in the seed extract, but they are not abundant in the extracts of other parts.

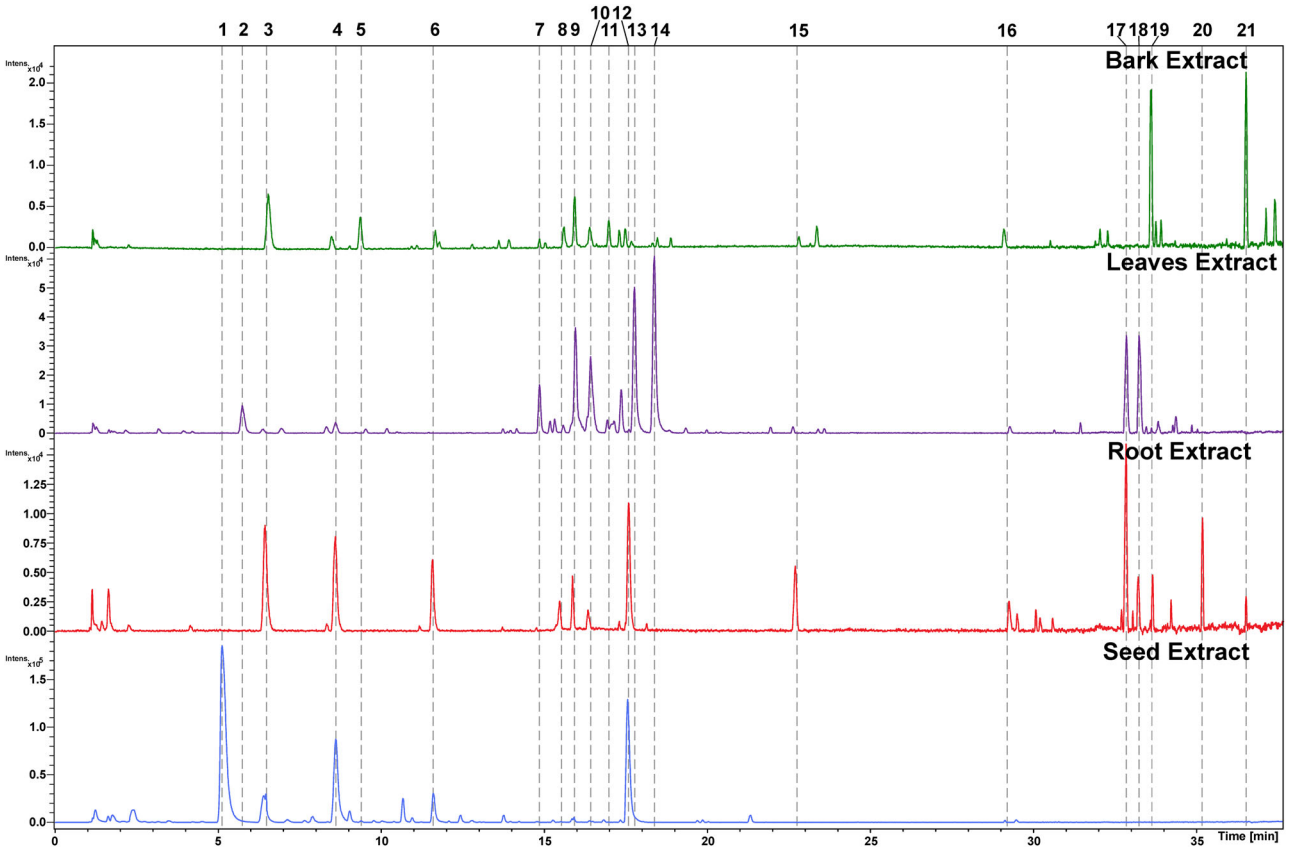

Fig. 4 Base peak chromatogram recorded within the positive ion mode of bark, leaves, root and seed extracts *E. fusca* used in allelopathic experiments on *L. sativa*, illustrating the chemical differences between the extracts depicting the metabolites identified by UFLC-DAD-MS

Table. 1 Compounds identified and relative intensity (•) of bark, leaves, root and seed extracts of *E. fusca* used in the allelopathic potential experiment on *L. sativa*. ID (identification number of each peak) RT (peak retention time)

ID	RT	Identification	Class	Bark	Leaves	Root	Seed
1	5,1	O-hexosyl-erysopine	Erythrinic alkaloids	–	–	–	•••••
2	5,8	Unknown	–	–	••	–	–
3	6,5	N-methyl tryptophan	Amino acid derivative	••	–	••	–
4	8,6	Hypaphorine	Amino acid derivative	–	•	••	•••••
5	9,4	Unknown	–	••	–	–	–
6	11,6	Erythratine	Erythrinic alkaloids	–	–	••	••••
7	14,8	Vicenin-2	Flavone	•	•••	–	–
8	15,5	Erythrinine/Oxo- erythrinine	Erythrinic alkaloids	•	•	•	–
9	15,9	Schaftoside	Flavone	••	••••	••	••
10	16,4	Isoschaftoside	Flavone	•	•••	•	•
11	17	Flavanone derivative	Flavanone	••	•	–	–
12	17,7	Erythraline	Erythrinic alkaloids	–	–	••	•••••
13	17,9	Vitexin	Flavone	–	••••	–	–
14	18,4	Isovitexin	Flavone	–	••••	–	–
15	22,8	Daidzein	Isoflavone	•	–	••	–
16	29,1	Sesquiterpene derivative	Sesquiterpene	••	••	••	••
17	32,9	Triterpene saponin	Saponin	–	••••	•••	–
18	33,2	Triterpene saponin	Saponin	–	••••	••	–
19	33,6	Prenylated pterocarpan	Pterocarpan	••••	–	••	–
20	35,2	Unknown	–	–	–	••	–
21	36,5	Sandwicensin	Pterocarpan	••••	–	••	–

Flavonoids are essential for many biological functions in plants, protecting plant tissues from UV light, participating in several plant interactions and also playing an important role in plant survival (Mierziak et al. 2014). These metabolites can be found in all parts of plants and they have been related to the allelopathic potential of several species (Weston and Mathesius 2013; Mierziak et al. 2014). In this respect, flavonoids *C*-glycosyl have demonstrated allelopathic potential, influencing seed germination, radicle growing and consequently reducing the establishment of the affected plants (de Bertoldi et al. 2009; Hooper et al. 2010, 2015; Weston and Mathesius 2013; Mierziak et al. 2014). Regarding this, *C*-glycosyl flavones found in root exudate of some species of *Desmodium* (Fabaceae), stimulated the seed germination of *Striga* spp. (Orobanchaceae), a parasitic plant, but inhibited the radicle growth, being used in the field to control the establishment of this plant parasite (Hooper et al. 2010, 2015). Hooper et al. (2015) described *C*-glycosyl flavones, such as isoschaftoside, vitexin, 2″ -*O*-glucosylvitexin, vicenin-2 and isoschaftoside in the root exudates (Hooper et al. 2015).

In the same way, de Bertoldi et al. (2009) have tested the allelopathic potential of a *n*-butanol fraction from *Avena sativa* L. (oat) on the germination of several weeds. This fraction was composed of *C*-glycosides flavone and saponins, but for the allelopathic tests these metabolites were tested separately, and only the fraction containing flavonoids demonstrated allelopathic potential, inhibiting the germination of all tested weeds at extract concentrations higher than 6.7 mg/mL. This concentration was higher than the active extract observed in our results. Furthermore, it is essential to emphasize that in our study we used a crude extract, and the results obtained for leaves extracts at the highest concentration (5 mg mL^{-1}) on the percentage of germination and formation of normal lettuce seedlings, as well as on the germination speed index and seedling speed indexes were statistically equal to the herbicide 2,4-D, which means that this treatment was highly deleterious to seed germination and development, highlighting the allelopathic potential of *E. fusca* metabolites. Wu et al. (2009) found similar pattern; leaves extract of *Mikania micrantha* showed stronger allelopathic potential on percentage of germination, speed germination index and shoot height of other species.

Bark and roots of *E. fusca* accumulated prenylated pterocarpans; pterocarpans are a subclass of isoflavones, that been described as critical allelopathic compounds for several species (Tsanuo et al. 2003; Weston and Mathesius 2013). Kato-Noguchi (2003) describes the allelopathic effect of the pterocapan pisatin on the Lettuce model. We believe that this class contributes to the observed activity of bark and roots, once the presence of other phytotoxic compounds in these extracts is low.

It is also important to note that, in Pantanal, *E. fusca* formed monodominant stands, showing a histosol with high levels of organic matter, a layer of leaf litter, and very homogeneous rotten plant matter mostly composed of *E. fusca* leaves. Palm, Sanchez (1990) observed that the leaves of *Erythrina* sp. showed significantly faster decomposition and nutrient release than other legume species analyzed. Also, we observed a superficial water table in these areas, suggesting that the soil essentially remains waterlogged, even in the dry season, which may allow the compounds present in the litter to persist there. Besides this, the *C*-glycosyl flavones found in leaves are more stable to enzymatic and chemical hydrolysis, remaining longer on the soil. Also the presence of saponins may assist in the dissipation of flavonoid. In this way, the continuous leaching of the litter, mainly composed of *E. fusca* leaves, may influence the germination and development of other species. Root and bark also have contact with the water during the flood season, and they can also transfer some metabolites to water, but as they were not decomposed, this translocation is probably lower.

Another relevant results observed in this study was the effect of seeds extracts, which is rich in erythrinic alkaloids. This extract did not influence on germination or seedlings parameters, but it reduces the length of the root after germination. Oliveira et al. (2013) tested extracts of seeds of *E. velutina* prepared using 50 g of seed in 500 ml of water in two distinct temperatures, and they observed a reduction in the germination of *L. sativa* at the higher concentrations. These authors also reported high percentage of abnormal seedlings (Oliveira et al. 2013). Another experiment performed with crude alkaloid fraction obtained from seeds of *E. amaericana* on germination of common bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) demonstrated no significant effect on the percentage of germination (García-Mateos et al. 2002), but they did not evaluate defective seedlings. Despite some extracts of *Erythrina* spp. did not affect germination, the disturb caused on the seedling and plant development may reduce the plant establishment. In this way, seeds of *E. fusca* are also abundant on the soil, since this species produces large quantity of seeds, which falling around the mother tree, remaining on the soil during the raining season. Although the metabolites found in the seeds affect only the root length, this result can decrease the seedlings fitness and consequently can reduce establishment of other plants, being an vital competition factor.

Thus, it is interesting to note that, the effects of *E. fusca* on the bioindicator species were most deleterious when this contact occurred before germination. When the contact occurred during the seedling phase (seeds germinated at 3 days) the effects were less drastic and only the most concentrated extracts affected the seedling growth, in a low proportion. For these compounds to be effective in nature,

they must be produced in large quantities and released to the soil through active secretion by the rhizosphere or by leaching of leaves, bark, roots, or seeds. As a result, taking into account the particularities of each type of soil, the concentrations must be sufficiently high in the soil to reach inhibitory levels (Wink and Latz-Brüning 1995). We observed that for all parts of the plant at concentration 5 mg mL^{-1} we detected an effect on growth and development of the roots of *L. sativa*, which means that, at high concentrations, all tissues of *E. fusca* have allelopathic potential.

In conclusion, we observed that all parts tested of *E. fusca* had adverse effects on the germination or development of *L. sativa*. Increasing the concentration of extracts led to a reduction of *L. sativa* germination and seedlings traits, mainly when the contact occurs before germination. The leaves had the highest potential (equal to herbicide 2,4-D), and this species has intense leaves loss and deposition on the litter, which may increase the leaching and release of allelopathic compounds in the soil. The presence of distinct classes of metabolites could compensate for the high concentration necessary to observe the allelopathic activity, once these compounds could act together with a synergistic interaction. Consider that, we can suppose that the dominance of *E. fusca* may be facilitated by this allelopathic potential, but studies are needed to evaluate the effect of it in co-occurring native species and possible synergistic effect.

Acknowledgements We thank Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for support. Universidade Federal de Mato Grosso do Sul (UFMS) and Programa de Pós-Graduação em Ecologia e Conservação (PPGEC) for financial support. We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), which provided scholarships to DG. This work was supported by Instituto Nacional de Ciência e Tecnologia em Áreas Úmidas (INAU) funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico and Ministério da Ciência, Tecnologia e Inovação (CNPq no. 573990/2008-5). We thank Êmelli LF de Souza for the help during the experiments. We also thank the anonymous reviewers for their important contributions during the paper submission process.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individuals included in the study.

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