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PHYTOTOXIC ACTION OF *Machaerium amplum* BENTH. LEAVES EXTRACT

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ABSTRACT

The indiscriminate use of herbicides can guide to some environmental damages. Thus, the search for alternative bioherbicides from natural sources with biodegradable properties and less toxicity to the microbiota is essential. The focus of this work is the chemistry and phytotoxic study of the hydroethanolic extract of *Machaerium amplum* Benth. leaves. The chemical study by FIA-ESI-IT-MS^n experiments allowed the detection of apigenin and luteolin *C*-glycoside derivatives, quinic acid, the A-type proanthocyanidin dimer and trimer, and a saponin Soyasapogenol B type. Phytotoxicity assays were performed with the extract and a group of fractions against tomato and onion seeds under the parameters of germination, hypocotyl growth, and root length. The extract showed a 70% of inhibition effect at 0.8 $mg.mL^{-1}$ on tomato root length. For the onion seeds, the percentual of inhibition was 65% at 0.4 $mg.mL^{-1}$. All groups of fractions tested showed significant inhibition on tomato seedling root length at 0.8 $mg.mL^{-1}$. The flavonoids fraction showed interesting activity at 0.4 $mg.mL^{-1}$. The same fraction showed a percentage of inhibition above 50% at 0.2 $mg.mL^{-1}$ on onion seedling root length. This activity may be associated with the *C*-flavones presence in this fraction. According to the ecotoxicity test with *Artemia salina*

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Leach, the extract presents no toxicity at the concentrations tested, and no antimicrobial activity against *Fusarium graminearum*, *F. oxysporum* and *Aspergillus flavus*, thus reinforcing the potential of the extract.

Keywords: *Machaerium*, Fabaceae, Fitotoxicity, *C*-flavones, Sustainability

1. INTRODUCTION

World agriculture has approximately 40% of loss of all products due to pests, which are even responsible for the fall of biodiversity, declining the number, variety of species, and the genetic variability [1]. The chemical intervention with herbicides on weed control allows the increase of productivity and cost reduction [2, 3]. The indiscriminate use of synthetic herbicides can lead to soil and water systems contamination, causing damage to the health of the population and considerable ecosystems changes [4, 5]. Soil contamination can affect all the active biological processes, responsible for nutrient demands for the plants [6]. Thus, the search for sustainable and economically viable products to the weeds control is indispensable [7]. Sustainable weed management includes a set of choices such as crop rotation, crop competitiveness tillage, biological control agents, and bioherbicides [8]. Bioherbicides obtained from plant extracts and microorganisms, can offer to humans and the environment, less toxicity and provide an interesting target specificity for the development of commercial products [9, 10]. Research involving bioherbicides development from natural origin promotes plant health preservation, which is essential within the current environmental context. Additionally, they minimize the use of toxic and polluting herbicides, improving environmental problems, and thus promoting more ecologically sustainable cities, as provided in Sustainable Development Goals (SDG 11). As suggested in SDG 3, the use of bioherbicides can collaborate in benefits to the health and welfare of the population, reducing the direct or indirect contact of the population's exposition to toxic substances. So, this supports what is expected in SDG 2, promoting more sustainable agriculture, with a healthier and safer food supply [1]. Natural compounds of plants with herbicidal action can be released into the environment, either directly through root pathways in the soil or through volatile substances through the air. According to the literature, there are several classes of natural products with this property such as phenols, terpenoids, alkaloids, flavonoids, and polyacetylenes [11, 12, 13]. Spinasterol, spinasterone, and spinasterol glycopyranoside isolated from the stem extract of *Moutabea guianensis* Aubl showed phytotoxic activity [14]. Aqueous extracts of *Artemisia absinthium* L. and *Psidium guajava* L. leaves reduced germination and seedling development by causing stimulation of antioxidant enzyme activity in *Parthenium hysterophorus* L. [15]. The aqueous extract of *Solanum lycocarpum* St. Hil. leaves promoted a delay in seed germination and root growth of *Sesamum indicum* L. [16]. The essential oil of *Eremanthus erythropappus* (DC) Macleisch leaves showed significant inhibition on seed germination of

Brassica rapa (L) with IC_{50}= 16.3 µL.mL^{-1}, and an inhibitory effect on radicle length of *Bidens pilosa* L. with IC_{50} = 16.3 µL.mL^{-1} [17]. According to Tahira [18], the phytotoxic action *of Machaerium eriocarpum* Benth. and *Machaerium hirtum* (Vell.) Stellfeld twigs and leaves is associated with the lupeol presence, which exhibited an important inhibition of germination, root growth, and lateral root emergence at the concentration of 100 ppm. The isovitexin isolated from *M. eriocarpum* showed inhibition of lateral root growth of *Cucumis sativus* at concentrations of 500 and 100 mg.L^{-1} [13]. These data can denote the potential of plant extracts to be employed on weeds [7]. Until now, there are no reports involving the study of *Machaerium amplum* Benth. species. In this sense, the chemistry and phytotoxic potential of this plant can contribute to the valorization and preservation of plant species of Brazilian biodiversity, as well as contributing to the search for plant extracts with bioherbicidal potential.

2. EXPERIMENTAL

2.1 Plant material

Leaves of *M. amplum* were collected at an urban square (coordinates 20º26'15 "S and 54º35'05"W), located in Vila Giocondo Orsi, Campo Grande - MS and authenticated by taxonomist Prof. Dr. Ângela Lúcia B. Sartori from the Federal University of Mato Grosso do Sul (UFMS). A voucher specimen (No. CGMS77482) was deposited at the CGMS Herbarium at UFMS and the study was registered in SISGEN under protocol #A466AA4.

2.2 Extract preparation and fractionation

The air-dried and powdered leaves (499.82 g) were extracted exhaustively with ethanol and water at a ratio of 70:30 (v/v) by percolation. After 24 hours, extract collection was started under a drip flow rate of 1.0-2.0 mL.min^{-1} [19]. The solvent was evaporated at 60 °C under reduced pressure to afford 70% EtOH extract (107.6 g). The yield (w/w) for the 70% EtOH extract from the dried powders of *M. amplum* leaves was 21.5 %. The crude extract (6.0 g) was fractionated on a Discovery® DSC-18 C18 silica column (5.3 cm x 6.0 cm di). The mobile phases used were 1.0 L of water, followed by 1.0 L of methanol/water mixture 1:1 (v/v), then 1.0 L of methanol/water 8:2 (v/v), and finally 1.0 L of methanol. Fifteen (250 mL) fractions were collected at a flow rate of 0.5 mL.min^{-1}. These fractions were grouped into 8 fractions group (F1-F8) according to chromatographic profile similarity checked by TLC on Si gel eluted with ethyl acetate/formic acid/ acetic acid/water (100:11:11:26, v/v) and revealed either with anisaldehyde sulfuric acid solution.

2.3 Determination of total phenols

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The total phenolic content of *M. amplum* extract was determined by the Folin-Ciocauteau method [20]. Using eight points standard curve of gallic acid solutions (87.5 $mg.L^{-1}$ to 700 $mg.L^{-1}$), the total phenolic content of the extract was determined in triplicate. The data was expressed obtaining the values in mass equivalent to gallic acid (EAG). The extract of *M. amplum* was prepared at a concentration of 1.0 $mg.mL^{-1}$ in methanol. For the determination of total phenols, 1560 µL of Milli-Q water, 40 µL of the sample, 100 µL of the Folin-Ciocauteau reagent, and 300 µL of a saturated sodium carbonate solution were added in microtubes. After 2 h of reaction, aliquots of 200 µL from each microtube were added to a 96-well plate for reading at 760 nm. The absorbance values of the samples were extrapolated on the standard curve of gallic acid, obtaining the values in mass equivalent to gallic acid (EAG).

2.4 Determination of total flavonoids

The reaction with aluminum chloride was used to the determination of total flavonoids. A solution of concentration 50 $mg.mL^{-1}$ of aluminum chloride was applied. To construct the rutin standard curve, ten dilutions were prepared at concentrations of 3.0 $\mu g.mL^{-1}$ to 60 $\mu g.mL^{-1}$ starting from a stock solution of 0.1 mg mL^{-1}. The extract of *M. amplum* was prepared at a concentration of 1.0 $mg.mL^{-1}$ in methanol. For the determination of total flavonoids, 0.5 mL of the sample and 0.5 mL of aluminum chloride solution were added in microtubes. After 15 min of reaction, 200 µL aliquots from each microtube were added to a 96-well plate for reading at 420 nm. The absorbance values of the samples were extrapolated on the rutin standard curve, obtaining the rutin equivalent mass values.

2.5 Analysis by high-performance liquid chromatography with diode array detection (HPLC-PDA)

HPLC-PDA analyses were performed employing an Agilent 1260 Chromatograph equipped with a 60 mm flow cell (model 1260) coupled to a UV detector. The chromatographic separation was performed on a Zorbax Eclipse Plus C-18 column (4.6 x 150 mm - particle 3.5 µm). The analyses were performed at a constant temperature of 45 ^{o}C, a flow rate of 1 $mL.min^{-1}$, and with an injection volume of 3 µL. The composition of the mobile phase employed was 0.1% acetic acid in water (A) and acetonitrile (B). The mobile phase used was in gradient mode, starting with a mixture of 90% acidified water with 0.1% acetic acid and 10% acetonitrile until 6 min, and then followed with a linear gradient reaching 100% acetonitrile in 60 minutes.

2.6 HPLC-MS and FIA-ESI-IT-MS^n Analysis Instrumentation

Mass spectrometry analyses were performed on a LTQ FLEET mass spectrometer (HPLC-PDA-ESI-IT-MS^n, Thermo Scientific®) equipped with a direct sample insertion device via continuous

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flow injection analysis (FIA). For chromatographic separation a RP18 reverse phase column, Acquity UPLC® BEH C-18 (2.1 x 50 mm 1.7 µm), was used. The chromatographic separation was performed in gradient mode: 0-2 min (5-40% ACN); 2-6 min (40-50% ACN); 6-9 min (50-100%) and 9-12 min (100%) under a flow rate of 0.350 mL.min^{-1}, capillary temperature 280 °C, nitrogen as mist gas and vacuum of 1.14 Torr. The mobile phases used were acidified water (A) and acetonitrile (B) acidified with 0.1% formic acid. The studied matrices were analyzed in electrospray ionization (ESI) mode and the multi-stage fragmentations (MS^2, MS^3, and MS^n) performed on an ion-trap (IT) type interface. The negative mode was chosen for the generation and analysis of the first-order mass spectra (MS) as well as for the other multi-stage experiments (MS^n) under the following conditions: capillary voltage -40 V, spray voltage -5 kV, capillary temperature 280 °C, and carrier gas (N_2) with the flow rate 60 (arbitrary units). The acquisition range was *m/z* 50-2000, with two or more scanning events performed simultaneously on the LTQ XL mass spectrometer.

The first event was a full scan of the mass spectrum to acquire the data of the ions in the established *m/z* range. The other MS^n events were performed from the data of this first scan for preselected precursor ions with collision energies of 25 and 30% of the total energy of the instrument. The FIA-ESI-IT-MS^n was performed under the following conditions: nitrogen as a mist gas; capillary temperature set at 350 °C; vacuum of 1.14 Torr and flow rate 5 µL.min^{-1}. The software Xcalibur (Thermo Scientific®) was used for the acquisition and processing of the spectrometric data.

2.7 Phytotoxic assay

The 70% EtOH extract and the fraction groups F1, F4, F5, F7, and F8 were submitted to the phytotoxic assay using three Standard Target Species (STS): lettuce (*Lactuca sativa* L.) tomato (*Lycopersicon esculentum* Mill.) and onion (*Allium cepa* L.). Bioassays were conducted on the parameters of germination rate, hypocotyl length, and root length using six-well microplates following the procedure previously described by NOVAES [21].

The samples were dissolved in DMSO and diluted using 2-[N-morpholino] ethanesulfonic acid (MES) buffer (10 mM, pH 6) to a DMSO concentration of 0.5%. The sample concentrations were 0.8 mg.mL^{-1}, 0.4 mg.mL^{-1} and 0.2 mg.mL^{-1}. Each well of the microplate received a sheet of filter paper (3.2 cm), 1.0 mL of samples, ten achenes, and then the microplate was capped and sealed with parafilm M. A MES buffer solution (10 mM, pH 6) containing 0.5% DMSO was used as negative control.

The microplates were incubated in a BOD Incubator (Biochemical Oxygen Demand), in a photoperiod of 12 hours at a temperature of 25 ºC for 7 days for tomato and 8 days for onion.

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After this period the microplates were taken to the freezer (-20 ºC) for 24 h to facilitate initial growth measurements after thawing.

Statistical analyses were performed in GraphPad prism 8. Tests for normality were performed. This was followed by Welch's test and Dunnett's T3 posthoc test. The means were compared with the controls only. The results are expressed as a difference in percentages from the control. GraphPad Prism 8 was also used for processing the graphs.

2.8 Ecotoxic Assay

The ecotoxic assay with *Artemia salina* Leach was performed according to the methodology proposed by Meyer [22]. The eggs of *A. salina* (Maramar) and sea salt (iodine-free) were purchased from specialized aquarium stores. Artificial seawater (2.0 L) was prepared by dissolving 38 g of sea salt per L of deionized water, followed by filtration. Part of the solution (1.6 L) was used in the hatching process of 50 mg of *A. salina* eggs, which mixture was kept in a plastic tray (42.0 x 29.0 x 7.0), under artificial lighting (40 W), temperature of 26 and 28 °C and constant oxygenation for 48 h. The nauplii used in the experiment were instar II because instar I nauplii are not fully formed and feed on the yolk juice [23].

The extract and group of fractions tested were prepared at concentrations of 1000, 100, 10, and 1 $\mu L.mL^{-1}$ according to the dilution process, proposed by McLaughlin [24]. The assay was performed in triplicate, totaling 12 test tubes of the different concentrations tested and their respective repetitions, and 1 tube for the positive control. The samples were taken to the fume hood for 48 h for the total elimination of the solvent, then resuspended in 50 µL of DMSO and 4.0 mL of artificial seawater, in which 10 nauplii of *A. salina* were inserted with the help of a Pasteur pipette. Next, the volume of each test tube was adjusted to 5.0 mL with the addition of saline water. The positive control contained saline water and 50 µL DMSO. The test tubes were kept under artificial light for 24 hours, and then with the aid of a Zeiss Stemi 2000-C stereomicroscope with a zoom range of 0.65x -5.0x, the dead were counted. The nauplii, which after 10 seconds of observation showed no movement, were considered dead.

2.9 Antimicrobial assay

The strains of the phytopathogenic fungi used were *Fusarium graminearum* (Schwein.) Petch, *Fusarium Oxysporum* (Schlecht) Snyder & Hansen, and *Aspergillus flavus* Link.

The antimicrobial assays followed the disc-diffusion protocol proposed by Benitez [25]. The assay was performed in duplicate, using 6 Petri dishes of 90 mm diameter and 20 mL of BDA (Potato Dextrose Agar) medium (Sigma Aldrich) specific for fungal growth. After the culture medium

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solidified, 1x107 CFU.mL^{-1} of the fungi selected for the assay were plated. After that, a sterile 6 mm paper disk was positioned in previously determined points and numbered 1 to 5. On these disks, 10 µL of the concentration of 4096 µg. mL^{-1} of the 70% EtOH extract and the groups of fractions F1, F4, F5, and F7 were applied separately. DMSO was used for the control. The plates were then capped and sealed with parafilm and incubated for 6 days at ± 30 °C in an incubator, after which the halos formed were measured.

3. RESULTS AND DISCUSSION

To evaluate the content of compounds classes in the 70% EtOH extract, spectrophotometric experiments were performed to determine the content of total phenols and total flavonoids (Table 1). The extract of *M. amplum* showed a high content of total phenols (183 mg EAG/g of extract), values higher than those of other bioactive plant extracts such as *Torilis leptophylla* L. with a content of 121.9 mg.g^{-1} [26], while *Zanthoxylum armatum* DC. seeds was 167.7 mg.g^{-1} [27]. The fruit extract of *Phoenix dactylifera* L. showed a content of 153 mg.g^{-1} [28](ALFARIS et al., 2021). Moreover, the content of flavonoids was expressed in the form of rutin equivalent, and the crude extract of *M. amplum* showed a content of 63.1 (mg.g^{-1} extract), higher than bioactive extracts such as the methanolic extract of *T. leptophylla* with a content of 59.6 mg.g^{-1} [26]. The crude extract of *Cariniana domestica* (Mart.) Miers. barks showed a content of 13.7 mg.g^{-1} [29].

Table 1: Determination of the content of total phenols and total flavonoids in the hydroethanolic extract of *M. amplum* leaves

Sample	Total phenol content (mg EAG/ g of extract)	Total flavonoid content (mg Rutin/ g of extract)
70% EtOH extract	187 ± 24	63.1 ± 3.9

In order to obtain more information about the compounds present in the crude extract of *M. amplum*, experiments by FIA-ESI-IT-MS^n were performed. In the full-scan mass spectrum, it is possible to observe the presence of at least 8 precursor ions of deprotonated molecules ([M - H]$^-$). For the identification of the different secondary metabolites present in the extract (Table 2), MS^n experiments were performed for each of the ions, according to the recommendations of Murray [30].

Quinic acid

This compound was identified by the presence of the [M-H]$^-$ at 191 and the fragment ions at 173; 147; 111. Quinic acid was observed previously in the extract of *M. hirtum* twigs [31].

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Type A proanthocyanidin

Two Type-A proanthocyanidin derivatives were detected in the 70% EtOH extract. Compounds 5 and 8 with $[M - H]^-$ at *m/z* 575 and *m/z* 863 were tentatively assigned as Type-A proanthocyanidin dimer and Type-A proanthocyanidin trimer, respectively.

Type-B proanthocyanidin and prodelfinidin have been detected in the extract of *Machaerium Floribundum* Benth. twigs [32]. However, it is the first report of type A proanthocyanidin in the *Machaerium* genus.

***C*-Flavone derivatives**

Three apigenin derivatives were identified by the $[M-H]^-$ at 431, 563, and 593. The losses of 90 and 120 units from each molecular ion indicate the presence of *C*-glycosides in the flavonoid skeleton. The two luteolin derivatives were observed by the $[M-H]^-$ at 447 and 609. These classes of flavonoids were observed before in the extract of other *Machaerium* species [13,31].

Table 2: Secondary metabolites identified through ESI-MS^n analyses of the hydroethanolic extract of the leaves of *M. amplum* Benth.

Number	*m/z* $[M-H]^-$	MS^n (*m/z*)	Identified compound	Literature
1	191	173; 147; 111	Quinic acid	[33, 34]
2	431	341; 311	Apigenin-*C*-hexoside	[31, 35]
3	447	429; 357; 327	Luteolin-*C*-hexoside	[31, 35]
4	563	443; 431; 341; 311	Apigenin-*C*-hexose- *C*-pentoside	[31, 35]
5	575	449; 423; 407; 289	Type A proanthocyanidin dimer	[36]

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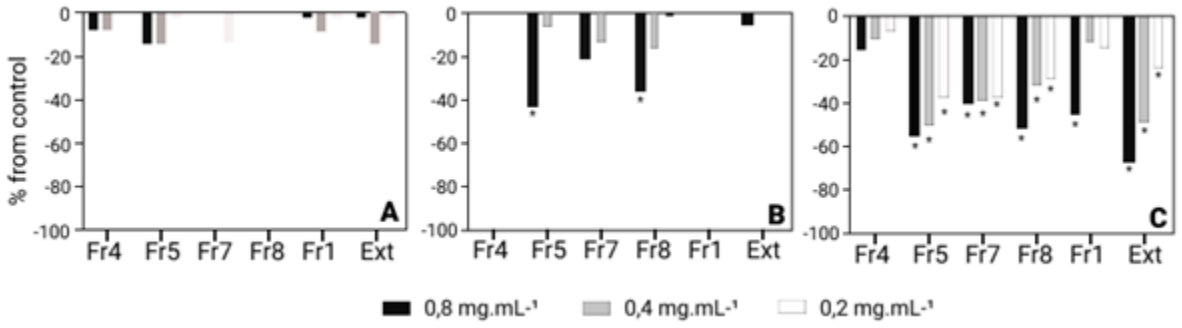
6	**593**	**473; 431; 341; 311**	**Apigenin-*C* –hexose- *C*-hexoside**	**[31, 35]**
7	**609**	**519; 489; 399; 369**	**Luteolin-*C*-hexose – *C*-hexoside**	**[31, 35]**
8	**863**	**711; 575; 531; 451; 411; 289**	**Type A proanthocyanidin trimer**	**[36, 37]**
9	**941**	**923; 879; 733**	**AcGlu-hexose-deoxihexose - Soyasapogenol B**	**[38]**

In the assay involving lettuce seeds, the extract and fraction groups showed no activity when compared to the control. For tomato seeds (Figure 1), all tested samples showed significant results. The extract showed a 70% inhibition effect at the concentration of 0.8 mg.mL^{-1} on root length. The groups of fractions F5, Fr7, and Fr8 showed significant phytotoxic action at the three concentrations tested, under the parameter of root length inhibition. The fraction group Fr5 showed a higher phytotoxic potential when compared to the other fractions, showing inhibition values of 55%, 50% and 40% at the concentrations of 0.8 mg.mL^{-1}, 0.4 mg.mL^{-1} and 0.2 mg.mL^{-1}, respectively.

Figure 1: Inhibition (%) of 70% EtOH extract and group of fractions on germination rate (A), hypocotyl length (B), and root length (C) of tomato seedling.



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In the phytotoxic assay using onion seeds (Figure 2), the extract and the three groups of fractions tested showed activity at a concentration of 0.8 $mg.mL^{-1}$. Only the Fr5 group of fractions showed phytotoxic potential at the three concentrations tested. At the concentration of 0.4 $mg.mL^{-1}$, the percentage of inhibition of hypocotyl length was 55%. The tested extract and fraction groups showed significant phytotoxicity results for the root length inhibition parameter. The fraction group Fr5 showed significant result inhibiting 65%, 60% and 55% at the concentrations of 0.4 $mg.mL^{-1}$, 0.8 $mg.mL^{-1}$ and 0.2 $mg.mL^{-1}$, respectively. In the case of the crude extract of *M. amplum*, the significant result for root length inhibition was at the concentration of 0.2 $mg.mL^{-1}$ with a percentage of 41%.

In order to know the composition of the fraction group Fr5, given its phytotoxic activity, HPLC-PDA and HPLC-MS analyses were performed. The ultraviolet spectra of the chromatogram indicate the presence of at least twelve flavonoids. According to HPLC-MS analysis of the Fr5 fraction group, the retention time peak at 1.49 min, can be identified as being Apigenin-*C*-hexose-*C*-hexose (6). According to the literature, *C*-glycosylated flavonoids have been evaluated for phytotoxic activity. The hydroethanolic extract as well as isovitexin, a *C*-glycosylated flavone isolated from the leaves of *M. eriocarpum*, presented phytotoxic activity inhibiting by 100% the growth of lateral roots of cucumber seeds [13]. The phytotoxic action of the fraction group Fr5 may be associated with the presence of *C*-flavones in the *M. amplum* extract. Flavones-*O*-glycosides can exert an allelopathic effect on weeds and soil bacteria in continuously released from rice tissues to paddy soil in a specific quantity [39]. Until now, the mechanism of action of the flavonoids in allelopathy is still undefined. According to Berhow [40], it is possible the influence of flavonoids on the cell growth inhibition, disorders on the ATP production and even preventing the auxins function. The proposals of other mechanisms are changes in the permeability of the membrane, hindering nutrient absorption, cell division, and elongation [41]. In some cases, flavonoids promote a wave of ROS, activating the Ca^{2+} signal cascade and guiding to the root system death [42].

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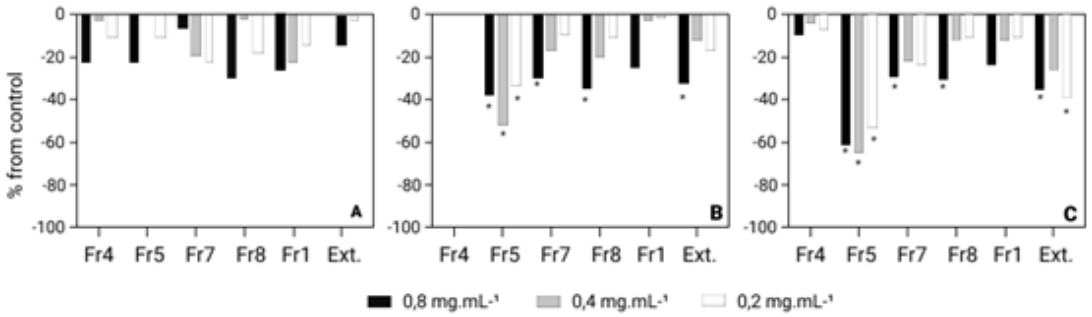
Figure 2: Inhibition (%) of *M. amplum* fractions and extract on germination rate (A), hypocotyl length (B), and root length (C) of onion seedling.



Plant extracts and their secondary metabolites have been studied by their biological potentiality with effects as synthetic insecticides and herbicides, having biodegradability in the environment, and being safer to non-target organisms [43]. However, García [44] relate the inhibition growth of the microalgae *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen et Skulberg after 160 h by the polyflavonoids exposition. These compounds were isolated from the bark of *Pinus radiata* and acted on the nutrient deficit or by the changes in the medium conditions after the growth phase. The alkaloids from *Symphytum officinale* L., *Senecio vernalis* Waldst. & Kit. and *Tussilago farfara* L. show genotoxic, carcinogenic effects and toxicity against two aquatic organisms, *A. salina* e *Daphnia magna* Straus with lethality induction of > 90% [45]. Ferraz [46], critically gather works about the ecotoxicological effects of natural products on different organisms such as phytoplankton, macroalgae, aquatic and terrestrial vertebrates, plants, and also soil microorganisms. In this sense, is important to evaluate the non-toxicity of plant extracts with biological potential. So, in this work, the 70% EtOH extract of *M. amplum* leaves was tested at the concentrations of 1000 μg.mL^{-1}, 100 μg.mL^{-1}, 10 μg.mL^{-1}, and 1 μg.mL^{-1}. Nauplii of *A. salina* Leach are bioindicators to the presence of toxic substances present in plant extracts through a rapid and inexpensive bioassay [24]. According to Meyer [22], substances in different concentrations, which present DL50>1000 μg.mL^{-1} will be considered non-toxic against *A. salina*, and toxic when they present DL50<1000 μg.mL^{-1}. Thus, it can be said that the extract of *M. amplum* is not toxic against the nauplii of *A. salina* because it presents LD50 greater than 1000 μg.mL^{-1}.

Some fungal species have an important ecological role, producing important mycotoxins to maintain the soil environment and host, such as *Fusarium* and *Aspergillus* species [47, 48]. Soil is the most important part within the boundaries of a natural or managed ecosystem because it can maintain the plant's productivity, animals, promote healthy plant growth, as well as water quality in the environment, degrade environmentally harmful substances, and promote the nutrient

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storage and cycling [49]. In agricultural ecosystems, the removal of natural vegetation for the insertion of high productivity crops causes significant changes in the soil microbial community, affecting important ecological functions [50]. Some microorganisms in soil are essential to plant species, soil type, macrofauna, and including to land crop management [51]. The imbalance caused in the soil promotes the frequent emergence of some microorganisms, which in high quantities become pathogenic to crops of interest. Thus, root and vascular diseases arise in agriculture by the increase of pathogenic microorganisms population [52]. These soil-borne diseases can cause significant reductions in the yield of various crops [53]. In this sense, it is important to consider plant extracts with phytotoxic action with low or without effect against the fungus *F. graminearum*, *F. oxysporum*, and *A. flavus*, which are microorganism presents in the soil microbiota. According to preliminary results, the extract and fraction groups of *M. amplum* tested showed no activity against these fungi. Thus, it shows the possible potentiality of the extract as a biodegradable bioherbicide and safe for the microbiota present in the soil.

4. CONCLUSIONS

The chemical study of the extract by FIA-ESI-IT-MS^n of the hydroethanolic extract of *M. amplum* allowed the detection of *C*-glycosylated flavones derived from apigenin and luteolin, type A proanthocyanidins dimer and trimer, quinic acid, and a saponin Soyasapogenol B AcGlu-hexose-deoxyhexose. Phytotoxic assays indicated activity of *M. amplum* extract inhibiting root growth of tomato and onion at the three concentrations tested. The results are more significant for tomato seeds. The best observed phytotoxic data involves the flavonoid fraction with a higher percentage of root length inhibition, which may be associated with the presence of *C*-flavones. The extract shows no ecotoxicity and no antimicrobial action against the fungi present in the soil microbiota: *F. graminearum*, *F. oxysporum*, and *A. flavus*. These data indicate the potentiality of fraction group Fr5 and the crude extract of *M. amplum* as a biodegradable, non-toxic, and sustainable potential phytotoxic.

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