

Phytotoxic study of glycosylated flavonols from *Annona coriacea* (Annonaceae) on cultivated species and an invasive grass.

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Abstract

Annona coriacea Mart. is a Brazilian native species whose phytotoxicity was described, although there is no data about the compounds responsible for it. The aim of this study was bioprospecting *A. coriacea* in relation to phytotoxicity on the elongation of wheat elongation coleoptiles and on germination and initial growth of standard target species (lettuce and tomato) and a weed (*Urochloa decumbens* (Stapf) R.D. Webster). Thus, the leaf ethanolic extract was subjected to chromatographic separation furnishing the isolation of 11 flavonols: **1** - Quercetin-3-*O*-gentiobioside, **2** - Quercetin-3-*O*-robinobioside, **3** - Rutin, **4** - Hyperin, **5** - Isoquercitrin, **6** - Biorobin, **7** - Nicotiflorin, **8** - Keioside, **9** - Narcissin, **10** - Cacticin and **11** - Isorhamnetin-3-*O*-glucoside. This is the first report of wheat coleoptile bioassay to all these compounds and it is also the first phytotoxicity results for **1**, **2**, **6**, **8** and **10**. Compounds **5**, **7** and **11** showed elevated phytotoxicity in wheat coleoptiles bioassay (IC₅₀ 0.22 mM, r^2 0.97; IC₅₀ 0.48 mM, r^2 0.93; IC₅₀ 0.28 mM, r^2 0.92, respectively). No correlation was found between the structure of the compounds and their activity. Compounds **5** and **11** were also tested on diaspores. Compound **11** significantly reduced the germination of *U. decumbens* in almost all concentrations, with values between 50% and 65%. The broad distinction concerning the complexity of the biological systems was suggested as an explanation for the distinct effects observed for coleoptile and germination and initial growth assays.

1. Introduction

The Brazilian neotropical savanna (named Cerrado) is one of the most diverse environments in the world, with considerable vegetation heterogeneity. Nowadays, this domain has been suffering from strong deforestation, fragmentation and invasion of exotic species (Sano et al. 2008). Cerrado has about 12,000 plant species, but currently only 71 (0.6%) had their phytochemistry studied (Novaes et al. 2013a). Among these studies, several biological activities were evaluated and strong phytotoxic, molluscicidal, insecticide, fungicide and antibacterial activities were described. Annonaceae is one of the richest families among the wood components of the Cerrado (Batalha and Mantovani 2001). *Xylopia aromatica* (Lam.) Mart. and *Annona coriacea* Mart. are the most studied species probably because of their high abundances (Novaes et al. 2016; Gatti et al. 2017). *A. coriacea*, popularly known as 'araticum', is a perennial shrub with edible fruits (Lorenzi 2008), native from the Brazilian savanna, found in southeast, south, and northeast areas of Brazil.

Chemically, acetogenins (Yu et al. 1994; Silva et al. 1997, 1998; Alves et al. 2014), polyphenols (Júnior et al. 2016), mono and sesquiterpenes (Siqueira et al. 2011), alkaloids (Machado et al. 2013) and flavonoids (Júnior et al. 2016; Novaes et al. 2018) are among the compounds already identified in *A. coriacea*. Biological activities have already been described for extracts. Leaf methanolic extract of the species showed insecticide (Freitas et al. 2014) and phytotoxic (Formagio et al. 2010) activities, while ethanolic extract exhibited antiprotozoal (Toledo et al. 2011), phytotoxic (Novaes et al. 2016), fungicide (Almeida-Apolonio et al. 2018) and cytoprotector (Júnior et al. 2016) actions. Ethanolic extracts of barks and flowers showed antioxidant (Camilo et al. 2017)

and fungicide (Almeida-Apolonio et al. 2018) effects, respectively. Alcoholic extracts of fruits and seeds showed insecticide (Costa et al. 2012), antiproliferative and anticholinesterase (Formagio et al. 2015), cytotoxic and enzymatic inhibitor (Brandão et al. 2011; Benites et al. 2015).

Allelopathy is a phenomenon actually defined as any direct or indirect effect caused by allelochemicals (secondary, or even primary, metabolites) from plants on other plants or microorganisms (Weir et al. 2004). This is an important ecological mechanism which influences plant dominance and succession, community formation, climax vegetation, crop management and productivity (Latif et al. 2017). The allelochemicals can be present in many plant organs and can be released in the environment by decomposition of residues, volatilization and root exudation (Weston and Duke 2003). Studies on this area can be performed in the field (Inderjit and Weston 2000), as well as in laboratories. These latter studies are frequently called phytotoxic activity and can be used to provide indicatives of allelopathy in the field, including the identification of the allelochemicals responsible for this interaction (Inderjit and Weston 2000). These allelochemicals

can be evaluated as mixtures or may be purified and tested as isolated compounds. Therefore, the assays will determine whether biological effects are synergistic or related to a single compound, respectively (Reigosa et al. 2013). In both cases, the samples can be used as natural herbicides. These herbicides from natural source are water soluble and free from halogenated molecules, have alternative paths of action, specific interactions with target plants, and are less dangerous to the environment (Macías et al. 2008).

Novaes et al. (2016) had studied the phytotoxic activity of extracts of four species of Annonaceae, including *A. coriacea*, native to the Brazilian savanna. The ethanol leaf extract of this species showed strong phytotoxic activity on germination and initial growth of two monocots, onion and *Urochloa decumbens* (Stapf) R.D. Webster (Poaceae), and moderate inhibitory activity on the germination and initial growth of lettuce and on the elongation of wheat coleoptiles. The presence of 11 glycosylated flavonols has been recently described in this extract (Novaes et al. 2018). Weston and Mathesius (2013) suggested that flavonoid glycosides as well as aglycones can be released in the soil by decomposition or exudation, and may exert activity in microbes, plants and animals. Therefore, since crude extract of *A. coriacea* presented phytotoxic effects over some species, we hypothesized that some leaf phytotoxic compounds could act as allelochemicals in the soil and could, at least in part, explain the persistence of *A. coriacea* in plantations, besides be used as natural-origin herbicides. Thus, the main goals of this study were the bioprospection of some fractions of ethanolic extract from leaves of *A. coriacea* concerning their phytotoxicity, the isolation and purification of some compounds, and the examination of their potential bioactivity.

2. Material and Methods

2.2. Extraction, isolation and identification of compounds of *A. coriacea*

Leaves of five individuals of *A. coriacea* Mart. were collected in the reforestation area of Instituto Florestal at Itirapina – SP – Brazil (-22°23'52 S, -47°85'02 W). The voucher (SPF 213417) of the species was deposited at the Herbarium of the Universidade de São Paulo (SPF), in Brazil.

Leaves were completely dried at 40 °C. The crude extract was obtained by maceration with ethanol (2.5 L: 1 Kg) during seven days (Novaes et al. 2016). The extract was filtered, completely dried using a rotary evaporator (25 g) and fractionated in an open silica column (0.06–0.02 mm) at atmospheric pressure. The eluent series in increasing polarity order was: hexane 100%, hexane/ethyl acetate 80/20, 60/40, 40/60, and 20/80%, ethyl acetate 100%, ethyl acetate/methanol 80/20, 60/40, 40/60, and 20/80% and methanol 100%. All fractions were concentrated and analyzed by thin layer chromatography (TLC), in chromatographic plates (0.25 mm thickness) with fluorescent indicators (Alugram Sil G/UV 254, Machery Angel). Plates were observed under UV light at λ 365 nm and revealed with sulfuric anisaldehyde and heated at 150 °C. Fractions were combined in nine groups: A (0.70 g), B (4.9 g), C (0.85 g), D (0.78 g), E (1.5 g), F (0.93 g), G (5.14 g), H (5.93 g) and I (2.56 g).

All groups were assayed. Since group G was significantly active at all concentrations tested, reaching over 60% of inhibition at the highest concentration, further fractionation was performed. The isolation and identification were performed as described in Novaes et al. (2018) through semi-preparative HPLC - DAD (Agilent 1200), using Eclipse XDB C18 column (250 mm x 9.4 mm id, 5,0 μ m particle) and a gradient of 0.1% acetic acid and acetonitrile as mobile phase, yielding 11 pure flavonoids: **1** - Quercetin-3-*O*-gentiobioside (375.6 mg), **2** - Quercetin-3-*O*-robinobioside (82.8 mg), **3** - Rutin (100.8 mg), **4** - Hyperin (26.1 mg), **5** - Isoquercitrin (96.4 mg), **6** - Biorobin (3.1 mg), **7** - Nicotiflorin (21.4 mg), **8** - Keioside (70.1 mg), **9** - Narcissin (650 mg), **10** - Cacticin (3.5 mg) and **11** - Isorhamnetin-3-*O*-glucoside (78 mg). The structure elucidation of compounds were done through UV/Vis spectroscopy procedures (Mabry et al. 1970), acid hydrolysis (Markham 1982), and ^1H and ^{13}C NMR analysis.

2.3. Wheat coleoptile elongation bioassay

Wheat seeds (*Triticum aestivum* L.) were sown in Petri dishes lined with filter paper, moistened with water, and grown in the dark at 25 ± 1 °C for 4 days (Barbero et al. 2010). After this period, etiolated seedlings were selected and placed in a Van der Weij guillotine, under green light. The apical 2 mm of the epicotyl were cut off and discarded. The following 4 mm of the coleoptiles were cut and selected for bioassays. The groups and the isolated compounds were diluted in phosphate-citrate buffer solution containing 2% sucrose and 0.5% DMSO (Nitsch and Nitsch 1956), at pH 5.6, to the final bioassay concentrations of 0.8, 0.4 and 0.2 mg mL⁻¹ for the groups and 1 mM, 0.3 mM, 0.1 mM and 0.03 mM for isolated compounds, usual in this bioassay (Novaes et al. 2013b; Rial et al. 2014). Five coleoptiles and 2 mL of each sample dilution were placed into a test tube (three tubes per dilution). Test tube of negative control without any sample, and positive control, using commercial herbicide glyphosate at same concentrations replacing the samples were also done. All tubes were placed in a roller tube apparatus (STUART SB2) at 20 rpm for 24 h at 25 °C in the dark. After this period, coleoptile elongation was measured and the length evaluated as percentage differences from negative control.

2.4. Germination and seedlings initial growth

Bioassays were performed as Macías et al. (2000), modified, and conducted using six-well microplates in which four wells were used as replicas, each one containing 8 diasporas of lettuce (*Lactuca sativa* L., cv. Grand Rapids), tomato (*Solanum lycopersicum* L., cv. IPA6) and *U. decumbens*. The wells were lined with filter paper and moisturized with 1 mL of group G and isolated compounds diluted in buffer, composed by 10⁻² M 2-[N-morpholino]ethanesulfonic acid (MES), 1 M NaOH (pH 6.0) and 0.5% DMSO, at 0.8, 0.4 and 0.2 mg mL⁻¹ for group G and 1 mM, 0.5 mM, 0.25 mM, 0.12 mM and 0.06 mM for isolated compounds. Negative (1 mL of buffer) and positive controls, using commercial herbicide glyphosate at same concentrations replacing the samples were also run. The six-well microplates were sealed with parafilm and incubated at 25 °C in B.O.D. (FANEM 347-CDG), with photoperiod of 12/12h light/dark. Bioassays took 8 days. After this period, measures of germination rate, and shoot and root length were performed and presented as percentage differences from negative control.

2.5. Statistics

The experimental design in the laboratory was completely randomized. The statistical tests were performed using the free software Bioestat 5.0. The data normality was analyzed by Lilliefors test. Significant differences between results from negative control and those from the test samples were paired evaluated by Mann-Whitney (when average values were not normally distributed) and T test (when the average values were normally distributed), with the decision level at $p < 0.05$ (Zar 2010).

3. Results

All groups obtained from the ethanolic extract of *A. coriacea*, except groups B and F, showed significant phytotoxicity on the elongation of wheat coleoptiles (Fig. 1), at least at the highest concentration. The herbicide was much more active than any fraction and showed inhibitions between 90% and 80%. Among the groups with higher phytotoxicity, group G stood out because of two factors: (1) the highest inhibition percentage, over 60% at the highest concentration and, (2) the higher yield (5.14 g). Therefore, group G was chosen for continuing assays. Group G also inhibited around 70% of *U. decumbens* germination rate with concentrations of 0.8 mg mL⁻¹ and 0.4 mg mL⁻¹ (Fig. 2). However, no significant effect was observed on initial growth of seedlings (Fig. 2).

Based on the results of group G on *U. decumbens* germination, further potential allelochemicals isolation was carried out on it. The group G afforded 11 glycosylated flavonols, being five quercetin (1–5), two kaempferol (6–7) and four isorhamnetin derivatives (8–11), identified as: Quercetin-3-*O*-gentiobioside (1), Quercetin-3-*O*-robinobioside (2), Rutin (3),

Hyperin (4), Isoquercitrin (5), Biorobin (6), Nicotiflorin (7), Keioside (8), Narcissin (9), Cacticin (10) and Isorhamnetin-3-O-glucoside (11) (Novaes et al. 2018).

Wheat coleoptile elongation bioassay was performed with all isolated flavonols at 1 mM (Fig. 3). Only the compounds 5, 7, 11 and the herbicide inhibited significantly the coleoptiles elongation at this higher concentration. Therefore, three other low concentrations were also tested. The herbicide inhibited about 80% of wheat coleoptile elongation at all studied concentrations, while the inhibition was dose-dependent with the flavonols. Compounds 5 and 11 showed similar phytotoxicity, about 70% of inhibition at 1 mM. Both compounds were more active than 7. The lowest IC₅₀ was obtained for 5 (IC₅₀ 0.22 mM, r^2 0.97), followed by 11 (IC₅₀ 0.28 mM, r^2 0.92) and 7 (IC₅₀ 0.48 mM, r^2 0.93). Since compounds 5 and 11 showed the lowest values of IC₅₀ on the elongation of the coleoptiles, they were also tested on germination and initial growth of tomato (Fig. 4), lettuce (Fig. 5) and *U. decumbens* (Fig. 6).

None of the compounds have significantly affected the germination or the initial growth of tomato (Fig. 4). For lettuce, however, compounds 5 and 11 stimulated shoot initial growth at almost all concentrations (Fig. 5). This stimulation reached 60% in the case of compound 11 at 0.5 mM. Shoot growth of lettuce was significantly inhibited only by the intermediated concentrations of 5 (1 mM) and 11 (0.05 mM). None of the concentrations of 5 and 11 affected its shoot growth.

Compounds 5 and 11 presented completely different effects on *U. decumbens* (Fig. 6). Compound 5 had almost none effect on it, except for the significant increase of shoot growth when applied at 0.25 mM. By the other hand, compound 11 inhibited significantly the germination of this species by 50% and 65% in almost all concentrations, despite elevated standard-deviations. Compound 11 was significant stimulatory on shoot growth at 0.25 and 0.06 mM and on root growth at 0.06 mM.

Comparing the effects of compounds 5 and 11 on coleoptile elongation and germination/initial growth assays, although both presented significant inhibition on the former (Fig. 3), only compound 11 exhibit some effect on the later assay using *U. decumbens* seeds (Fig. 6).

The herbicide presented a dramatic effect on the three target species. The germination of the three target species was inhibited in 100% in all concentrations of it, except the lowest one (Fig. 4, Fig. 5, Fig. 6). At 0.06 mM, the herbicide reduced germination of tomato in 70% and inhibited 100% of *U. decumbens* and therefore there is no result of its influence on shoot and root growth (Fig. 6). No significant effect of it was observed for lettuce (Fig. 5). However, for both tomato and lettuce, the few diaspores germinated presented no shoots and strongly reduced roots, reaching 75% and 90% of lower length, respectively, in the presence of the lowest concentration of the herbicide (Fig. 4, Fig. 5).

4. Discussion

Almost all groups at 0.8 mg mL⁻¹, presented higher inhibition percentage than that previously observed for the leaf crude extract (around 40% - Novaes et al. 2016), specially groups D, E, G and I. Although group G showed strong phytotoxicity on the elongation of wheat coleoptiles and germination rate of *U. decumbens*, no significant effect was observed on initial growth of seedlings. This species is an aggressive weed in Brazil, which besides its invasiveness in fields, also shows easy adaptation to the soils of the Brazilian savanna (Alvim et al. 1990) and allelopathic potential (Souza et al. 2003). Novaes et al. (2016) observed over 70% of inhibition of germination and also on shoot and root lengths when diaspores of *U. decumbens* were grown with crude leaf extract of *Annona coriacea*. The difference of results between the leaf crude extract and group G on the growth of the weed seedlings could be probably because some active compounds responsible for this effect may have been placed in other groups after fractionation of the extract.

The good germination results of Group G on *U. decumbens* justified its prospection for allelochemicals which could be used as natural origin pesticides in future. Since 2009, Brazil is the largest consumer of pesticides in the world (INCA,

2015). The indiscriminate and often misguided use of these products have been responsible for intoxication of human beings, especially workers who make their application in the field, and biological communities (INCA 2015). Thus, the search for natural pesticides, which are biodegradable and do not produce contaminants such as synthetic products, is of fundamental importance.

The group G afforded 11 glycosylated flavonols, as presented before (Novaes et al. 2018). The flavonol occurrence in Annonaceae species was already described by Santos & Salatino (2000). Compounds **5**, **7** and **11** significantly affect the elongation of wheat coleoptiles. Novaes et al. (2013b), Nebo et al. (2014), Watanabe et al. (2014), and Marsni et al. (2015) described phytotoxic activity of many flavonoids on the elongation of wheat coleoptiles. Most of the compounds showed effects between 0–70% of inhibition, even in the highest concentrations, as 1 mM. Some aglycones, however, were very active. The flavone (2-phenyl-4H-1-benzopyran-4-one) showed almost 100% inhibition in concentrations between 0.1 mM and 1 mM (Nebo et al. 2014), while heliannone B showed 100% inhibition at 1mM, but had strong reduction of activity in concentrations lower than 0.3 mM (Marsni et al. 2015). Here, the three compounds with the highest activity were, respectively, a quercetin, a kaempferol and an isorhamnetin glycosides, and their sugars were a glucose (**5** and **11**), and a glucose connected to a rhamnose (**7**), what is also present in other compounds without activity. Therefore, at least in this work, there was no correlation between the structures and the activities.

Compounds **5** and **11** affected the elongation of wheat coleoptiles close to those exhibited by the herbicide. These compounds did not show important results on tomato and lettuce germination and seedling growth. Compounds **5** and **11** presented completely different effects on *U. decumbens*: compound **5** had almost no effect on it, and compound **11** significantly affected its germination, showing elevated standard-deviations. High standard-deviations are expected for results using *U. decumbens* since it is a wild species and present wide intrinsic variation. At first, germination should be less affected by phytotoxic compounds than plant growth because of the barrier provided by seed, especially in those of larger endosperm (Soltys et al. 2012). However, this was not our case. Reduction of weed germination is especially important to farmers interested in their control. Other authors have already reported the same effect on this weed (Novaes et al. 2016; Rial et al. 2014).

One possible explanation for this difference between the results of compounds **5** and **11** could be related to the distinct complexity of the biological systems in both assays. While coleoptile assay evolves the activity of a single tissue with less differentiated cells, germination and initial growth assays are developed on more complex systems, evolving the development of a new individual, which could also show complex defense responses against stressful environment (Gniazdowska et al. 2015), in our case, the presence of an allelochemical.

Phytotoxicity of the compounds **5** and **11** have been studied before on standard target species. Parvez et al. (2004) observed a decrease of 70% on the initial growth of *Arabidopsis thaliana* in the presence of **5**. Contradicting our results, Almeida et al. (2008) observed 60% of inhibition of lettuce root elongation when exposed to compound **5**. For these authors, flavonoids with a catechol group, like compound **5**, are responsible for changes on cellular membrane permeability and modify the radicular lengthening needed for root protrusion. Compound **11**, isolated from leaves of *Melilotus neapolitana* Ten. (Fabaceae) by Esposito et al. (2008) showed no effect on coexisting species of Mediterranean herbaceous plant community, *Petrorhagia velutina* (Guss.) P.W.Ball & Heywood (Caryophyllaceae), *Dactylis hispanica* Roth, and *Phleum subulatum* (Savi) Asch. & Graebn. (Poaceae).

Although some effect of compounds **5** and **11** were observed on germination/initial growth with the three target species used (tomato, lettuce and *U. decumbens*), they were much less expressive than those previously found with ethanolic crude leaf extract on *Annona coriacea* (Novaes et al. 2016). With the crude extract, germination inhibition was around 60–70% and the initial development of shoots and roots were also reduced for all the three target-species. These differences between the results could be due to the joint action of the compounds when they are together in the extract. Joint action is the set of interactions that can occur when compounds are mixed and they can show synergic, additive or antagonist

activities (Inderjit et al. 2002). These phenomena have been studied in elongation of wheat coleoptiles and antioxidant assays (Garcia et al. 2015; Rial et al 2016; Galindo et al. 2017), but there is no data proven it in germination and growth bioassays. Garcia et al. (2015) showed that binary mixtures of polymethoxyflavones of citrus showed synergistic effects on the elongation of wheat coleoptiles and antioxidant assays, whilst Rial et al. (2016) showed that the joint action of sesquiterpene lactones isolated from *Cynara cardunculus* L. (Asteraceae) were, predominantly, additive of inhibitory activity on the coleoptiles. To be more certain of additive or synergic activities between **5** and **11**, experiments of binary mixtures of the compounds, with different variations of concentrations, as performed by these authors, would be necessary, especially in germination and growth bioassays.

Concluding, the groups D, E, G and I of *A. coriacea* extract showed higher inhibitory activities on elongation of wheat coleoptiles than that observed for the crude extract. Eleven flavonols were isolated from group G and, as far as we know, this is the first report of wheat coleoptile elongation bioassay to all the compounds and it is also the first phytotoxicity results for five of them. There was no apparent correlation between the structure of the compounds and their activity in the present study. While isoquercitrin (**5**) and isorhamnetin-3-*O*-glucoside (**11**) showed elevated phytotoxicity in wheat coleoptiles elongation bioassay, they had no effect or were mainly stimulatory to germination and initial growth of lettuce and tomato. Only **11** was inhibitory to *U. decumbens* germination. The higher structural complexity of seeds, when compared to wheat coleoptile, is suggested as a possible explanation for distinct effects of isolated flavonoids on coleoptile elongation and germination/initial growth assays. There were also differences between the activity of the isolated compounds and the crude extract of *A. coriacea* on the target plants and it could have resulted from joint action of the compounds, which shall be tested in further experiments.

Declarations

Authors' contributions and Conflict of Interests:

PN and DYACS conceptualized and designed the study, critically revised the manuscript, and actively contributed to data discussions. PN prepared and fractionated the extracts, isolated the compounds, conducted the phytotoxicity assays. MJPF identified the compounds. JCL identified *A. coreacea* individuals. All authors critically reviewed and approved the final version of the manuscript. The authors declare that they do not have any conflict of interests.

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Figures

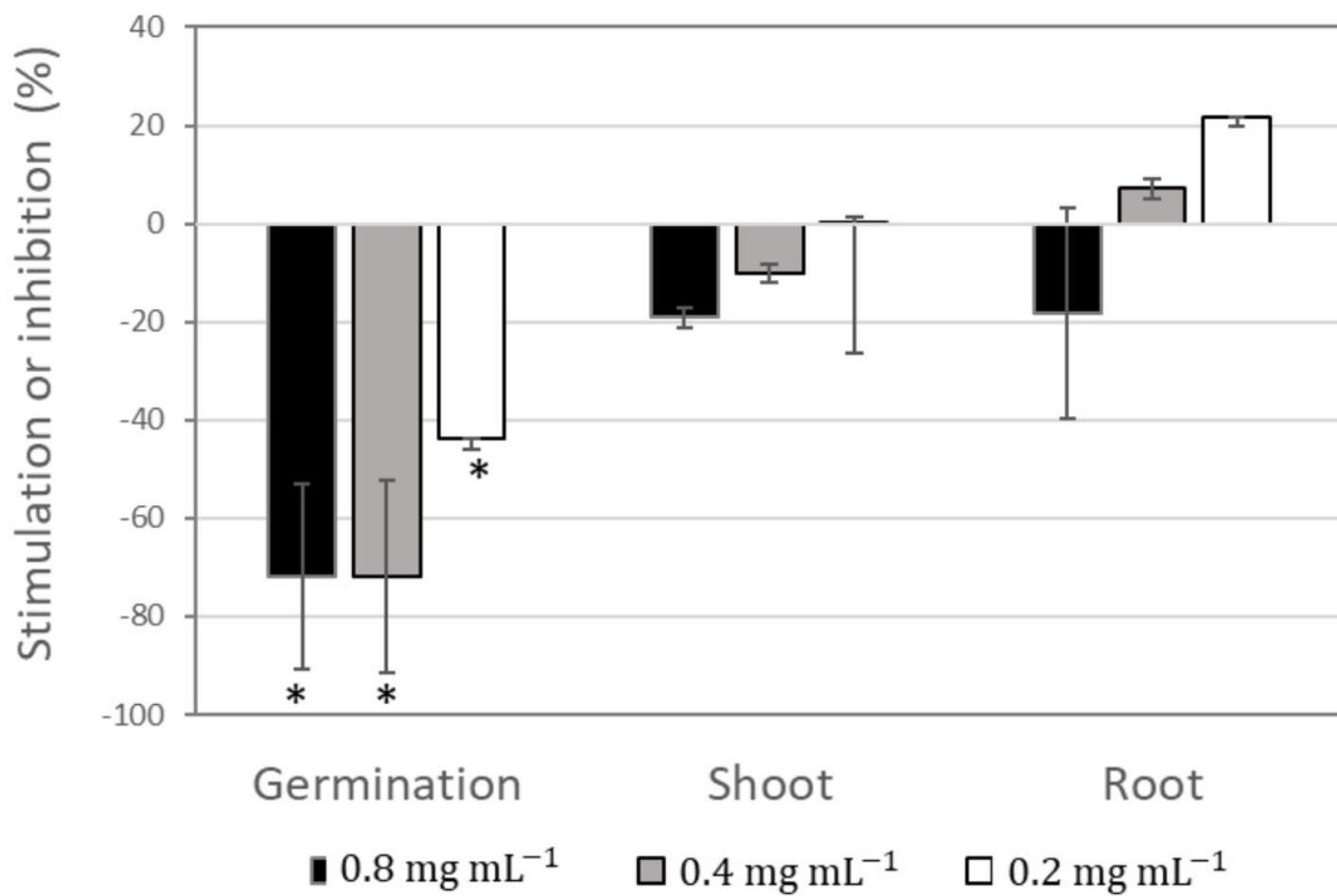


Figure 1

Percentage of stimulation/inhibition of wheat coleoptile elongation threatened with groups (A to I) obtained after column chromatography of ethanolic extract of *Annona coriacea* and the herbicide, in relation to negative control. Asterisks indicate significant differences in relation to negative control ($p < 0.05$)

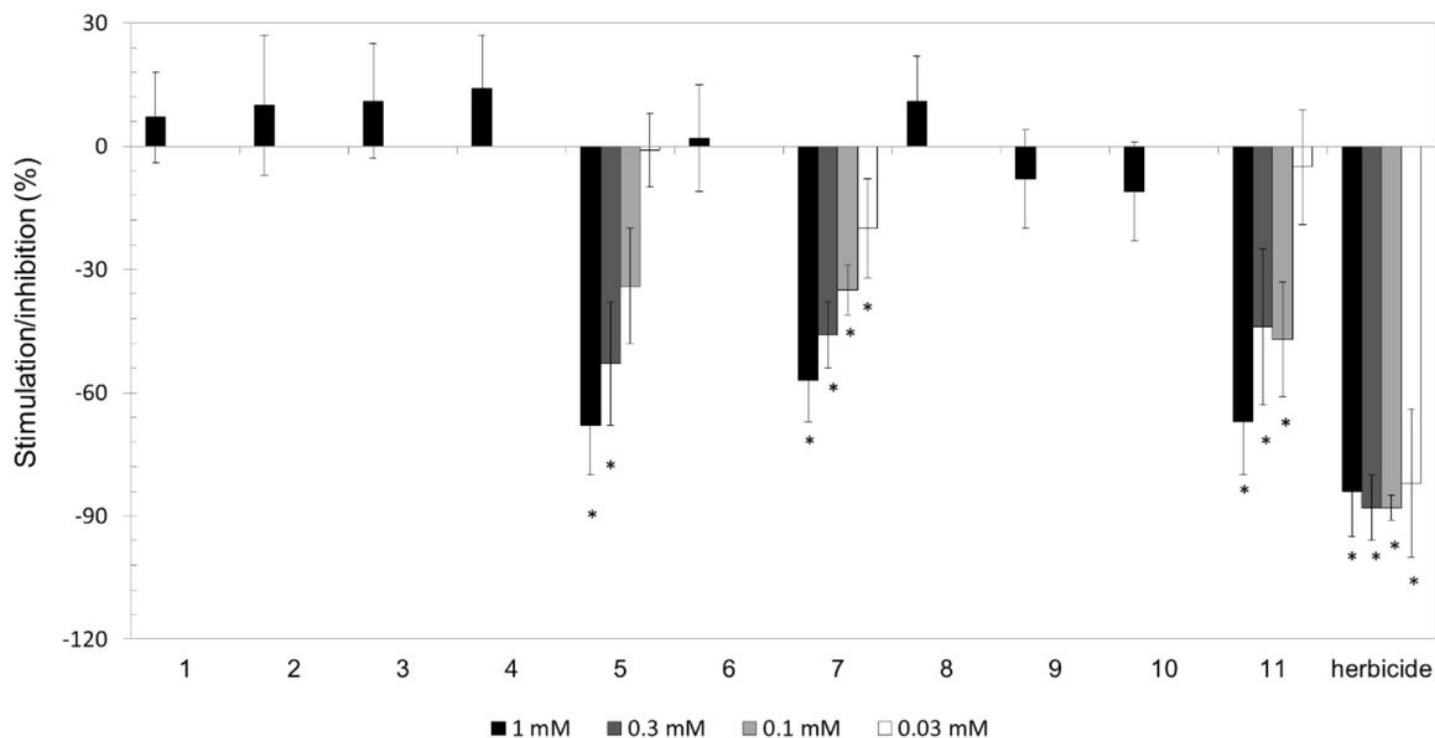


Figure 2

Percentage of stimulation/inhibition of germination and initial growth (shoots and roots) of *Urochloa decumbens* threatened with group G obtained after column chromatography of ethanolic extract of *Annona coriacea*, in relation to negative control. Negative results correspond to inhibition, while positive results correspond to stimulation. Asterisks indicate significant differences between treatments and negative control ($p < 0.05$)

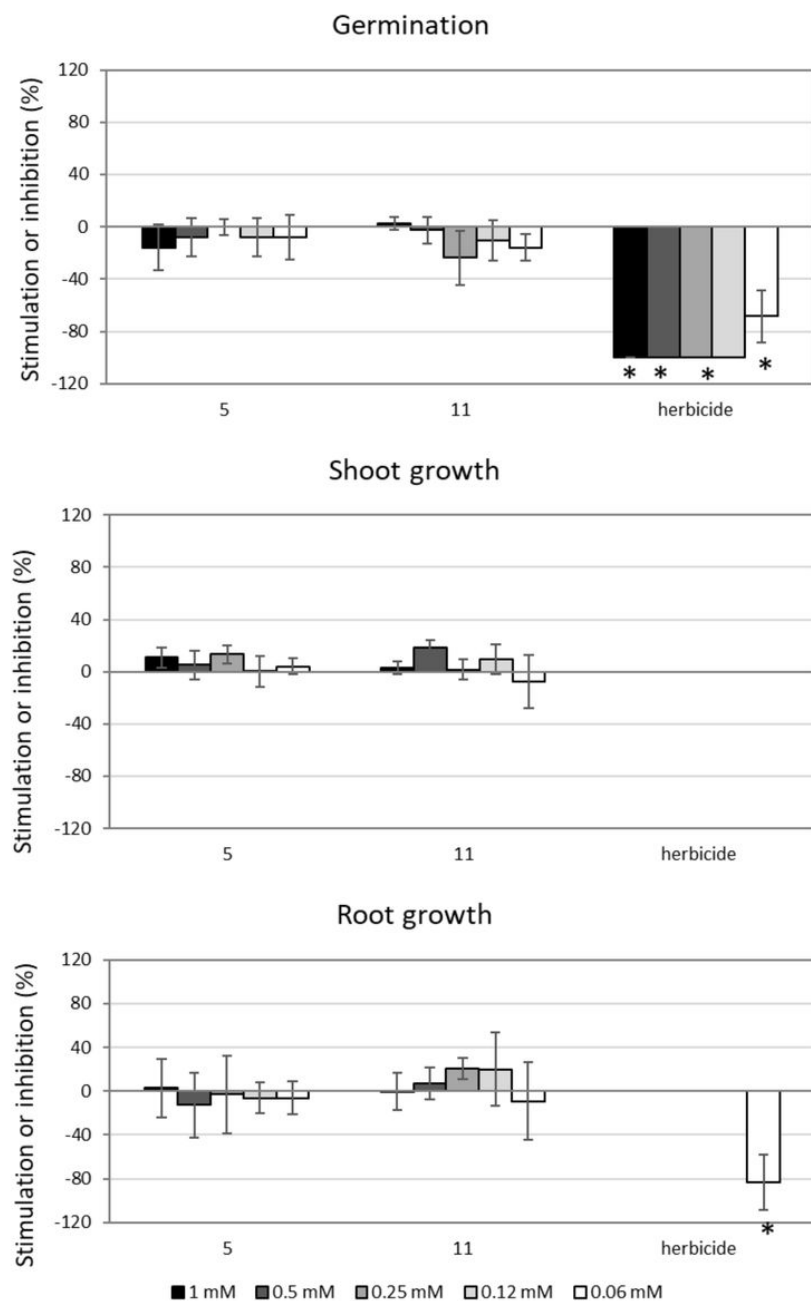


Figure 3

Percentage of stimulation/inhibition of wheat coleoptile elongation threatened with the flavonols isolated from group G obtained after column chromatography of ethanolic extract of *Annona coriacea* and the herbicide, in relation to negative control. Negative results correspond to inhibition, while positive results correspond to stimulation. Asterisks indicate significant differences between treatments and negative control ($p < 0.05$). **1** (Quercetin-3-*O*-gentiobioside), **2** (Quercetin-3-*O*-rabinobioside), **3** (Rutin), **4** (Hyperin), **5** (Isoquercitrin), **6** (Biorobin), **7** (Nicotiflorin), **8** (Keioside), **9** (Narcissin), **10** (Cacticin) and **11** (Isorhamnetin-3-*O*-glucoside)

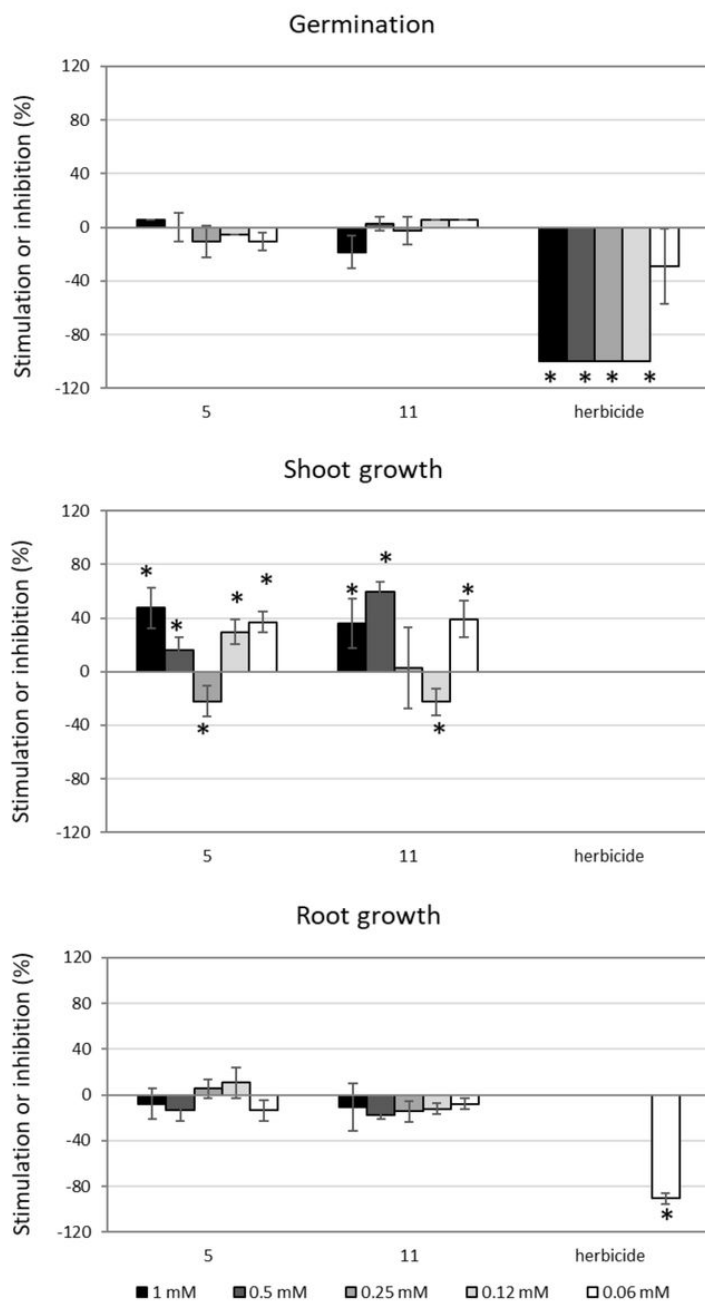


Figure 4

Percentage of stimulation/inhibition of germination and initial growth (shoots and roots) of tomato threatened with the flavonols **5**(Isoquercitrin) and **11**(Isoramnetin-3-*O*-glucoside) isolated from ethanolic extract of *Annona coriacea* and the herbicide, in relation to negative control. Negative results correspond to inhibition, while positive results correspond to stimulation. Asterisks indicate significant differences between treatments and negative control ($p < 0.05$)

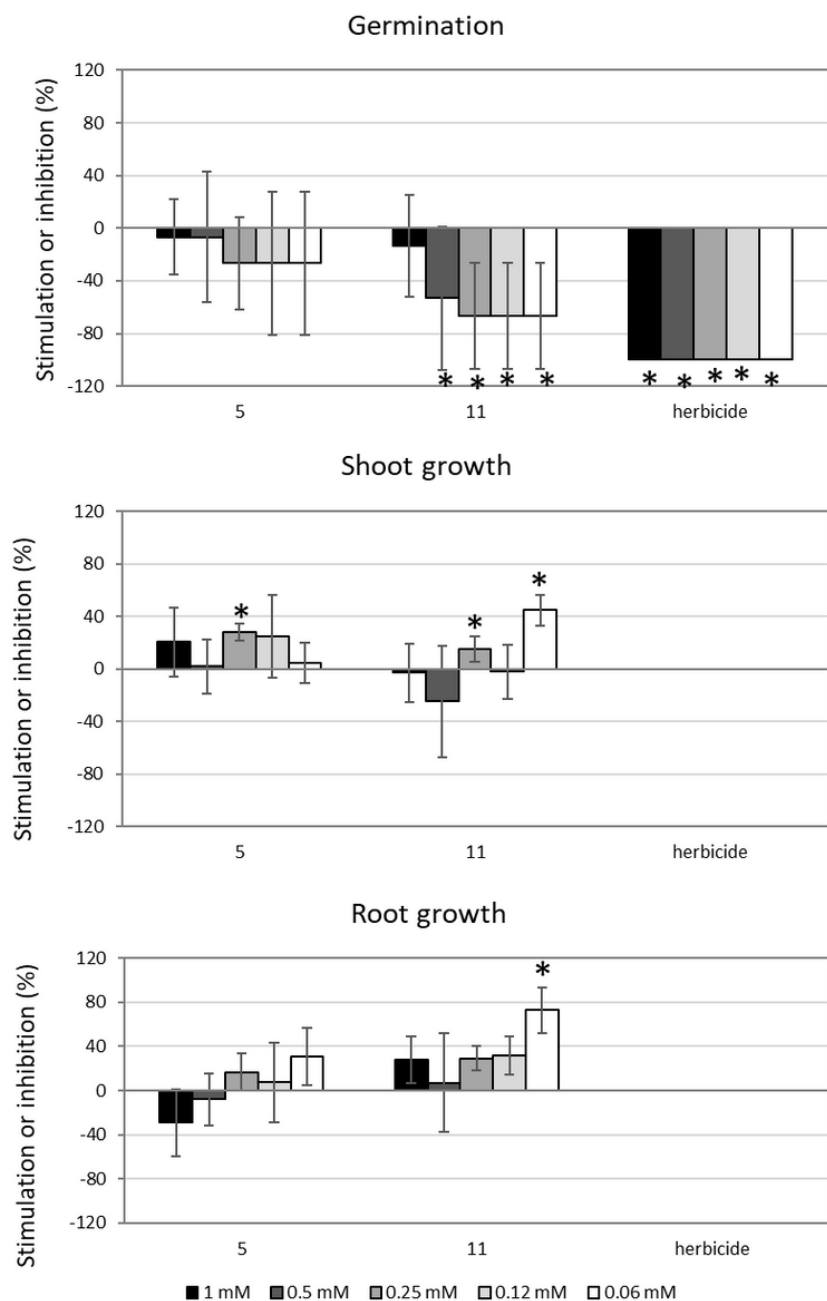


Figure 5

Percentage of stimulation/inhibition of germination and initial growth (shoots and roots) of lettuce treated with the flavonols **5**(Isoquercitrin) and **11**(Isoramnetin-3-*O*-glucoside) of the ethanolic extract of *Annona coriacea* and the herbicide, in relation to negative control. Asterisks indicate significant differences between treatments and negative control ($p < 0.05$)

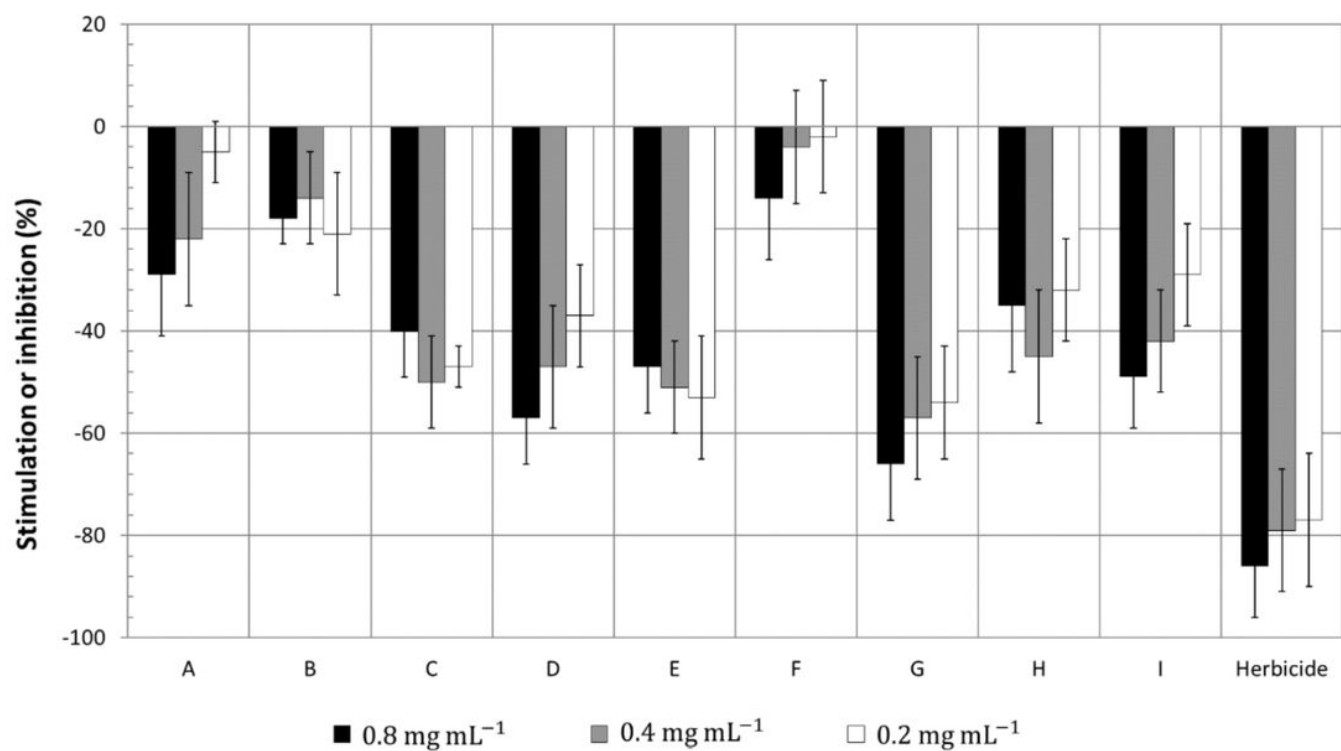


Figure 6

Percentage of stimulation/inhibition of germination and initial growth (shoots and roots) of *Urochloa decumbens* threatened with the flavonols **5** (Isoquercitrin) and **11** (Isoramnetin-3-*O*-glucoside) of the ethanolic extract of *Annona coriacea* and the herbicide, in relation to negative control. Asterisks indicate significant differences between treatments and negative control ($p < 0.05$)