UNIVERSIDADE D
COIMBRA

Daniela Mendes Duarte

RESEARCH ON ENVIRONMENTALLY SUSTAINABLE HERBICIDES FOR WEED CONTROL IN URBAN AREAS

Dissertação no âmbito do Mestrado em Biodiversidade e Biotecnologia Vegetal orientada pela Doutora Paula Lorenzo e pela Professora Doutora Cristina Galhano e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

Julho de 2021

Faculdade de Ciências e Tecnologia da Universidade de Coimbra

RESEARCH ON ENVIRONMENTALLY SUSTAINABLE HERBICIDES FOR WEED CONTROL IN URBAN AREAS

Daniela Mendes Duarte

Dissertação no âmbito do Mestrado em Biodiversidade e Biotecnologia Vegetal orientada pela Doutora Paula Lorenzo e pela Professora Doutora Cristina Galhano e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

Julho de 2021

1 2 9 0

UNIVERSIDADE D
COIMBRA

ACKNOWLEDGEMENTS

Firstly, and as it should be, I want to thank my thesis advisors Dr. Paula Lorenzo and Prof. Dr. Cristina Galhano that had an incredible patience with me and helped me and taught me a lot. Dr. Paula was present in every assay working alongside me, shared tips and work methods that I will treasure for life. Dr. Cristina, was one of the kindest people I met along the way. Thank you for always believing in and supporting my work and for your valuable suggestions and reviews. And obviously, thank you to Prof. Dr. Paula Castro that made everything possible since the beginning by starting this collaboration. To the three of you, thank you so much for presenting me with new projects and challenges that made me push myself out of my comfort zone and for being present and interested people.

Thank you to Cláudia, that has an incredible knowledge and was always available to help and share interesting books and papers. Also, thank you to the team at the Inproplant that kindly gave us an amazing work place for our experiments.

To the most important part of my life, my family, that was there every step of the way. Without you this would not had been possible.

I also want to thank Diogo, that saw me at my worst but always made me appreciate the good side of things and was proud of every little step I took. Thank you for not giving up.

I could not end this without mentioning my university family, Sara and Beatriz, that even though our contact was reduced the past years, they were an essential part of my academic life. And also my friends, Adriana, Robalo, Cristiana, Joana, Inês, Gonçalo, Taborda, Joana, Ricardo that even without realizing it contributed a lot for my mental sanity throughout the years and sometimes, just a message was everything I needed. Thank you so much.

Everyone knows I am really bad at expressing my feelings, but what I mean is that I really appreciate every single one of you and I am really lucky for having crossed paths with you.

TABLE OF CONTENTS

ABSTRACT

Urban areas are expanding throughout the world and questions about the impacts on biodiversity have been raised. One of the problems found in urban areas is weed control using herbicides based on synthetic chemicals which are extremely hazardous both to public health and to the environment. However, their use is needful, not only for agricultural production, but also for urban spaces maintenance. Public concern with the environment has been increasing and legislation regarding the use of chemical substances has been more restrictive. Therefore, it urges to find alternative sustainable solutions which are currently lacking in the market. For this purpose, several studies are focusing on natural herbicides based on plants. As the most frequent and closest contact between people and chemical products occurs in urban areas, this study aims to assess the herbicidal potential of plant aqueous extracts (dried *Acacia dealbata* bark and fresh *Oxalis pes-caprae)* and of an agrifood waste (spent coffee grounds) on the urban weed species: *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa*. For that, *in vitro* and pot assays were performed to test the effect of the extracts on seed germination and on plant growth. Results showed that *Oxalis pes-caprae* and *Acacia dealbata* bark extracts were the most effective ones at reducing seedling growth and development, mainly affecting germination and radicle length. Results depended on weed species and extract concentration. It also seems that soil have neutralized the herbicide effect of the extracts. Therefore, our study reinforces the need of carrying out studies under conditions as close as possible to the natural ones. Based on our results, further studies should be conducted to better know the potential of *Oxalis pes-caprae* and *Acacia dealbata* bark extracts as selective preemergence bioherbicides.

KEYWORDS: *Acacia dealbata* bark; Bioherbicides; *Oxalis pes-caprae;* Spent coffee grounds; Urban weeds.

RESUMO

As áreas urbanas estão em expansão por todo o mundo e diversas questões acerca dos impactos na biodiversidade têm sido levantadas. Um dos problemas encontrados nestes locais é o controlo de infestantes urbanas com recurso a herbicidas à base de químicos de síntese que são extremamente nocivos, tanto para a saúde pública, como para o ambiente. No entanto, a sua utilização é indispensável, não só para a produção agrícola, mas também para a manutenção de espaços urbanos. A preocupação da população com o ambiente tem sido crescente e a legislação sobre o uso de substâncias químicas tem-se tornado mais restrita. Assim, é crucial a procura de soluções alternativas sustentáveis que faltam atualmente no mercado. Diversos estudos têm-se focado em herbicidas naturais à base de plantas. Como o contacto mais frequente e próximo entre a população e estes produtos químicos ocorre nas áreas urbanas, este estudo tem como objetivo avaliar o potencial herbicida de extratos aquosos de plantas (casca seca de *Acacia dealbata* e *Oxalis pes-caprae* fresco) e de um resíduo agroalimentar (borra de café) em infestantes urbanas: *Achillea ageratum, Conyza canadensis* e *Dittrichia viscosa.* Para tal, foram realizados ensaios *in vitro* e em vaso para testar os efeitos dos extratos na germinação e no crescimento das plantas. Os resultados demonstraram que os extratos de *Oxalis pes-caprae* e de casca seca de *Acacia dealbata* foram os mais eficazes na redução do crescimento e desenvolvimento das plântulas, afetando principalmente a germinação e o comprimento da radícula. Os resultados dependeram da espécie de infestante e da concentração do extrato. O solo parece ter um importante efeito de neutralização dos efeitos dos extratos. Também parece que o solo neutralizou o efeito herbicida dos extratos. Portanto, o nosso estudo reforça a necessidade de se realizarem estudos em condições tão próximas quanto possível, às naturais. Com base nos nossos resultados, mais estudos devem ser realizados, para melhor conhecer o potencial dos extratos de casca de *Acacia dealbata* e de *Oxalis pes caprae* como bioherbicidas seletivos de pré-emergência.

PALAVRAS-CHAVE: Bioherbicidas; Borra de café; Casca de *Acacia dealbata*; Infestantes urbanas; *Oxalis pes-caprae*.

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

I.INTRODUCTION

1. Urban areas

Nowadays, urban areas, besides covering only about 1 % of all habitable land, are expanding throughout the world, both in size and number (Ritchie & Roser, 2013, 2020). In 2018, the number of cities with at least 1 million inhabitants was 548, which is expected to increase to 706, in 2030 (UN, 2018). This has been raising questions about the impacts on biodiversity because they are often located at critical ecosystem junctions or in areas known as biodiversity hotspots (Ruth & Coelho, 2007; Seto et al., 2012; Rastandeh et al., 2017). These impacts may be direct, mainly referring to habitat loss and biotic and abiotic changes, or indirect, such as the high dependence on ecosystem services and the produced urban waste (McDonald et al., 2019).

Some researchers have shown that the inclusion of biodiversity in urban planning plays an important role influencing people's lives (Giles-Corti et al. 2005, Barton & Pretty, 2010; Nilon et al., 2017). The interaction with nature makes people more aware of its conservation and has positive effects on physical and psychological health, social cohesion and sense of belonging, crime reduction, and economic advantages (Kuo & Sullivan, 2001; Elmqvist et al., 2015; Shanahan et al., 2016; Lee et al., 2017; Aerts et al., 2018).

Therefore, it is important that cities become planned spaces allowing biodiversity and anthropogenic coexistence (Gaston et al., 2013; Aronson et al., 2017). To achieve that, green spaces and asphalted areas must be harmoniously integrated, since they are both essential for cities growth and development (Davies et al., 2019).

1.1. Urban weeds

Of the 250 000 existing plant species, around 3 % show an invasive behaviour and less than 0.3 % are major world weeds (Holm, 1978; Westbrooks, 1998). Weeds are plants that grow spontaneously in unwanted places, being often pioneer species (Clements & Jones, 2021). They are characterized by a highly competitive capacity and persistence, thriving in disturbed habitats, and producing abundant seeds, frequently not useful to humans (Zimdahl, 2007).

In urban areas, weeds are perceived as a problem from an environmental point of view as well as from a public health and aesthetic perspectives. Weeds are capable to

interfere with native vegetation, causing soil erosion, and may promote substrate establishment that aggravates the whole problem (Ward et al., 1999; Kristoffersen et al., 2008). Furthermore, they interfere with public wellbeing by appearing spontaneously in pavements and causing asphalt damage, indirectly causing water flow disturbance, interference with activities such as bike riding and field sports and may reduce visibility contributing to traffic-accidents (Ruiz-Avila & Klemm, 1996; Benvenuti, 2004; Zimdahl, 2007; Melander et al., 2009; Keken et al., 2019). Additionally, they are considered a human health hazard, given that some people are allergic to the plant itself and/or their pollen, and are seen as aesthetically unappealing in public places (Zimdahl, 2007; Gadermaier et al., 2014; Bonthoux et al., 2019a). All these problems embody extra expenses for the government, either because of weed control costs or because of the money spent on damage repair (EPA, 1974; Marble et al., 2017; McLeod, 2018). Another negative consequence associated with urban weeds is related with their currently most used control method: by applying herbicides based on synthetic chemicals (Bonthoux et al., 2019b). We can also consider that these products are used to maintain turfgrass and green city spaces (Fuller & Gaston, 2009). In fact, these urban green infrastructures, including lawns and parks, cover up to 40 % of core European cities (Maes et al., 2019).

2. Herbicides

Herbicides, a subdivision of pesticides, are defined as phytotoxic chemicals used to destroy or inhibit the growth of several plants, mainly weeds (Gupta, 2011). Among pesticide, herbicides are the leading group in terms of tons produced, total treated area, and sales revenue (Gruber et al., 2011; Holt, 2013).

To be effective, an herbicide should contact the plant or seed, be absorbed, move to the action site, and accumulate in enough quantity to induce a response (Beckie et al., 2000). Usually, this response is achieved through enzymes' inhibition or through a general interference with proteins that support fundamental pathways for plant survival (Flamini, 2012; Zulet et al., 2013; Lonhienne et al., 2018). Nonetheless, an herbicide can be categorized in different ways according to some characteristics:

a) Their action timing: they can act during preemergence (seed germination may be compromised) or they can act during postemergence (they are applied when the plant is completely formed) (Vats, 2015). Postemergence herbicides are still separated in two categories: contact (the chemical does not move inside the plant after contact application an only damages the portion it contacts) (Sherwani et

al., 2015) and systemic (the chemical is translocated through the plant vascular system) (Singh & Sharma, 2008).

b) Their mode of action or the way they affect the plants: lipid and amino acid biosynthesis inhibitors, plant growth regulators, photosynthesis inhibitors, nitrogen-metabolism inhibitors, pigment inhibitors, cell-membrane disruptors and seedling-growth inhibitors (Sherwani et al., 2015).
c) And their smaller or larger action spectrum when it comes to the plants they affect: non-selective herbicides that damage all types of vegetation, and selective herbicides that are more specific to a particular plant and when applied do not harm other types of vegetation (Das & Mondal, 2014).

2.1. Pros and cons of synthetic herbicides

Herbicides are definitely an essential element for agricultural production and public spaces' maintenance (Gianessi, 2013; Hahn et al., 2020). In addition, they are relatively easy to apply and many different formulations are available (Zimdahl, 2007). However, these synthetic products contain dangerous active chemicals that can cause several problems. The most concerning one is their possible toxicity for human and animal health (Flamini, 2012; Barchanska et al., 2017; Zhu et al., 2018).

The continued use of these products can lead to "superweeds" emergence - resistant plants that have a diminished response to a specific compound (Zimdahl, 2007; Gaines et al., 2020), water contamination and other challenges on account of these persistent chemicals due to environmental pollution by volatilization, leaching, and runoff (Flamini, 2012; Li et al., 2018; Wilms et al., 2020). In addition, non-target species may be compromised due to mortality or population reduction caused by non-specific herbicides, and, if those species are edible, dangerous chemicals may be bioaccumulated in food chain (Flamini, 2012; Sikorski et al., 2019).

It is also known that herbicides and its application have a high cost. On the other hand, expenses to develop new products that fulfil all the requirements imposed by an increasingly strict law have been rising (Zimdahl, 2007; Green, 2014).

2.2. Glyphosate as an example

Glyphosate is one of the most globally used active substances as herbicide (Duke, 2017). This non-selective herbicide has been used in agricultural fields since

1974 (Benbrook, 2016). In Portugal, around 1600 tons are sold annually for agriculture and urban purposes, thus, being the bestselling herbicide on the country (DGAV, 2017) and the most used in the world (Duke, 2017). According to the World Health Organization, glyphosate has been classified as *probably carcinogenic* for humans because it has proven to be carcinogenic on lab animals (Quercus, 2016). Other studies linked glyphosate to other health problems, such as teratogenic effects (Paganelli et al., 2010), hormonal deregulation (Romano et al., 2010), hepatic and renal toxicity (Beuret et al., 2005; Jayasumana et al., 2014) and even autism (Beecham & Seneff, 2015). Furthermore, formulations with glyphosate frequently include adjuvants that may increase product hazard and are not shown on the label of commercial herbicides (Mesnage et al., 2015).

From an environmental perspective, glyphosate residues are present in water, soil, and plant products because this herbicide is fairly resistant to degradation (Van Bruggen et al., 2018; Zhao et al., 2018; Helander et al., 2019; Medina-Pastor & Triacchini, 2020). There are also reports suggesting its negative effect on bees (Balbuena et al., 2015) and links to the decline of Monarch butterfly populations in North America (Thogmartin et al., 2017).

On the European Union (EU), glyphosate use is authorized at least until 2022 (European Commission, 2017). However, there are an increasing number of reports on countries that are banning this product or expanding restrictions on its use. In 2016, local administrations in Spain banned its use for weed control in public parks and in 2019, it was totally banned in Austria and Vietnam (Kanthal et al., 2020). Other restrictions were also applied in France, Czech Republic, Italy, and the Netherlands (Kanthal et al., 2020).

2.3. The present and the future

On the EU, herbicides are part of the “Plant Protection Products” (PPPs) and are under the Regulation (EC) No 1107/2009 that coordinates the approval of active substances and its commercialization and under the Regulation (EC) No 396/2005 that deals with residues maximum limits definition on food products for humans and animals. Also, as a complement, there is the Sustainable Use Directive (SUD) (Directive 2009/128/EC) that aims to reduce potential hazards of pesticides promoting the use of alternative approaches and techniques, also known as Integrated Pest Management (IPM) (Kudsk & Mathiassen, 2019).

The European Commission, in collaboration with the European Food Safety Authority (ESFA) and all Member States drafts annual reports on the use of these chemicals and on the implementation of national action plans (European Commission, 2020b; Medina-Pastor & Triacchini, 2020). In the last years, legislation regarding these synthetic products has been stricter. The number of approved active substances in the EU drastically decreased. In fact, a reduction of more than 50 % has been observed (https://ec.europa.eu/assets/sante/food/plants/pesticides/lop/index.html).

EU pesticides' laws are considered the most stringent around the world. This fact was supported by a study showing that 72 approved pesticides in the USA are banned or in the process of being discontinued in the EU (Donley, 2019). Moreover, 25 % of the banned pesticides in the EU are free to use in the USA (Donley, 2019). When it comes to the turfgrass sector and urban spaces in general, this fact is very clear (Hahn et al., 2020). For instance, in Germany, only two herbicides can be used on golf courses (German Golf Association, 2017). In Great Britain, the maximum allowable load of herbicide active ingredients that may be applied to public areas, residential lawns, sports field, golf courses, etc., suffering a 64 % reduction from 2006 to 2012 (Department for Environment, Food and Rural Affairs, 2015). In Holland, the turfgrass industry has agreed to accept a complete ban of all pesticides by 2020 (Government of the Netherlands, 2017).

Regarding market, in 2016, the global agrochemical sales of the six largest firms was up to 32 billion euros and, in Europe, the profit was around 12 billion euros (European Commission, 2020a). Since then, those firms became the "big four": Syngenta and ChemChina, Dow and Dupont, Bayer and Monsanto (European Commission, 2020a). In 2014, the process to obtain a new PPP was estimated to cost 250 million euros and take about 10 years. Thus, we can see the important role that biotechnology and evolution play in improving some life aspects (European Commission, 2020a).

Recent trends in weed management point to an increase in the quantity of herbicides used, a decline in herbicide productivity (the amount of crop produced per herbicide input), and to a rising problem due to herbicide resistant weeds in farms leading to more expenses (Davis & Frisvoldb, 2017; Heap, 2014). There has also been an increasing public concern with environmental protection and with the search for organic and pesticide free products in the EU. As a result, the organic farming area already increased 70 % in the last decade (Czaja et al., 2015; Cordeau et al., 2016; European Commission, 2019).

The “perfect” herbicide described as able to control weeds selectively, non-toxic to non-target species and humans, persistence in soil but not beyond a given time, easily and quickly degraded to harmless products, active at very low rates and not leachable nor volatile is still not available (Zimdahl, 2007). We all know how hard it has been to fulfil all these requirements, thereby, the search for sustainable alternative solutions to meet farmers, countries and individuals’ needs is crucial.

3. Biopesticides

Nowadays, research has focused on the biopesticides development as an alternative solution (Damalas & Koutroubas, 2018). It is believed that these products neither negatively impact ecosystems, nor represent a risk for public health (Benvenuti et al., 2017; Kaab et al., 2020). Natural products such as essential oils, agricultural by-products, plant extracts, and living organisms (bacteria, fungi and insects) showing potential phytotoxicity are probably more environmentally friendly than synthetic products (Saini & Singh, 2019). This view comes to meet the goals established on ONU’s 2030 Agenda for Sustainable Development, mainly goal 11: Sustainable cities and communities – delineates that until 2030 the negative environmental impact per capita must be reduced and also, by then, “enhance inclusive and sustainable urbanization and capacity for participatory, integrated and sustainable human settlement planning and management” and “ provide universal access to safe, inclusive and accessible, green and public spaces” (ONU, 2015). Goal 15 also states: Life on land is extremely important given that biodiversity is in danger, therefore, natural herbicides appear as a solution to “reduce the degradation of natural habitats, halt the loss of biodiversity” (ONU, 2015).

These natural control products can be classified according to the active substance and its use. Thus, following the division used by Abbey et al. (2019), based on the active substance, biopesticides can be: microbial, biochemical, and plant-incorporated protectants. Regarding its use, they can be named bioinsecticides, biofungicides, bioherbicides and bionematicides.

a) Microbial: This includes fungi, bacteria and viruses and substances obtained from them (Duke & Dayan, 2018). They act by suppressing pest’s development through the production of a specific toxin. The most known and used microbial pesticide is the bacterium *Bacillus thuringiensis* (Bt) (Chandler et al., 2011; Czaja et al., 2015; Kumar & Singh, 2015; Abbey et al., 2019).

b) Biochemical: natural substances capable of a non-toxic pest's control by interfering with mating, growth and/or population build-up. They may have their origin in plants, animals or insects and plant secondary metabolites are an example (e.g. pyrethrins [Silvério et al. 2009]) (Chandler et al., 2011; Abbey et al., 2019; Liu et al., 2021).
c) Plant-Incorporated protectants: compounds produced by plants from genetic material that has been added to them. For example, a specific Bt pesticidal protein gene, when introduced into the plant genetic material, induces the pesticidal protein synthesis, making it resistant to pest's infection (Fujimoto et al., 1993; Chandler et al., 2011; Abbey et al., 2019; Liu et al., 2021).

3.1 Bioherbicides

Bioherbicides are products that have its origin in either living organisms or their natural metabolites, being applied as conventional herbicides to control weed populations, while keeping the biopesticide's requirement of not degrading the environment (Radhakrishnan et al., 2018). Most bioherbicides have been targeted toward agronomic weeds, but they are also useful to control weeds in non-agronomic areas (recreational areas, forests, pavements, lawns, gardens, etc.) (Hoagland et al., 2007).

3.1.1. Plants as a source of chemical compounds

The search for natural herbicides has had several starting points, being the study of plants one of the most common (Bordin et al, 2020). Plants are one of the richest organisms in organic compounds. Up to now, more than 2 140 000 secondary metabolites are known, and 80 % of them have its origin in plants (Berdy, 2005; Thirumurugan et al., 2018). These compounds play important roles in regulating metabolism, growth, cell signalling and many other biochemical processes (Chen et al., 2020).

Some secondary metabolites do not play a direct role on the plant's development, but they are crucial in defence mechanism against microorganisms, animals, and other plants (Flamini, 2012). Therefore, they display the so-called phytotoxicity activity – an impact on species growth or fitness (Werrie et al., 2020).

Allelopathy is the most interesting phenomenon for the search of natural herbicides based on plant compound (Soltys et al., 2013; Macías et al., 2019). Allelopathy happens when one plant interferes with the development of others by releasing allelopathic compounds (a type of secondary metabolites) (Zimdahl, 2007). Allelochemicals with negative effects have the capacity of inhibiting cell division, of preventing hydrolysis of nutrient reserves, and of disturbing the electron transport in photosynthesis (Balke, 1985; Irshad and Cheema, 2004; Hejl & Koster, 2004). These bioactive metabolites are released from plants via volatilization, foliar leaching, root exudation or decomposition of residues and leaf litter (Ben-Hammouda et al., 2001; Bonanomi et al., 2006; Kumar et al., 2009). They also have a very promising activity profile: wide biological activity at low concentrations, high selectivity and sensitivity on species, and they may perform new modes of action at different target sites (Macias et al., 2001). In addition, according to recent studies, Asteraceae and Poaceae are probably the most allelopathic plant families (Sánchez-Moreiras et al., 2003; Li et al., 2019;). Therefore, they are good contenders to be the base for natural herbicides or used in addiction to synthetic chemicals to reduce their dosage (Flamini, 2012).

3.1.1.1. Plant extracts

Because of the previously stated, there has been a huge interest on plant extracts as a source of natural chemicals as some studies have shown an herbicidal effect by direct spraying on weeds (Omezzine et al., 2011; Sbai et al., 2016; Jelassi et al., 2016). Plant extraction consists of using an appropriate solvent to separate active plant compounds (alkaloids, flavonoids, terpenes, steroids, glycosides and others) from inert or inactive material (Abubakar & Haque, 2020). The type of solvent used depends on factors such as the type of plant material to be extracted, bioactive compounds nature, and the solvent availability. The most popular ones are water, methanol, ethanol, hexane, and dichloromethane (Abubakar & Haque, 2020). Water is a recurrent polar solvent due to its capacity of dissolving a wide range of substances, being cheap and non-toxic (Das et al., 2010). Aqueous plant extracts have been exhibiting good inhibitory activity on root and shoot growth of seedlings and also on seed germination (Imatomi et al., 2015; Kapoor et al., 2019). The higher the concentration of these extracts the more visible and intense is the effect (Radhakrishnanv et al., 2018; El-Wakeel et al., 2019; Chen et al., 2020).

3.1.1.2 Agri-food and forest waste as a potential source of bioactive compounds

Another interesting source of biocompounds that may act as biopesticides to pest control is agri-food waste (Santana-Méridas et al., 2012; Vardanega et al., 2015; Balasubramanian & Tyagi, 2017). Food waste, as defined on the Special Report - Combating Food Waste: an opportunity for the EU to improve the resource-efficiency of the food supply chain from the European Court of Auditors, “refers to any product or part of a product grown, caught or processed for human consumption that could have been eaten if handled or stored differently” (Storup et al., 2016). Agricultural waste refers to unwanted material resulting from agricultural activities such as manure, oil, silage plastics, fertilizer, farms, veterinary medicines, or horticultural plastics (Ramírez-García et al., 2019). As we know, agricultural and food waste, by-products, and co-products (stems, leaves, seeds, shells, pomace, bran, food that does not meet the quality standards) are produced throughout the entire agri-food sector, amounting on more than 250 MT/year (Panouillé et al., 2007; Fava et al., 2015). In the EU alone, 129 million tons of food is wasted per year (nearly one fifth of food production for human consumption) (Europen Commission, 2020a). This represents a negative impact both on the economy and on the environment (Vardanega et al., 2015). Therefore, the valorisation of these products contributes to a circular economy while reducing waste.

Bioactive compounds can be extracted from this waste while taking into account parameters such as solvent, time, temperature, mode of stirring and others (Santana-Méridas et al., 2012). When it comes to compounds with biopesticidal activity, there are a few studies addressing this subject. Did et al. (2011) reported the use of citrus peels to obtain limonene or essential oils to control mosquitoes. Moiteiro et al. (2006) studied the insecticidal and phytotoxic potential of friedelane triterpenes from cork processing waste, among others. Yamane et al. (2014) found that spent coffee grounds may have a short duration weed control capacity and Hardgrove & Livesley (2016) reported that horticultural plants grew poorly in response to spent coffee grounds, regardless of soil type and fertiliser addition. It has frequently been stated that spent coffee grounds have naturally toxic properties (Leifa et al., 2000; Mussatto et al., 2011; Pujol et al., 2013) and may interfere with plant growth via (1) biological N immobilisation and (2) phytotoxicity (Hardgrove & Livesley, 2016).

Waste is also produced when preventing, controlling, and eradicating aggressive invasive plants (van Wilgen et al., 2016). Recently, an idea emerged consisting of finding

potential uses for plant waste resulting from management actions as a way to partially recover invested funds and promote a sustainable strategy aligned with the principles of circular bio-economy (Schmidt et al., 2012; Brito et al., 2013; Brito et al., 2015). *Acacia dealbata* Link is one of the invasive species being managed worldwide (Richardson & Rejmanek, 2011; Lorenzo & Rodríguez-Echeverría, 2015). Some reports have shown that this species produces and releases allelopathic and/or phytotoxic molecules that interfere with the normal functioning of plants and soil microorganisms (Lorenzo et al., 2008; Lorenzo & Rodríguez-Echeverría, 2015; Aguilera et al., 2015a; Aguilera et al., 2015b). Its bark, the most common waste, is an abundant source of chemicals, and its extract may be studied as a source of natural herbicides in agriculture (Narwal, 2010; de Albuquerque et al., 2011; Lorenzo et al., 2016). *Oxalis pes-caprae* L. is another species that behaves as an invader in the Euro-Mediterranean area, thus requiring management actions which produce waste (Chawdhry & Sagar, 1974; Paspatis, 1985; Papini et al., 2017). However, this species also has a high potential to be the base of a natural herbicide, given that, several studies have already identified phytotoxic compounds and allelopathic capacity linked to weed management (Travlos et al., 2008; DellaGreca et al., 2009; Marisa et al., 2018).

3.1.1.3. Pros and cons of bioherbicides

One of the perks of studying secondary metabolites of living organisms is that it is more likely they show biological activity at lower concentration than chemical compounds synthesized in labs (Nascimento et al., 2000). This is because they result from an evolution of responses to biotic interactions (Duke et al., 2000). Natural compounds are usually water-soluble and non-halogenated molecules, thereby being less environmentally harmful, with usually shorter half-lives than synthetic chemicals (Duke et al., 2000; Soltys et al., 2013).

Moreover, we know there is way more of these bio compounds than we know of and the possibility to find one that has the properties we look for is very attractive (Shoemaker et al., 2005). Of the already discovered metabolites, there are a large number that has not been fully studied mainly on its phytotoxicity (Duke et al., 2000).

The look for new herbicides with different modes of action is also very appealing, given that evolution of resistant species to current herbicides has been increasing (Zimdahl, 2007; Pallett, 2016).

Although they have many positive aspects, we must not forget natural toxins are known to be some of the most powerful toxins in mammals, for instance, oleandrin, oleandrigenin, and other cardiac glycosides found in *Nerium oleander* L. makes it one of the most dangerous plants in the world (Duke et al., 2000; Farkhondeh et al., 2020). Many secondary metabolites also have a very complex structure, such as multiple chiral centres, which makes economical production more difficult and expensive (Duke et al., 2000). In addition, there are all the bureaucratic processes to create legislation that permit implementation of bio-based products (Bordin et al., 2020).

3.1.1.4. The present and the future

Nowadays, no more than 8 % of conventional herbicides derived from natural compounds (Dayan & Duke, 2014). On a global scale, there are only 13 natural herbicides in the market based on products extracted from microorganisms and other living organisms. Only a few of them contain active substances extracted from plants:

a) Beloukha® is obtained from rapeseed oil through a natural extraction process (Cordeau et al., 2016). It's active substance is the pelargonic acid and it incites plant cells dehydration (Godard et al, 2016). This herbicide is authorized in Portugal (DGAV, 2019).
b) BioWeed™ and BioSeed™ based on pine oil, works by destroying the outer coating of the plant and seed material when it contacts the plant material, causing cell collapse and desiccation (James et al., 2002).
c) Katoun Gold® has pelargonic acid extracted from sunflower as its base. It causes permeability disruption on the plant's cellular membranes. This product is intended for use on green spaces as: parks, paths, public gardens, sidewalks (Cordeau et al., 2016).

However, new biocompounds from plants with a promising bioherbicidal activity have been identified in recent studies. For instance, compounds from *Cynara cardunculus* L. var. *altilis* (DC*)* leaf extracts appear to have influence on species such as *Amaranthus retroflexus* L., *Portulaca oleracea* L., *Stellaria media* (L.) Vill and *Anagallis arvensis* L. (Scavo et al., 2020).

The first biopesticide appeared on the market in 1980 and, since then, there has been a raise, but only 10 % of that increase refers to natural herbicides (Charudattan, 2001). Recently, biopesticides comprised just a small share (5–6 %) of the total global

crop protection market (Dunham & Trimmer, 2018). Nevertheless, the growth prospects of the bioherbicides market are really attractive and suggestive, since in 2020 it represented 1.7 billion dollars and in 2029 it should reach around 6.3 billion dollars (Prophecy Market Insights, 2020). This is a great incentive to obtain funds to finance research projects on this area (Ndolo et al., 2020).

However, some issues still must be addressed. Research has to overcome certain challenges: development and methodological aspects, as synthesis difficulties due to complex structure of natural compounds, poor results in field trials, rapid degradation; certain application and environmental, biotic and abiotic factors, such as microflora, water stress and temperature fluctuations, and mammalian toxicity; and to commercialization issues, like cost effectiveness, and the competition that chemical compounds still represent (Li et al., 2019; Bordin et al., 2020).

4. Aim of the study

Taking into consideration that urban areas are the most suitable places to enhance the contact between population and chemical products, that the market lacks alternatives for more sustainable and environmentally friendly herbicides, the present work intended to explore the potential herbicidal activity of plant extracts and agri-food waste to valorise them and to promote circular economy. The specific objective of this study aimed at assessing the potential herbicidal effect of three aqueous extracts: bark and aboveground biomass from *Acacia dealbata* and from *Oxalis pes-caprae* management actions, respectively, and spent coffee grounds on germination, seedling growth and well-stablished plants of urban weeds. The selection of these extracts was based on previous phytotoxic results (Travlos et al., 2008; Cruz & Marques dos Santos Cordovil, 2015; Lorenzo et al., 2019).

II. MATERIALS AND METHODS

1. Weed seeds collection and preliminary germination tests

Seeds of different weed species were collected from pavements, particularly on sidewalks, parking spots, roadsides, and separators in iParque, a science and technology park located in Antanhol, Coimbra (40°10'46.1"N 8°27'58.5"W). Weed species were subsequently identified by a plant specialist of the Botanical Garden of the University of Coimbra. Most of them belonged to the Asteraceae family. Then, four weeds that showed the highest number of available seeds at visit time in iParque. were chosen to be included in this study: *Conyza canadensis* (L.) Cronquist (horseweed)*, Achillea ageratum* L. (sweet yarrow)*, Dittrichia viscosa* (L.) Greuter (false yellowhead) and *Cortaderia selloana (*Schult. & Schult.f.) Asch. & Graebn*.* (pampas grass).

After that, preliminary germination tests were conducted to evaluate the germination process of those species, as following described. For each plant species, two Petri dishes (9 cm diameter) were prepared by covering their bottom with filter paper which was later moistened with 2.5 mL of distilled water. Twenty-five seeds of each species were homogeneously distributed on the filter paper, and after being sealed with parafilm, to prevent evaporation, Petri dishes were randomly placed for incubation at 22/18 ºC and 12 /12 h photoperiod, for ten days. The number of germinated seeds were recorded. The species with the highest germination record and better performance, *Achillea ageratum* (86 %), *Conyza canadensis* (74 %) and *Dittrichia viscosa* (22 %), were selected to conduct subsequent herbicidal experiments.

2. Extracts preparation

Acacia dealbata bark was collected in Polo II, University of Coimbra (40°11'10.6"N 8°24'45.9"W) in the summer of 2019, dried at room temperature and cut into 4-7 cm portions. *Oxalis pes-caprae* fresh flowers, leaves and stems were collected in the Botanical Garden of the University of Coimbra on the 11th of March of 2020 and immediately used. Spent coffee grounds were collected in local coffees, while still damp, one or two days before the beginning of the experiments.

Each extract was prepared with a proportion of 40 g of plant material/waste added to 100 mL of distilled water, to obtain the 40 % concentration of the extract (Fig.1A). After 24 hours, at room temperature, the extract was filtered through a filter paper, Whatman

nº 1®, to Schott glass bottles and kept frozen in 500 mL aliquots, at -18 ºC pending use (Lorenzo et al., 2011).

Extracts were assayed at 40, 20 and 10 % concentrations, using distilled water (0 %) and the herbicide Podium (recommended dose: 2.5 mL product / 200 mL distilled water, 5 L ha^{-1}) as negative and positive controls, respectively. At the beginning of the experiments, defrosted 40 % extracts were diluted with distilled water to obtain extracts with 20 and 10 % concentrations (Fig. 1). Extracts pH were measured for each extract (Table 1.).

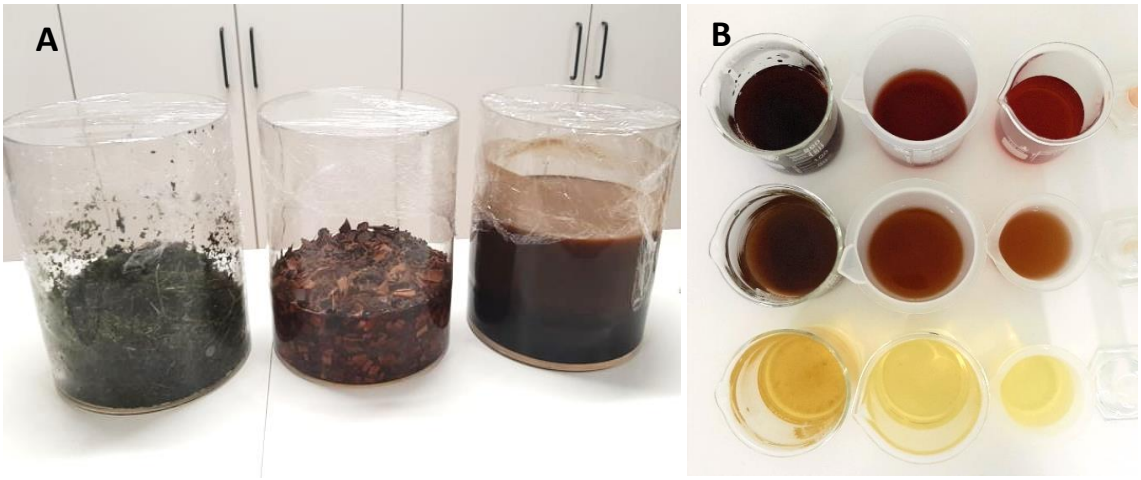

Figure 1. A) Extracts preparation to obtain the 40 % concentration. B) Aspect of the dilutions of the studied extracts. From top to bottom and left to right: *Acacia dealbata* bark extract at 40, 20 and 10 %, Spent coffee grounds extract at 40, 20 and 10 %, and *Oxalis pes-caprae* extract at 40, 20 and 10 %.

Table 1. Values of pH of different concentrations for each studied extract.

Treatment	**Concentration (%)**	**pH**
Spent coffee grounds extract	10	6.43
	20	6.35
	40	6.33
Acacia dealbata dried bark extract	10	5.53
	20	5.45
	40	5.43
Oxalis pes-caprae fresh extract	10	3.21
	20	3.03
	40	2.88
Distilled water	0	5.88

3. Experiment 1: Preemergence effect of extracts - *in vitro* assays

In order to study the effect of the three extracts, *Acacia dealbata* bark, *Oxalis pes-caprae* flowers, leaves and stem, spent coffee grounds, at three concentrations, 40, 20, and 10 %, on germination and early growth of the three urban weeds, *Achillea ageratum*, *Conyza canadensis,* and *Dittrichia viscosa*, an *in vitro* bioassay was set up, using two substrates, Whatman nº 1 filter paper and field soil. To compare the results, distilled water and the herbicide were used as negative and positive controls, respectively. There were five replicates for each extract dilution, as well as for controls, for the two substrates.

For each plant species, Petri dishes (9 cm diameter) were prepared by covering their bottom with one filter paper which was later moistened with 4 mL of extract/distilled water/herbicide. Twenty-five seeds of each species were homogeneous distributed on the filter paper, and after being sealed with parafilm, to prevent evaporation, Petri dishes were randomly placed in a growth chamber, at 26 ºC, with 12/12 h (light/dark), for nine, twelve, and fourteen days, for *A. ageratum*, *D. viscosa*, and *C. canadensis,* respectively, when cotyledons reached the lid (Fig.2A & B). After that, dishes were stored at -20ºC to stop the growth of seedlings during the measurement process. Then, the number of germinated seeds in each dish was recorded and the root and stem lengths for each seedling were determined (Fig. 2C).

A similar experiment was conducted using field soil from the iParque as substrate, instead of filter paper. The only different conditions were that Petri dishes (9 cm diameter) were filled with 20 mL of field soil and were later moistened with 5 mL of extract/distilled water/herbicide (Fig. 2).

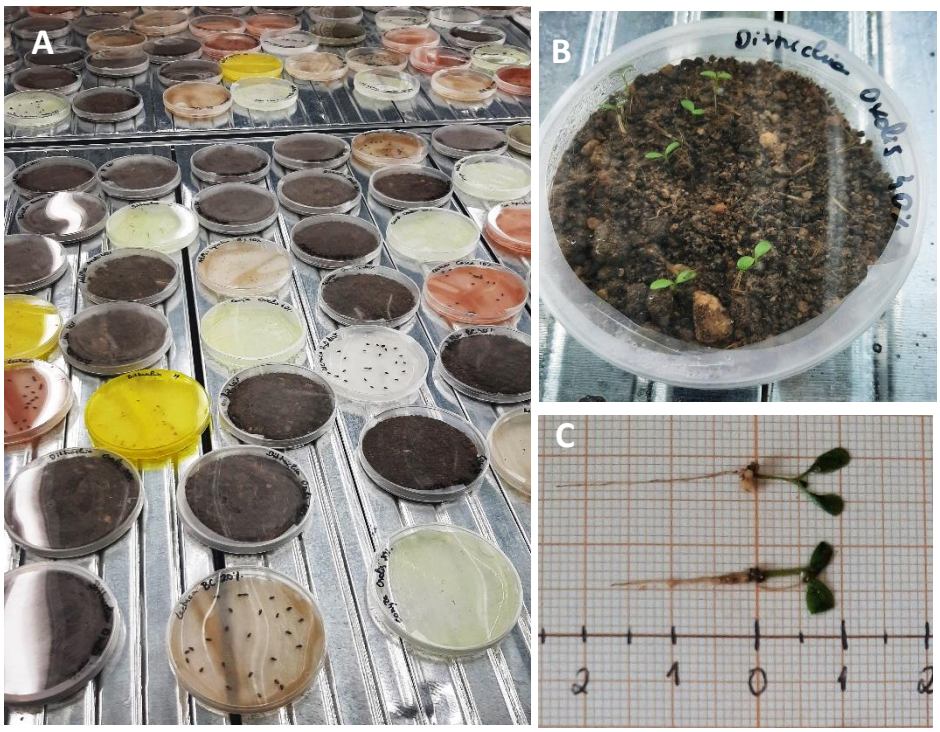

Figure 2. A) Petri dishes with the different treatments randomly placed in the growth chamber; B) Grown seedlings of *Dittrichia viscosa* in soil treated with *Oxalis pes-caprae* extract at 40 %; C) Measurement of two *Dittrichia viscosa* seedlings.

4. Experiment 2: Postemergence effect of extracts

4.1. Pot assays

A pot bioassay was also conducted to test the effect of the same extracts (*Acacia delabata* bark, *Oxalis pes-caprae* flowers, leaves and stem, and spent coffee grounds) at three concentrations (40, 20, and 10 %, plus controls) on the growth of well-established *A. ageratum*, *C. canadensis* and *D. viscosa* plants grown in field soil, under natural photoperiod in greenhouse conditions. For that, on the 9th of September of 2020, 120 mL pots were filled with field soil collected in iParque and ten seeds of *A. ageratum, C. canadensis* and *D. viscosa* were immediately sown in each pot. Then, they were watered with 50 mL of tap water to achieve soil saturation and were allowed to germinate and grow for eight weeks. During the growing period, pots were irrigated with around 3 mL of tap water, three times a week the hottest weeks, and once a week the coldest ones. Pots were also randomly rearranged once a week. On the 21st of October, one or two plants in highly - germinated pots were carefully removed, avoiding root damages, and transplanted into non-germinated pots assigned to the same species, allowing them to acclimate for two weeks. On the 6th of November, plants were well-established for the three weed species. Then, plants were thinned to obtain one per pot, attempting to maintain a homogeneous size (Fig. 3A). On the 8th of November, plants

were sprayed with 2.5 mL of respective extract or control, turning 90º each pot to cover every side of the plant (Fig. 3B). Before applying treatments, the number of leaves and maximum leaf length in each plant were recorded.

Extracts of the three concentrations, as well as controls were randomly assigned to plants and replicated ten times.

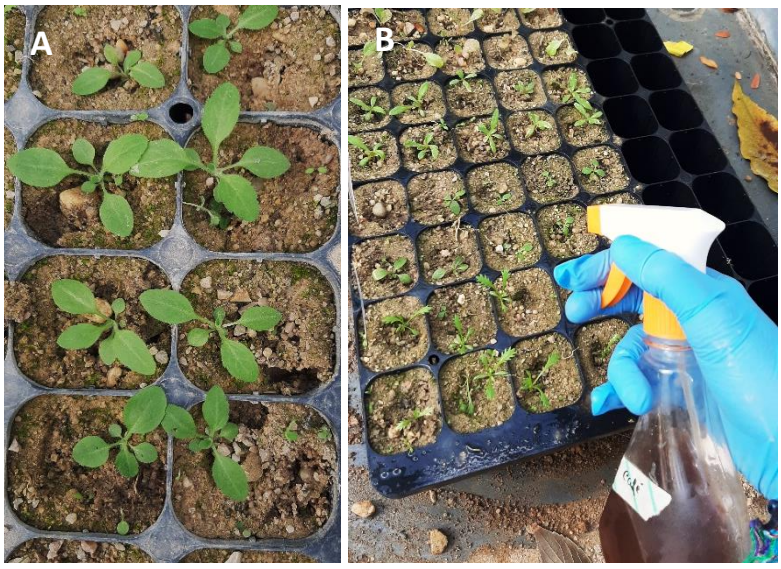

Figure 3. A) *Conyza canadensis* plants after thinning process. B) Spent coffee grounds extract application.

Two weeks and a half after treatments application, on the 26th of November, the assay was finished, as some leaf chlorosis and dead leaves in plants were observed (Fig. 4A). Number of dead plants was counted and the leaf number and maximum foliar length in each plant were also recorded (Fig. 4B). Then, the aboveground biomass was collected and dried at 50 ºC, for two weeks, to determine the dried weight for each plant (Fig. 4C & 4D).

4.2. Quantification of pigments

To determine if the extracts have any effect on the pigments' concentration present in the leaves of the weeds, the collected fresh leaves, were weighted and then macerated in the cold with TrisM and acetone (80 %) buffer with a 1:4 proportion (Fig.4E). The macerated was collected to Eppendorf microtubes, which were placed in the dark and on ice. Absorbances of the aliquots at 663, 537, 647 and 470 nm were registered, using the EnSpire® Multimode Plate Reader® (Fig.4F).

Chlorophyll a (**Chla)** and b (**Chlb**), anthocyanins and carotenoids concentrations (µmol of pigment/g of leaf fresh weight), were calculated with the following equations according to Sims & Gamon (2002) protocol:

$$\textbf{Chla} = \frac{(0.01373\ A663 - 0.000897\ A537 - 0.003046\ A647) \times extration\ volume}{sample\ weight}$$

$$\textbf{Chlb} = \frac{(0.02405\ A647 - 0.004305\ A537 - 0.005507\ A663) \times extration\ volume}{sample\ weight}$$

$$\textbf{Anthocyanins} = \frac{(0.08173A537 - 0.00697A647 - 0.002228A663) \times extration\ volume}{sample\ weight}$$

$$\textbf{Carotenoids} = \frac{(((\ A470 - (17{,}1\ x\ (Chla + Chlb) - 9.479\ x\ anthocyanins))\ /\ 119.26) \times extration\ volume}{sample\ weight}$$

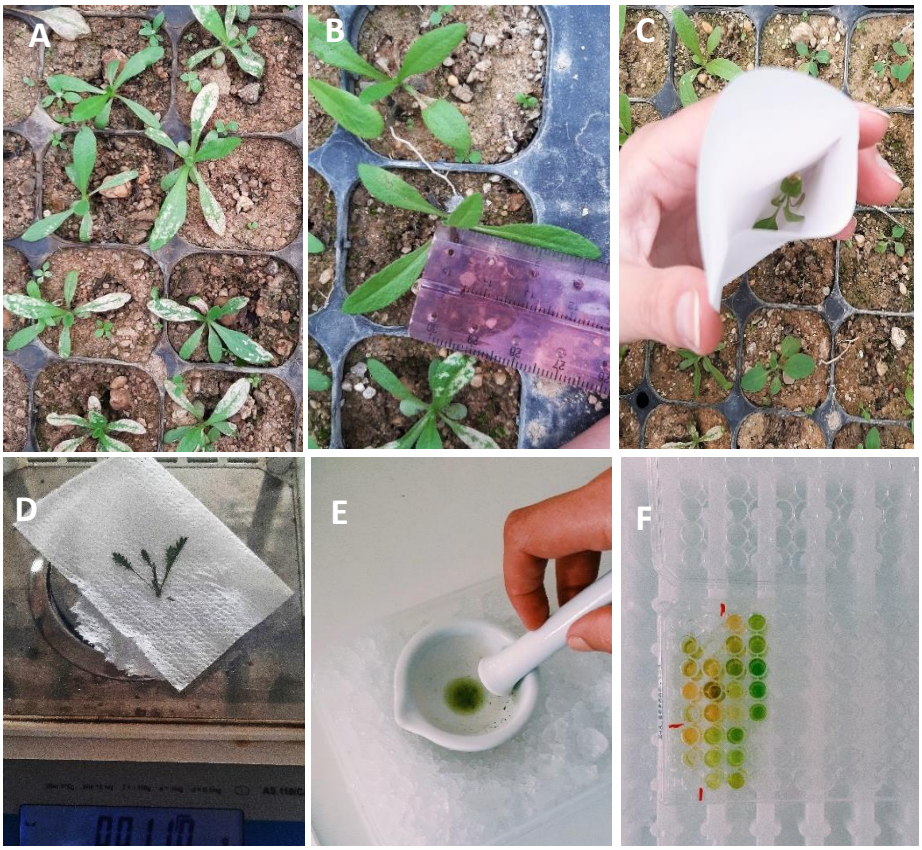

Figure 4. A) Chlorosis on *Dittrichia viscosa* plants; B) Measuring maximum foliar length of a *Dittrichia viscosa* plant; C) Plant collection to dry and freeze; D) Weighting an *Achillea ageratum* dried plant; E) Cold pigment extraction; F) Plate preparation with samples to absorbance reading.

5. Data analysis

In the preemergence assay, the effect of extracts (*Acacia dealbata* bark, fresh *Oxalis pes-caprae*, spent coffee grounds), concentrations (40, 20, and 10 % plus the controls distilled water, and herbicide) and substrate (filter paper and soil) on germination and seedling growth, was separately evaluated for each weed species. Firstly, a

statistical analyses to determine the effect of substrate on germination (Generalized Linear models, *glm()* function from the 'stats'; poisson error and link log) and seedling parameters (Linear Mixed Models, *lmn()* function from the 'nlme' package; Petri dish as random factor) was conducted. When substrate had a significant effect, statistical analyses were separately conducted for each substrate. Two-way Generalized Linear models (GLMs, *glm()* function from the 'stats' package) with poisson error and log link were used to test the effect of treatment, concentration and the interaction between these two factors on the number of germinated seeds for each substrate for the three weed species. We conducted the two-way Linear Mixed Models (LMMs, *lmn()* function from the 'nlme' package) with Petri dish as random factor to assess the effect of treatment, concentration, and the interaction between them on radicle and stem length of germinated seedlings for each substrate in all the weed species, except for the radicle length of *A. ageratum*.

For the postemergence assay, data were also separately analysed for each weed species. The effect of treatment, concentration, and the treatment x concentration interaction on the number of dead plants and leaf number increase was analysed using two-way GLMs using binomial error and cloglog link or using quasipoisson error and log link with the number of leaves before treatments application (NF0) as a covariate, respectively. We conducted two-way Liner Models (LMs, *lm()* function from the 'stats' package) to test the effect of treatment, concentration, and the interaction between these two factors on the foliar length increase using the maximum foliar length before the treatment application (CMF0) as a covariate. Finally, two-way GLMs with Gamma error and identity link were used to test the effect of treatment, concentration, and the treatment x concentration interaction on dried biomass using the leaf number increase INF) and foliar length increase (ICF) as covariates. When running the analyses, we detected inconsistencies between our results and the post-hoc outcomes. Thus, when covariables did not show significant differences, we removed them from the analysis: from the influence on dry biomass, the INF covariate on *Conyza canadensis* and the ICF covariate on *Dittrichia viscosa*.

For the analyses of pigment concentrations, for each weed species, we run a two-way Linear Model (LM) for Chlorophyll a, Chlorophyll b, and Carotenoids' concentration to test for the effect of treatment, concentration, and the interaction between them. The exception was the carotenoid content of *Conyza canadensis*, where we performed a two-way GLM with Gamma error and identity link to test for the effect of the same factors.

Statistical differences between groups were analysed using the *lsmeans()* and *cld()* functions from the 'lsmeans' and 'multcomp' packages by comparing the least-squares means obtained within each model. The level of significance was set at P≤0.05 for all the analyses. All statistical analyses were conducted in R (R Development Core Team 2015).

III. RESULTS

1. Preemergence effect of extracts - *in vitro* assays

The results obtained in the preemergence assay show that the substrate had a significant effect on germination, stem length and radicle length of *C*. *canadensis* (P<0.001, P<0.001, and P<0.001) and also of *D*. *viscosa* (P<0.001, P<0.001 and P<0.001) and germination and stem length of *A*. *ageratum* (P<0.001 and P<0.001), respectively, but not on the *A*. *ageratum* radicle length (P=0.08).

The effect of extracts

When the substrate was soil, the number of germinated seeds did not vary among extracts for any weed species (APPENDIX – TABLE 1, Fig. 5A). However, extracts significantly affected germination in filter paper as substrate (APPENDIX – Table1). In fact, germination was lower in the *Oxalis pes-caprae* extract for *A. ageratum* compared to the other two extracts (Fig. 5B). In addition, for C. *canadensis*, O. *pes-caprae* and *A. dealbata* bark extracts showed a 43.9 % and 40.2 % seed germination reduction, respectively, when compared to the spent coffee grounds extract (Fig.5B).

The extracts also had a significant effect on the radicle length in both soil and paper substrates, for all weed species (APPENDIX – TABLE 1). In *A. ageratum*, the radicle growth was lower in the *Oxalis pes-caprae* extract compared to the other two extracts, regardless the type of substrate (Fig. 6A). For the remaining species in soil, radicle length was significantly lower when treated with the spent coffee grounds extract (Fig. 6B). However, an opposite tendency was generally observed for this parameter when seedlings were grown in paper (Fig. 6C).

Regarding stem length, this parameter was affected by extracts in soil and paper, except for *D. viscosa* in paper (APPENDIX – TABLE 1, Figs. 7A, 7B). In soil, stem length was lower for *A. ageratum* seedlings treated with spent coffee grounds and with *O. pes-caprae* extracts, for *C. canadensis* seedlings grown in presence of *A. dealbata* bark and *O. pes-caprae* extracts, and for *D. viscosa* seedlings treated with the *A. dealbata* bark extract (Fig. 7A). However, in paper, the lowest stem length was found in the *O. pes-caprae* extract for *A. ageratum* and *C. canadensis* (Fig. 7B).

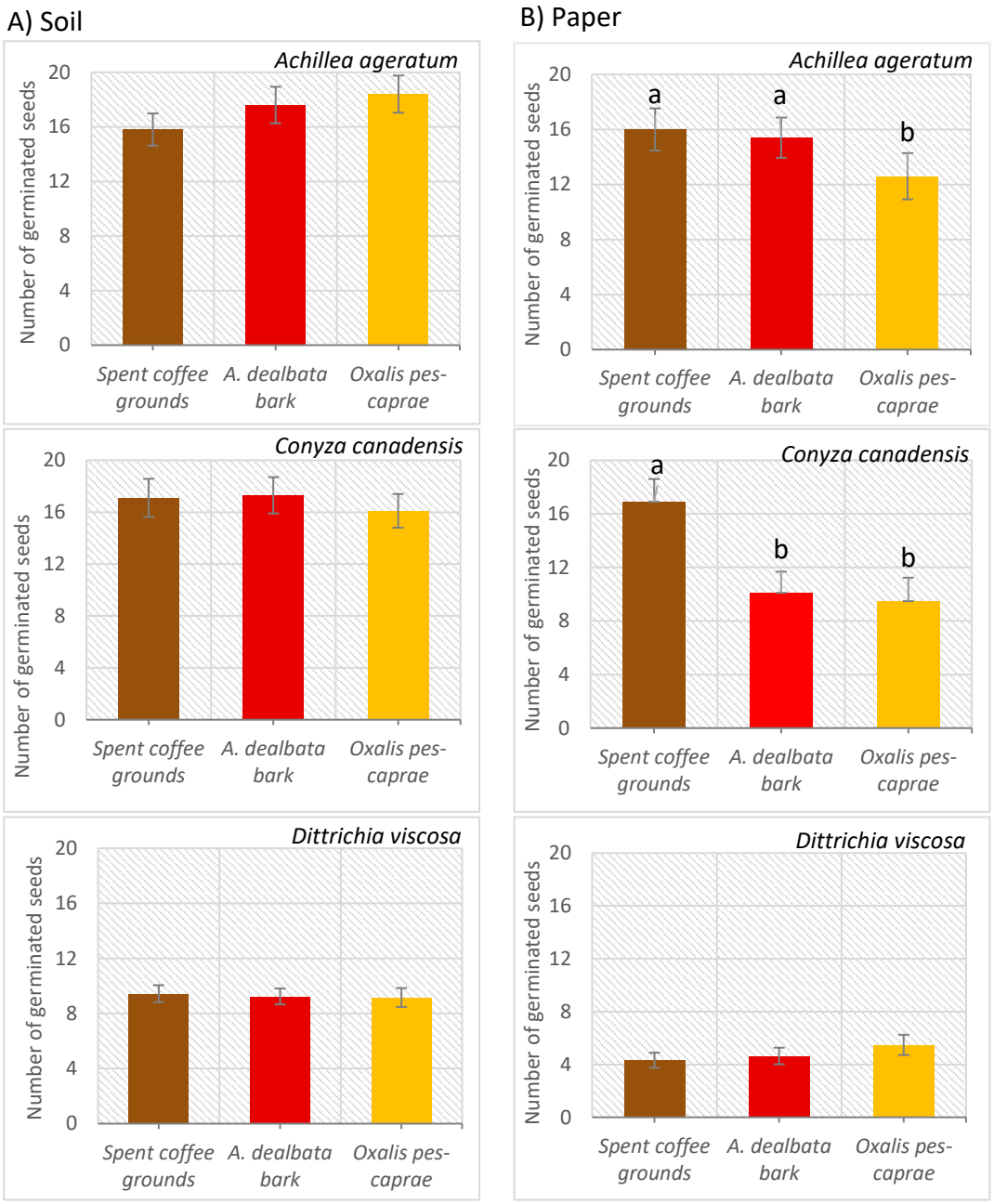

Figure 5. Effect of the extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) on seed germination of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* grown in soil (A) and paper (B). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear models).

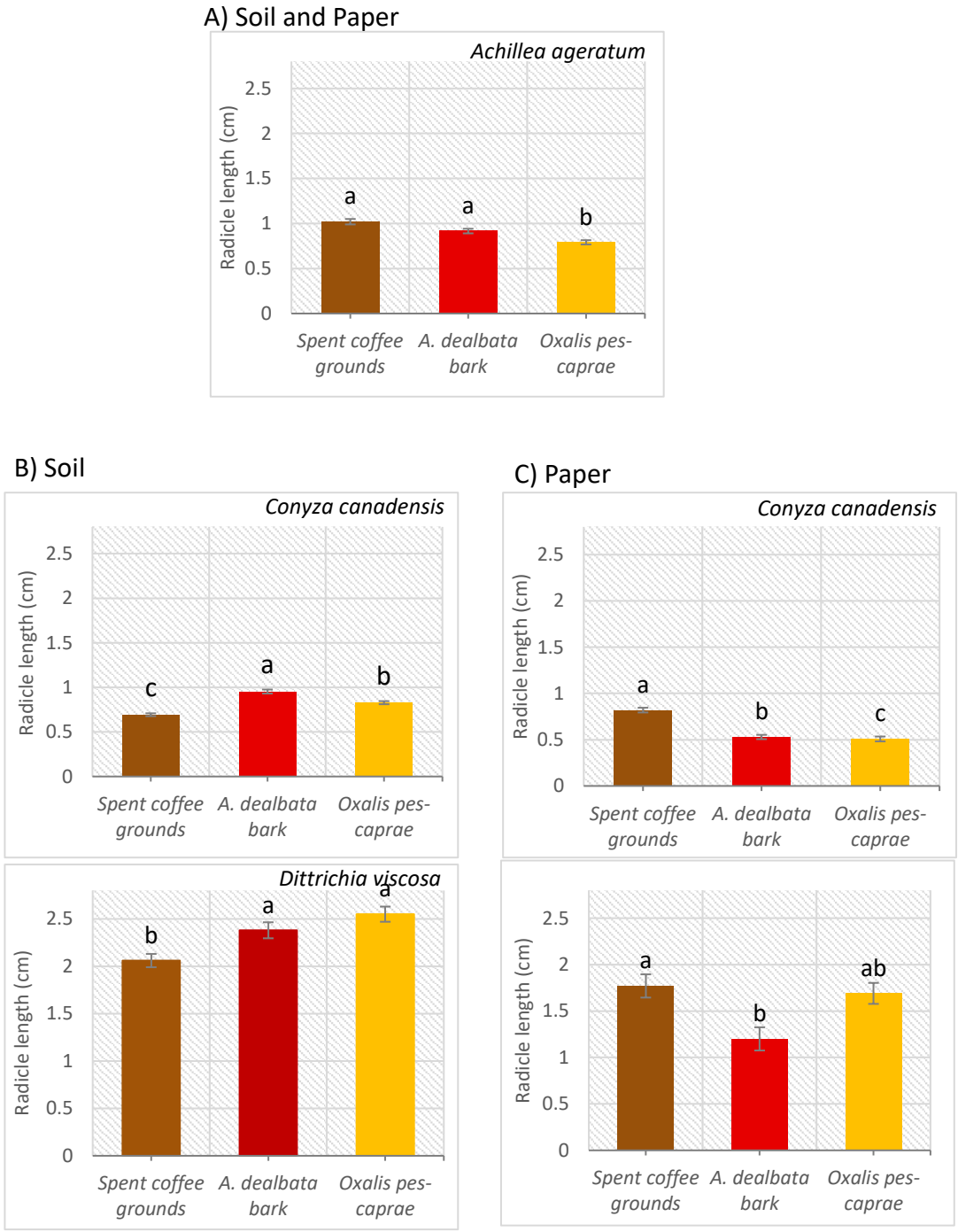

Figure 6. Effect of the extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) on the radicle length of *Achillea ageratum* seedlings in soil and paper (A) on the radicle length of *Conyza canadensis* and *Dittrichia viscosa* seedlings grown in soil (B) and paper (C). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Linear Mixed Models).

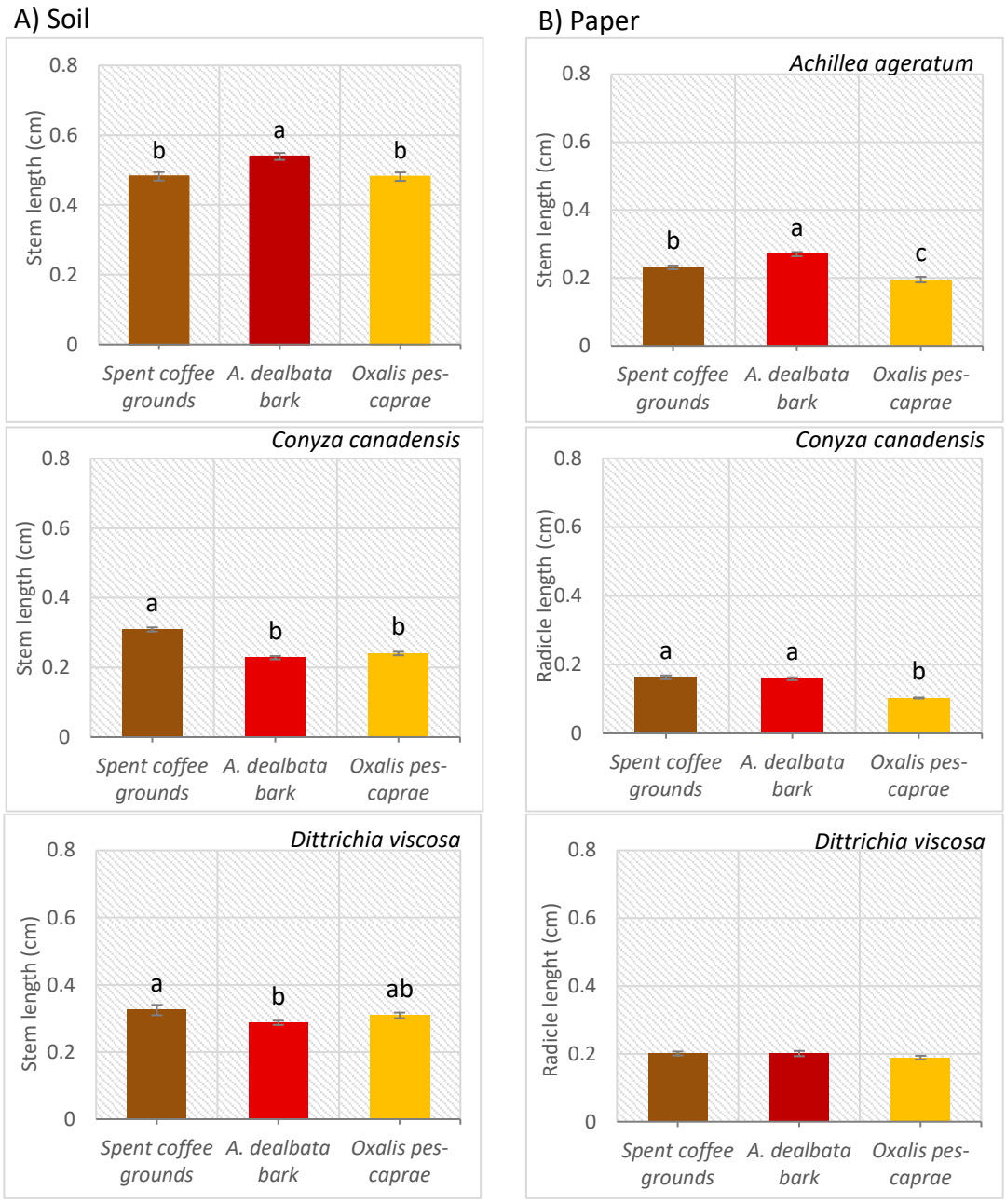

Figure 7. Effect of the extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) on the stem length of *Achillea ageratum*, *Conyza canadensis* and *Dittrichia viscosa* seedlings grown in soil (A) and paper (B). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Linear Mixed Models).

The effect of concentrations

The extracts concentration had a significant effect on seeds germination of all weed species, in both soil and paper substrates (APPENDIX – TABLE 1). In soil, only the herbicide application (the positive control) reduced seed germination comparing with distilled water (negative control) (Fig. 8A). In paper, germination seems more affected with concentration increase: the 40 % concentration reduced this parameter by 45.5 % in *A. ageratum*, 59.1 % in *C. canadensis* and 70.1 % in *D. viscosa*, having similar effect to the herbicide in the last two species (Fig. 8B). Additionally, extracts at 20 % also reduced germination of *C. canadensis* seeds (Fig. 8B).

Extracts' concentration affected radicle length of all species in both substrates (APPENDIX – TABLE 1), being herbicide the most effective treatment (Fig. 9). The radicle length of *A. ageratum* was reduced at higher concentrations (20 and 40 % with a radicle length reduction of 28.2 and 42.5 %, respectively), but did not reach the herbicide level (88.8 % reduction) regardless the substrate used (Fig. 9A). For this parameter, in soil and compared to distilled water, there was a significant stimulation at 10 % and a reduction at 40 % of *C. canadensis* (Fig.9B). The 40 % concentration reduced the radicle growth of *D. viscosa* (Fig.9B). For the paper substrate, the three concentrations assayed significantly reduced the radicle length compared to the negative control (Fig. 9C). The inhibitory effect was similar among concentrations for *C. canadensis*, but this effect increased with concentration for *D. viscosa*, achieving 86.7 % of reduction at 40 % (Fig. 9C).

For all the weed species, the stem length in soil was more affected by the herbicide and none of the extract concentrations reached its impact. The stem growth was also reduced by the 20 and 40 % concentrations in *A. ageratum*, and by all the concentrations in *C. canadensis* when compared with distilled water (Fig. 10A). This parameter was stimulated in *D. viscosa* seedlings at 10 and 40 % concentrations (Fig. 10A). In paper, we mainly found stimulatory effects, i.e., at 10 % for *A. ageratum,* and at any concentration for *C. canadensis* compared with distilled water (Fig. 10B). An inhibition was found in *D. viscosa* at 40 % (Fig. 10B). None of the effects reached the herbicide efficacy (Fig. 10B).

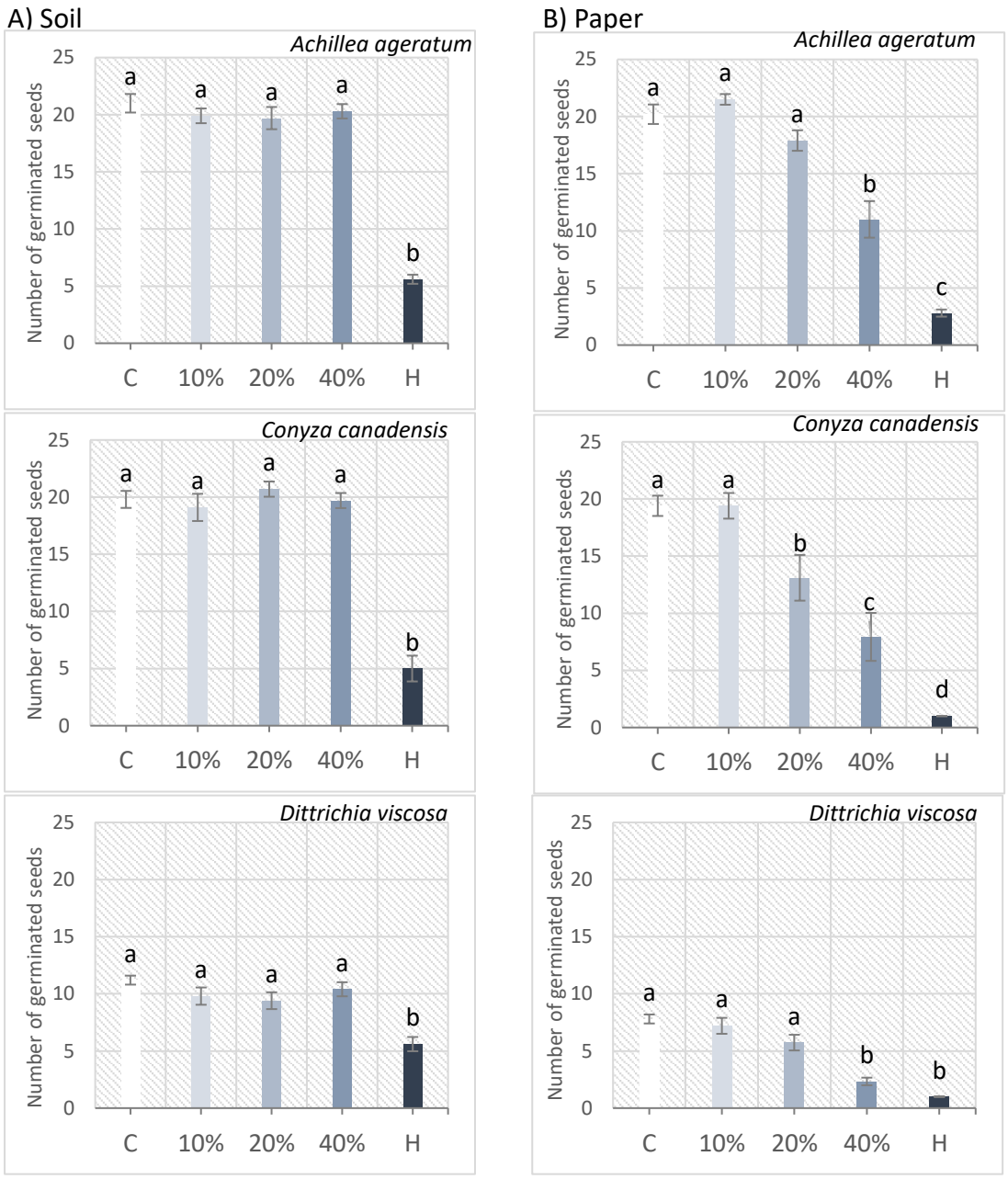

Figure 8. Effect of the concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the germination of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* seeds in soil (A) and paper (B). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

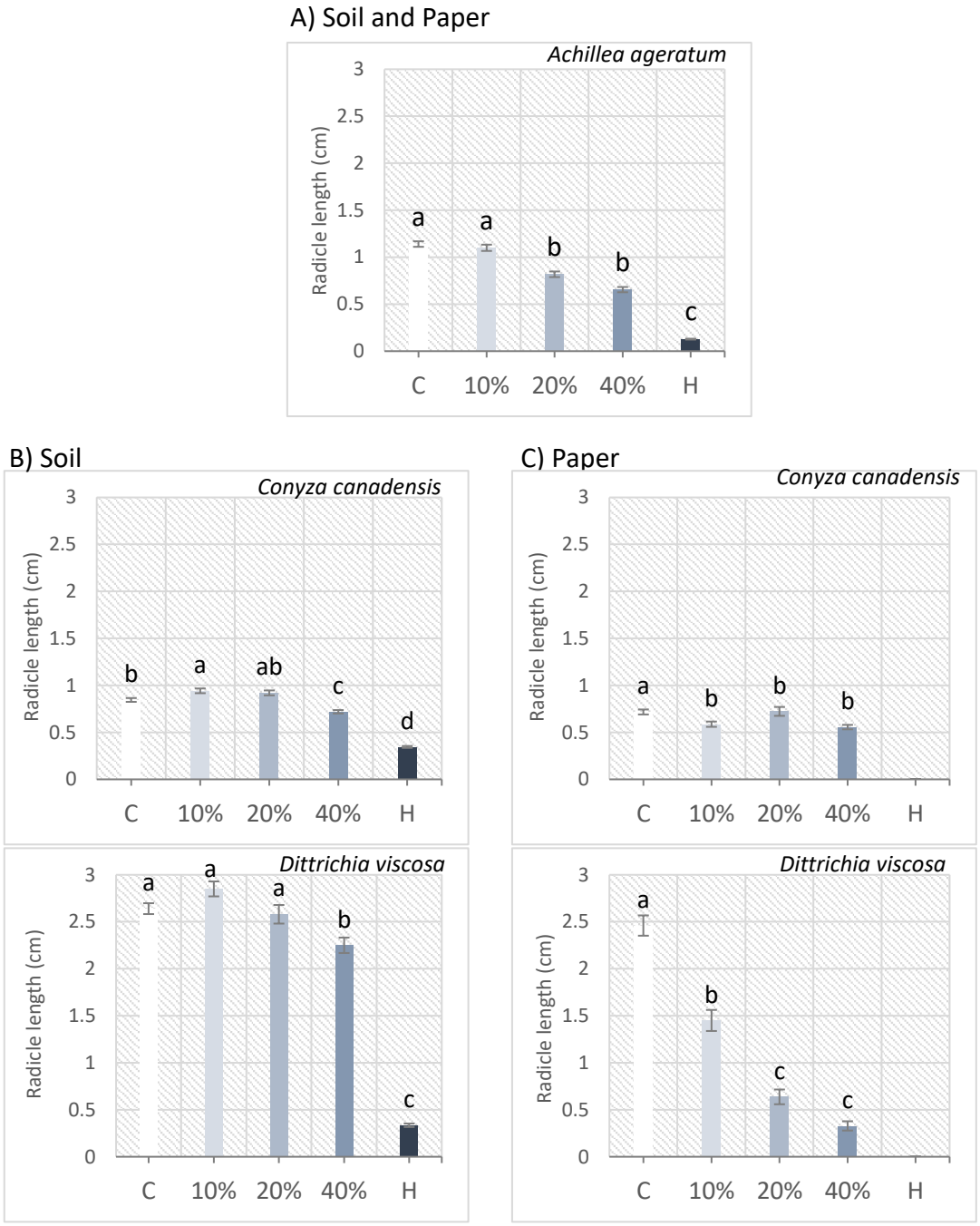

Figure 9. Effect of the concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the radicle length of *Achillea ageratum* seedlings grown in soil and paper (A), on the radicle length of *Conyza canadensis* and *Dittrichia viscosa* seedlings grown in soil (B) and paper (C). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Linear Mixed Models).

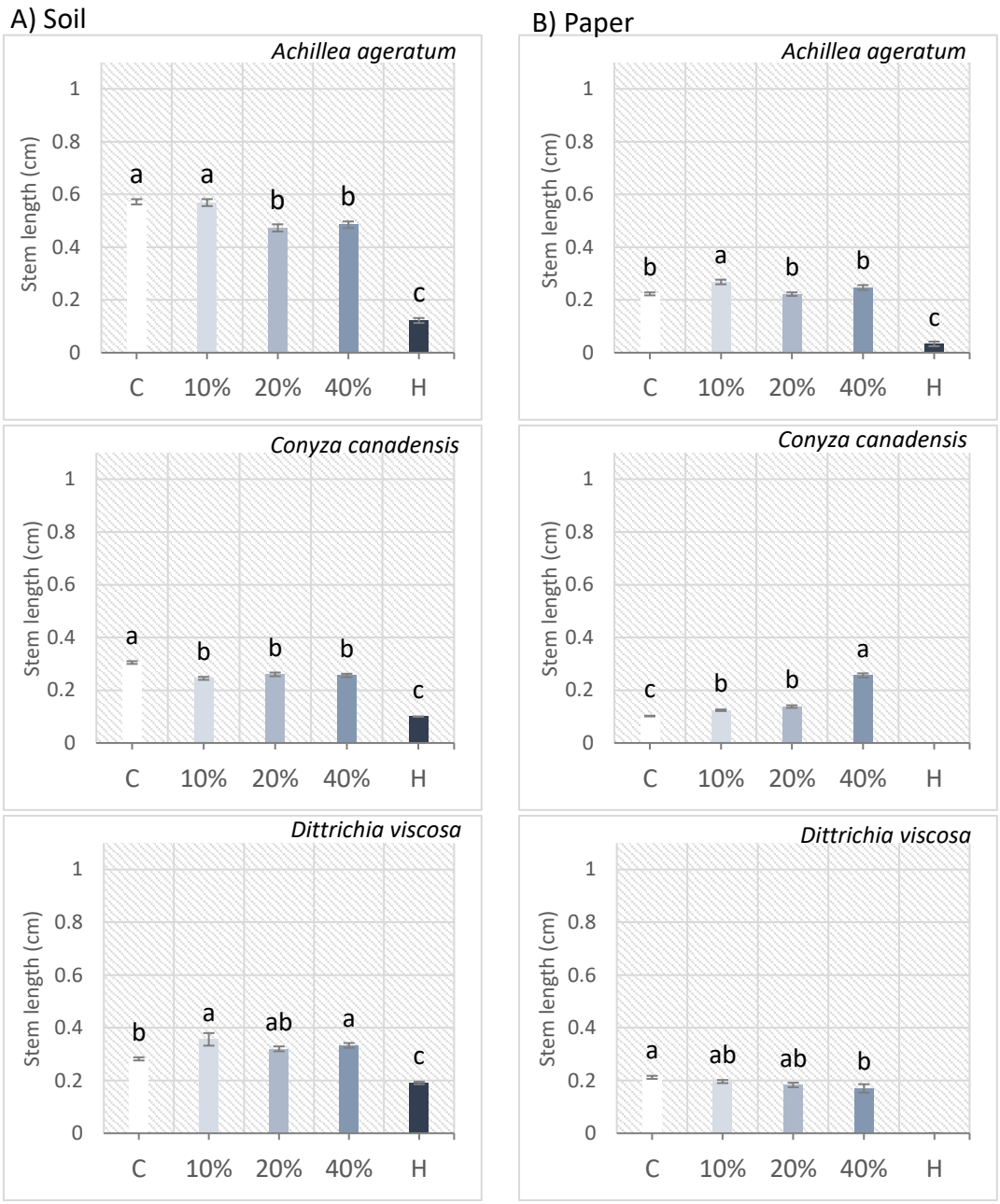

Figure 10. Effect of the concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the stem length of *Achillea ageratum, Conyza canadensis,* and *Dittrichia viscosa* seedlings grown in soil (A) and paper (B). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Linear Mixed Model).

The interaction between the two variables, extract and concentration

The interaction between extract and concentration did not have a significant effect on seed germination in soil, for all the weed species (APPENDIX – TABLE 1). In this

substrate, the number of germinated seeds followed the same trend in any plant species (Fig. 11A). On paper, the extract x concentration interaction significantly affected the germination of *A. ageratum* and *C. canadensis* (APPENDIX – TABLE 1). For *A. ageratum* species, germination was inhibited by the herbicide and by O*. pes-caprae* at 40 %, which had the same inhibiting effect as the herbicide (around 86.1 %) (Fig. 11B). A similar trend was found for *C. canadensis* seeds, herbicide had the highest inhibition (94.8 %) in the three extracts, accompanied by *A. dealbata* bark extract at 40 % (87.6 %) and *O. pes-caprae* extract at 20 and 40 % (76.3 and 87.6 %, respectively) compared to distilled water (Fig. 11B).

The radicle length was significantly affected by the interaction between extracts and concentration in all weed species for both substrates (APPENDIX – TABLE 1). For *A*. *ageratum* regardless the substrate, the radicle growth was stimulated when spent coffee grounds extract was applied at 10 % but inhibited when applied at 40 %. Radicle inhibition was also found when using *A. dealbata* bark at 10 % and *O. pes-caprae* at 20 and 40 % compared to distilled water (Fig. 12A). For the other two weed species the results varied according to extract and concentration, but herbicide always showed the highest inhibition compared with distilled water (Fig.12B, C). In soil, radicle length of *C. canadensis* was inhibited when treated with spent coffee grounds extract at 20 and 40 % but stimulated with *A*. *dealbata* bark at 10 and 20 % (Fig. 12B). For *D. viscosa*, spent coffee grounds and *A. dealbata* bark extracts at 40 % inhibited the growth and *A. dealbata* bark at 10 % showed stimulation (Fig. 12B). In paper, the *C*. *canadensis* radicle length was higher in spent coffee grounds at 10 and 20% than in distilled water, being significantly reduced by the same extract at 40 %, and also by *A. dealbata* bark extract at 10 and 20 % and by the three concentrations of the *O*. *pes-caprae* extract (Fig. 12C). For *D. viscosa*, we observed the previously identified tendency of a higher concentration having a more significant negative effect on the plant development: when using spent coffee grounds and *O. pes-caprae* extracts at 20 and 40 % and *A. dealbata* bark extract at 10, 20, 40 % (Fig.12C).

The interaction between extract and concentration significantly affected the stem length of *A. ageratum* and *C. canadensis* in soil, and of the three weed species in paper (APPENDIX – TABLE 1). In soil, this parameter was reduced in *A. ageratum* when using spent coffee grounds and *O. pes-caprae* extracts at 20 and 40 %, comparing with distilled water (Fig. 13A). In *C. canadensis*, spent coffee grounds extract at 20 % stimulated the stem growth, but it was inhibited by the three concentrations of *A. dealbata* and *O. pes caprae* extracts, although inhibition did not achieve the herbicide efficacy (Fig.

13A). In paper, *A. ageratum* stem growth was stimulated by applying *A. dealbata* bark at 10 and 20 % and inhibited by using *O. pes-caprae* at 20 % compared with distilled water (Fig. 13B). Stem length of *C. canadensis* was stimulated by spent coffee grounds extract at 20 and 40 % and by *A. dealbata* bark at 10, 20 and 40 % (Fig. 13B). Finally, in *D. viscosa*, we observed a stem length reduction when using *O. pes-caprae* at 20 % (Fig. 13B).

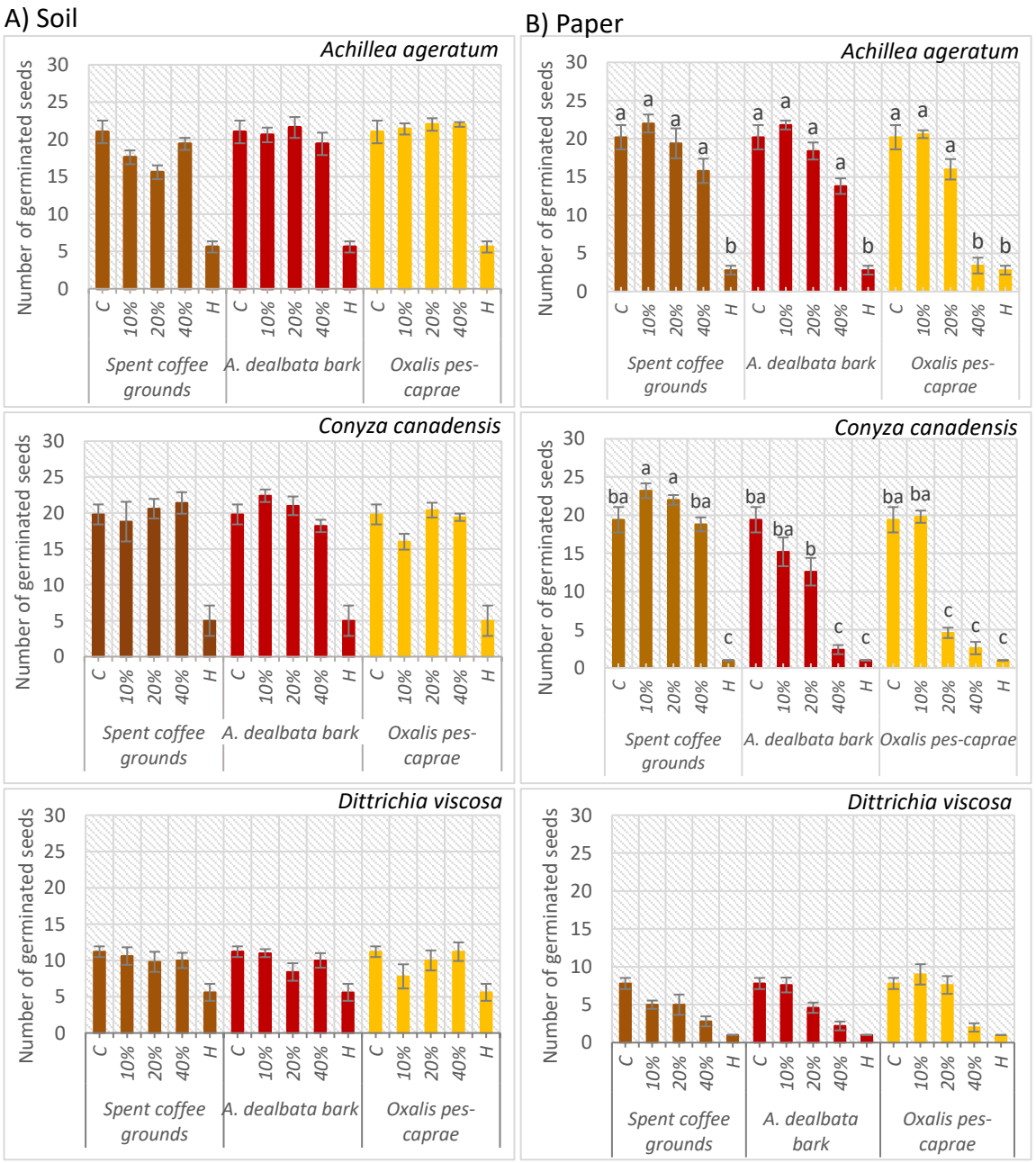

Figure 11. Effect of the interaction between extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) and concentrations (distilled water (c), 10 %, 20 %, 40 % and herbicide (H)) on the germination of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* seeds in soil (A) and paper (B). Mean values±SE are shown. n=25. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

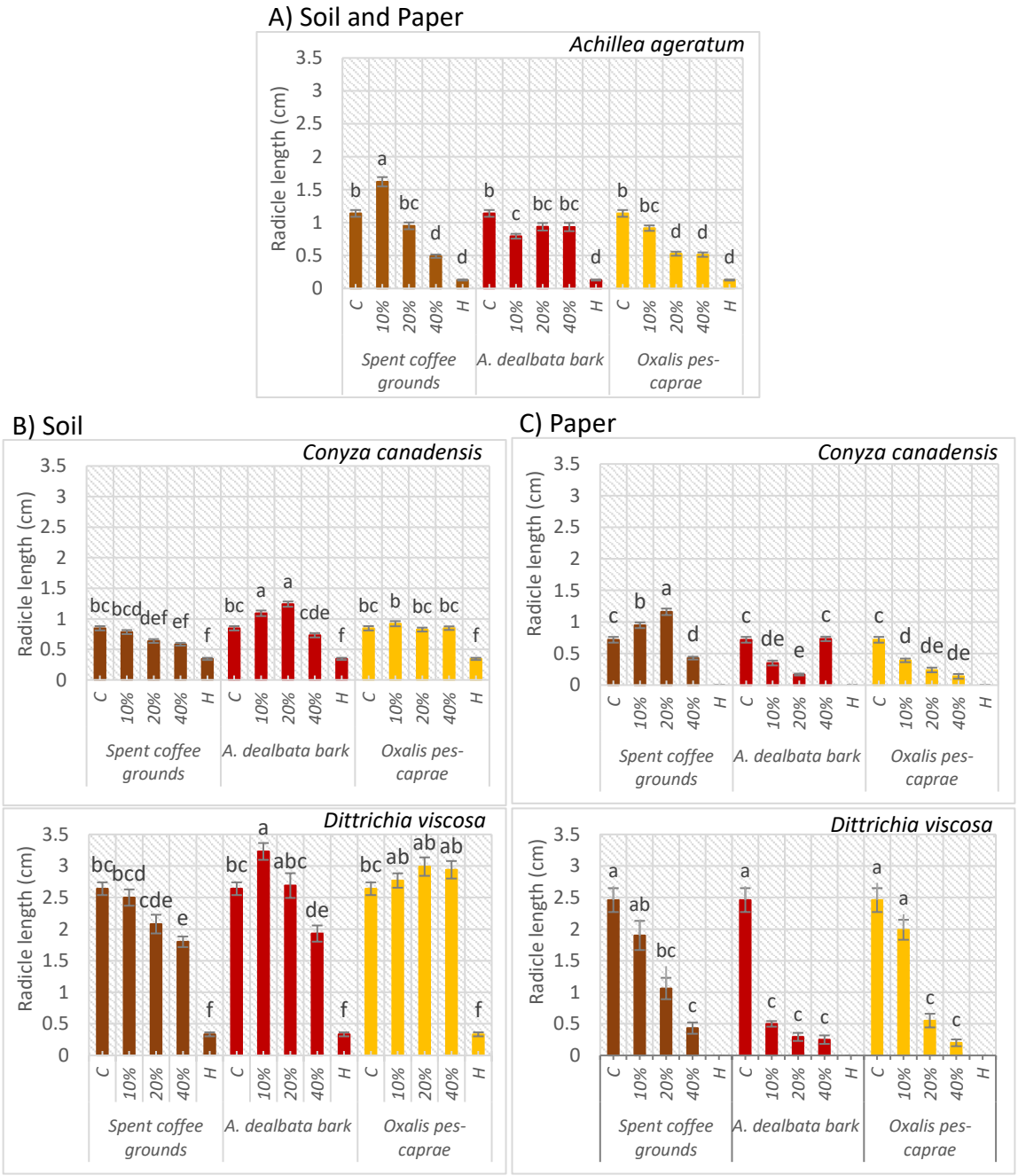

Figure 12. Effect of the interaction between extracts (Spent coffee grounds, *Acacia dealbata bark* and *Oxalis pes-caprae*) and concentrations (distilled water (c), 10 %, 20 %, 40 % and herbicide (H)) on the radicle length of *Achillea ageratum* seedlings grown in soil and paper (A), on the radicle length of *Conyza canadensis* and *Dittrichia viscosa* seedlings grown in soil (B) and paper (C). Mean values±SE are shown. n=20. Different letters indicate statistically significant differences (P≤0.05; Linear Mixed Models).

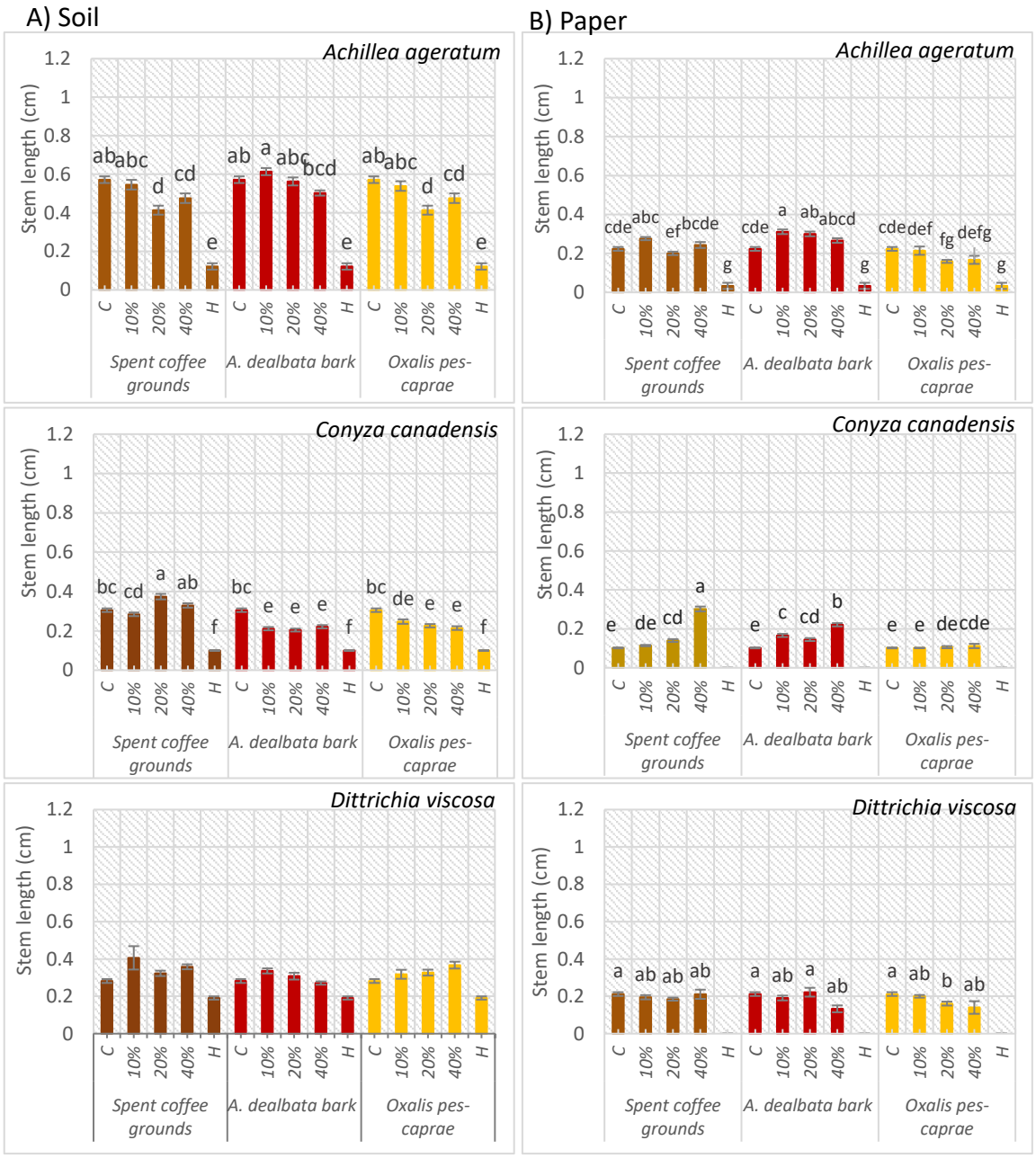

Figure 13. Effect of the interaction between extracts (Spent coffee grounds, *Acacia dealbata bark* and *Oxalis pes-caprae*) and concentrations (distilled water (c), 10 %, 20 %, 40 % and herbicide (H)) on the stem length of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* seedlings grown in soil (A) and paper (B). Mean values±SE are shown. n=20. Different letters indicate statistically significant differences (P≤0.05; Linear Mixed Models).

2. Post-emergence effect of extracts - pot assays

We observed that neither extract, concentration, nor interaction between them significantly affected the number of alive plants in any weed species (APPENDIX – TABLE 2). Treatments were not able to kill well-stablished plants in any species,

surviving almost all of them (Data not shown). However, treatments differentially affected plant growth.

The effect of extracts

The extract factor had a significant effect on the increase of leaf number and plant biomass of *D. viscosa* (APPENDIX – TABLE 2). Plants sprayed with *A. dealbata* bark extract showed lower increase of leaves number in *D. viscosa* compared with the other two extracts (Fig. 14), and lower plant biomass compared with the *O. pes-caprae* extract in the same weed species (Fig. 16). In the other cases, extracts did not show significant differences among them (APPENDIX – TABLE 2, Figs. 14, 15, 16).

In *D. viscosa* plants, concentration of chlorophyll a, chlorophyll b and carotenoids obtained the lowest value when *O. pes-caprae* was applied (APPENDIX – TABLE 2, Table 2).

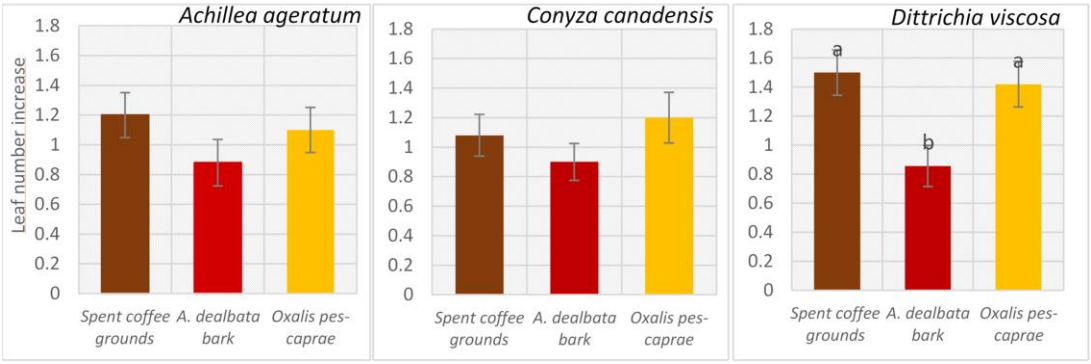

Figure 14. Effect of the extracts (Spent coffee grounds, *Acacia dealbata bark* and *Oxalis pes-caprae*) on the leaf number of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=10. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

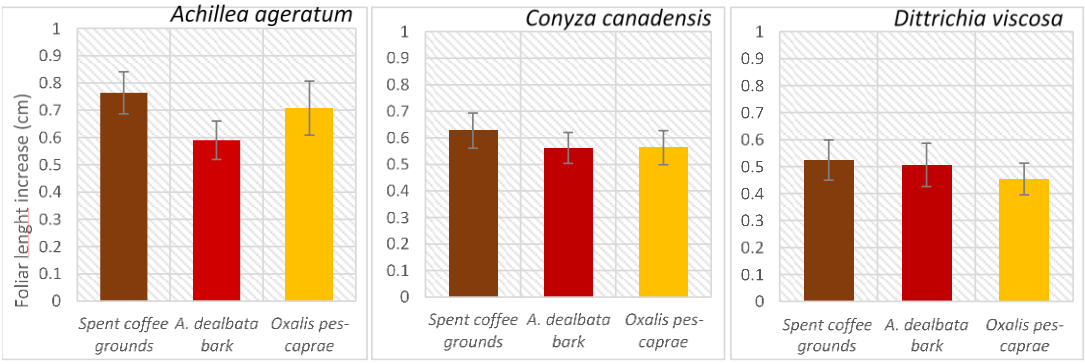

Figure 15. Effect of the extracts (Spent coffee grounds, *Acacia dealbata bark* and *Oxalis pes-caprae*) on the foliar length of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=10. Different letters indicate statistically significant differences (P≤0.05; Linear Models).

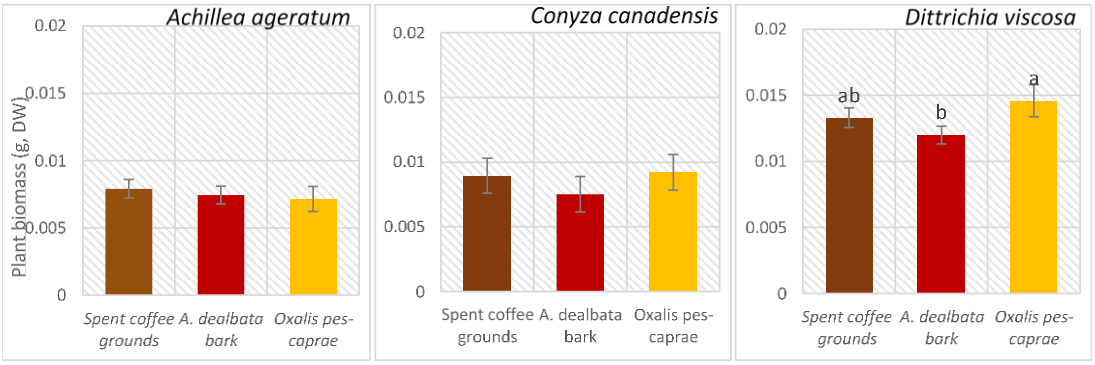

Figure 16. Effect of the extracts (Spent coffee grounds, *Acacia dealbata bark* and *Oxalis pes-caprae*) on the dry biomass of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=5. DW= Dry weight. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

Table 2. Mean comparisons of the pigment concentrations (Chlorophyll a – Chla, Chlorophyll b – Chlb and Carotenoids) of the three weed species (*Achillea ageratum, Conyza canadensis* **and** *Dittrichia viscosa*) grown in pots with the application of three extracts (Tr) – Spent coffee grounds, *A. dealbata* bark and *Oxalis pes-caprae*, at three different 10 %, 20 % and 40 % vs. herbicide (H) and vs. distilled water/negative control (C) and mean comparisons of the interaction between extracts and concentration (Tr x C). Mean values ± **SE** are shown.

Specie	Fixed effects	Variable	Chla (µmol/g)	Chlb (µmol/g)	Carotenoids (µmol/g)
Achillea ageratum	Tr	Spent coffee grounds (SCG)	0.809 ± 0.038	0.438 ± 0.025	0.510 ± 0.023
		A. dealbata bark (AB)	0.830 ± 0.050	0.486 ± 0.047	0.513 ± 0.022
		Oxalis pes-caprae (O)	0.732 ± 0.066	0.405 ± 0.038	0.461 ± 0.037
	C	C	0.900 ± 0.025 a	0.464 ± 0.020	0.523 ± 0.024
		10 %	0.894 ± 0.062 a	0.424 ± 0.031	0.505 ± 0.038
		20 %	0.931 ± 0.096 a	0.560 ± 0.085	0.505 ± 0.050
		40 %	0.718 ± 0.054 ab	0.439 ± 0.047	0.458 ± 0.039
		H	0.466 ± 0.046 b	0.315 ± 0.027	0.481 ± 0.028
	Tr × C	SCG - C	0.900 ± 0.047	0.464 ± 0.038	0.523 ± 0.044
		SCF – 10 %	0.955 ± 0.105	0.459 ± 0.053	0.520 ± 0.056
		SCF – 20 %	0.847 ± 0.062	0.529 ± 0.087	0.558 ± 0.075
		SCF – 40 %	0.807 ± 0.043	0.400 ± 0.029	0.461 ± 0.032
		SCF – H	0.466 ± 0.088	0.315 ± 0.052	0.481 ± 0.053
		AB - C	0.900 ± 0.047	0.464 ± 0.038	0.523 ± 0.044
		AB – 10 %	1.060 ± 0.096	0.514 ± 0.048	0.640 ± 0.061
		AB – 20 %	1.040 ± 0.199	0.828 ± 0.278	0.398 ± 0.037
		AB – 40 %	0.694 ± 0.048	0.410 ± 0.041	0.470 ± 0.033
		AB - H	0.466 ± 0.088	0.315 ± 0.052	0.481 ± 0.053
		O - C	0.900 ± 0.047	0.464 ± 0.038	0.523 ± 0.044
		O – 10 %	0.612 ± 0.080	0.267 ± 0.038	0.317 ± 0.042
		O – 20 %	0.946 ± 0.241	0.432 ± 0.103	0.517 ± 0.122
		O – 40 %	0.639 ± 0.176	0.524 ± 0.156	0.437 ± 0.130
		O – H	0.466 ± 0.088	0.315 ± 0.052	0.481 ± 0.053
Conyza canadensis	Tr	Spent coffee grounds (SCG)	1.590 ± 0.125	0.819 ± 0.066	1.000 ± 0.083
		A. *dealbata* bark (AB)	1.310 ± 0.122	0.668 ± 0.063	0.851 ± 0.085
		Oxalis pes-caprae (O)	1.400 ± 0.157	0.669 ± 0.076	0.828 ± 0.097
	C	C	0.859 ± 0.099 b	0.420 ± 0.054 b	0.481 ± 0.062 b
		10 %	1.660 ± 0.171 ab	0.865 ± 0.090 ab	1.050 ± 0.103 ab
		20 %	1.130 ± 0.170 ab	0.609 ± 0.096 ab	0.695 ± 0.111 ab
		40 %	1.780 ± 0.131 a	0.862 ± 0.059 a	1.050 ± 0.076 a
		H	1.780 ± 0.237 ab	0.860 ± 0.116 ab	1.250 ± 0.159 a

Specie	Fixed effects	Variable	Chla (µmol/g)	Chlb (µmol/g)	Carotenoids (µmol/g)
	Tr × C	SCG - C	0.859 ± 0.185	0.420 ± 0.100	0.481 ± 0.116
		SCF – 10 %	1.760 ± 0.370	0.889 ± 0.199	1.050± 0.222
		SCF – 20 %	2.240 ± 0.137	1.240 ± 0.028	1.440 ± 0.057
		SCF – 40 %	1.410 ± 0.069	0.717 ± 0.033	0.847 ± 0.037
		SCF – H	1.780 ± 0.453	0.860 ± 0.222	1.250 ± 0.304
		AB - C	0.859 ± 0.185	0.420 ± 0.100	0.481 ± 0.116
		AB – 10 %	1.700 ± 0.104	0.982 ± 0.063	1.280 ± 0.079
		AB – 20 %	0.526 ± 0.206	0.287 ± 0.129	0.316 ± 0.138
		AB – 40 %	1.700 ± 0.175	0.805 ± 0.080	0.978 ± 0.101
		AB - H	1.780 ± 0.453	0.860 ± 0.222	1.250 ± 0.304
		O - C	0.859 ± 0.185	0.420 ± 0.100	0.481 ± 0.116
		O – 10 %	1.570 ± 0.425	0.733 ± 0.212	0.811 ± 0.225
		O – 20 %	0.637 ± 0.119	0.295 ± 0.061	0.331 ± 0.068
		O – 40 %	2.230 ± 0.329	1.080 ± 0.143	1.350 ± 0.182
		O – H	1.780 ± 0.453	0.860 ± 0.222	1.250 ± 0.304
Dittrichia viscosa	Tr	Spent coffee grounds (SCG)	0.970 ± 0.063 ab	0.509 ± 0.028 ab	0.612 ± 0.030 ab
		A. dealbata bark (AB)	1.190 ± 0.127 a	0.593 ± 0.056 a	0.710 ± 0.057 a
		Oxalis pes-caprae (O)	0.625 ± 0.110 b	0.330 ± 0.053 b	0.403 ± 0.057 b
	C	C	0.975 ± 0.068 abc	0.475 ± 0.034 ab	0.506 ± 0.032 ab
		10 %	1.230 ± 0.213 ab	0.587 ± 0.101 ab	0.644 ± 0.105 ab
		20 %	1.350 ± 0.161 a	0.646 ± 0.079 a	0.720 ± 0.076 a
		40 %	0.693 ± 0.064 bc	0.353 ± 0.034 ab	0.391 ± 0.039 b
		H	0.404 ± 0.023 c	0.326 ± 0.021 b	0.613 ± 0.049 ab
	Tr × C	SCG - C	0.975 ± 0.117 b	0.475 ±0.063 ab	0.506 ± 0.059 ab
		SCF – 10 %	1.010 ± 0.076 b	0.528 ±0.050 ab	0.602 ± 0.053 ab
		SCF – 20 %	1.500 ± 0.132 ab	0.741 ± 0.051 ab	0.826 ± 0.045 ab
		SCF – 40 %	0.964 ± 0.063 b	0.474 ± 0.038 ab	0.514 ± 0.041 ab
		SCF – H	0.404 ± 0.042 b	0.326 ± 0.039 b	0.613 ± 0.092 ab
		AB - C	0.975 ± 0.117 b	0.475 ± 0.063 ab	0.506 ± 0.059 ab
		AB – 10 %	2.340 ± 0.430 a	1.100 ± 0.204 a	1.190 ± 0.202 a
		AB – 20 %	1.370 ± 0.133 ab	0.618 ± 0.047 ab	0.722 ± 0.063 ab
		AB – 40 %	0.871 ± 0.051 b	0.450 ± 0.022 b	0.524 ± 0.035 ab
		AB - H	0.404 ± 0.042 b	0.326 ± 0.039 b	0.613 ± 0.092 ab
		O - C	0.975 ± 0.117 b	0.475 ± 0.063 b	0.506 ± 0.059 ab
		O – 10 %	0.326 ± 0.143 b	0.133 ± 0.056 b	0.146 ± 0.061 b
		O – 20 %	1.170 ± 0.479 b	0.580 ± 0.232 ab	0.613 ± 0.229 ab
		O – 40 %	0.245 ± 0.078 b	0.136 ± 0.040 b	0.136 ± 0.035 b
		O – H	0.404 ± 0.042 b	0.326 ± 0.039 b	0.613 ± 0.092 ab

The effect of concentrations

The concentration factor significantly affected the increase in the number of leaves, the increase in the maximum foliar length and also the plant biomass of all the weed species (APPENDIX – TABLE 2, Figs. 17, 18, 19). As expected, the herbicide (positive control) showed the most inhibitory effect (Figs. 17, 18, 19). The increased number of leaves was reduced at 10 % in *A. ageratum*, by almost half, at 10 and 40 % in *C. canadensis*, and at all extracts concentration in *D. viscosa* (Fig. 17). In the case of the increase in maximum foliar length, we found a reduction of 53.8 % in this parameter at 10 % for *A. ageratum*, and of 34 and 42.9 %, at 10 % and 40 % for *C. canadensis* when compared with distilled water (Fig. 18). Finally, plant biomass followed the tendency of the parameters above: for *A. ageratum* and *D. viscosa*, biomass was lower at 10 and 40 % and for *C. canadensis*, at 40 % (Fig.19).

Concentration also influenced pigment quantities of the weed plants (APPENDIX – TABLE 2, Table 2). Chlorophyll a was inhibited with the herbicide use on *A. ageratum* plants (Table 2). However, for *C. canadensis* plants, there was an increase in the content of chlorophyll a, chlorophyll b and carotenoids at 40 %, and in carotenoids with the herbicide treatment compared to distilled water (Table 2) .

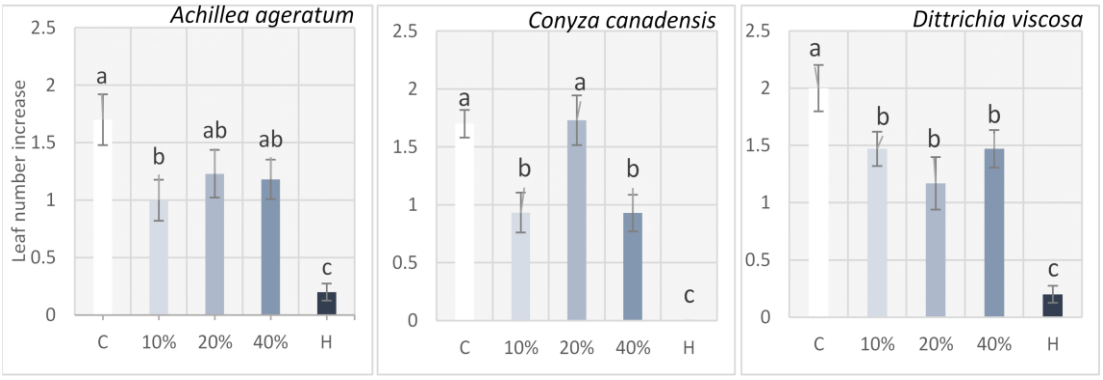

Figure 17. Effect of the concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the leaf number of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=10. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

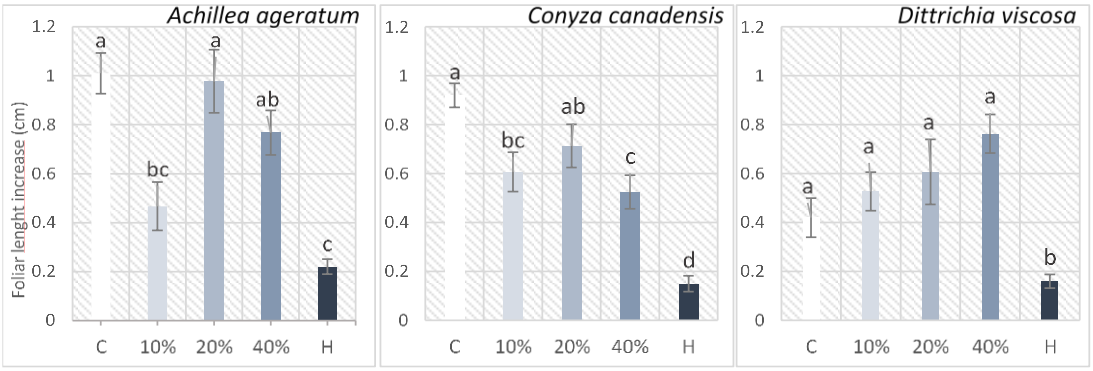

Figure 18. Effect of the concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the foliar length of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=10. Different letters indicate statistically significant differences (P≤0.05; Linear Models).

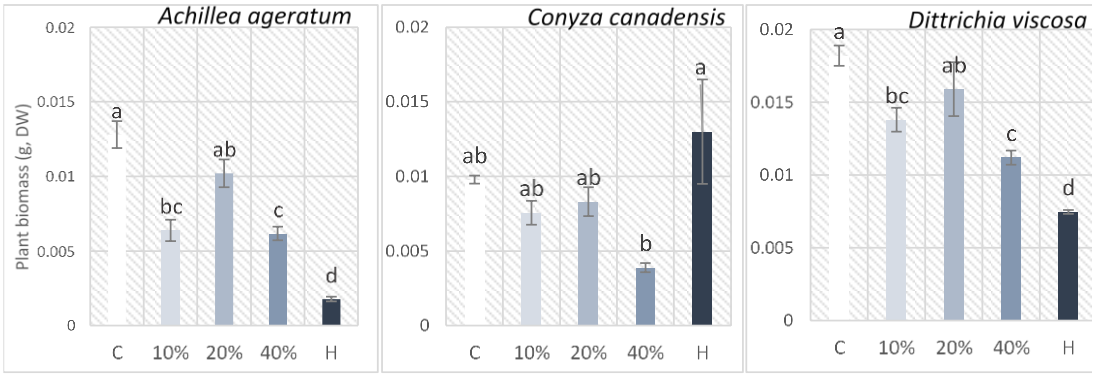

Figure 19. Effect of the concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the dry plant biomass of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=5. DW= Dry weight. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

The effect of the interaction between extracts x concentration

The extract x concentration interaction had a significant effect on the increase number of leaves for *C. canadensis* and *D. viscosa*, on the increase of the maximum foliar length for *A*. *ageratum* and for *C. canadensis*, and on plant biomass of *A. ageratum* and *D. viscosa* (APPENDIX – TABLE 2, Figs. 20, 21, 22). Statistical models also found a significant effect of covariates: the number of leaves previous treatment application affected the increased number of leaves in *C. canadensis* and *D. viscosa*; the maximum foliar length before treatment application had an effect on this parameter in treated

plants; the increase in the maximum foliar length and the increased number of leaves affected plant biomass of *A*. *ageratum* and *D. viscosa*, respectively (APPENDIX – TABLE 2).

As previously indicated, the herbicide treatment inhibited growth in almost all cases (Figs. 20, 21, 22). Although the increased number of leaves in *C. canadensis* and *D*. *viscosa* was affected by the factor's interaction, significant differences between distilled water and extract-concentration treatments was only found for *O. pes-caprae* extract at 10 %, in *C. canadensis*, and for *A*. *dealbata* bark extract at 20 %, in *D. viscosa*, where inhibition reached the herbicide level (Fig. 20). The increase in foliar length was reduced to herbicide values in plants of *A. ageratum* treated with *A. dealbata* bark extract at 10 %, and in plants of *C*. *canadensis* sprayed with *A*. *dealbata* bark at 10 % and with *O. pes-caprae* at 40 % concentration (Fig. 21). On the other hand, plant biomass was significantly decreased when using *O*. *pes-caprae* extract at 10 % in *A. ageratum* plants, and spent coffee grounds extract at 20 % and *O. pes-caprae* at 40 % in *D. viscosa* plants (Fig. 22).

Interaction between extracts x concentration only had significant effects on clorophylls a and b and on carotenoid concentrations of *D*. *viscosa* plants (APPENDIX – TABLE 2, Table 2). On this species, chlorophyll a concentration was higher with *A*. *dealbata* bark extract at 10 % application (Table 2). However, the content of Chlorophyll b and carotenoids were not significantly different from the negative control (distilled water treatment) (Table 2).

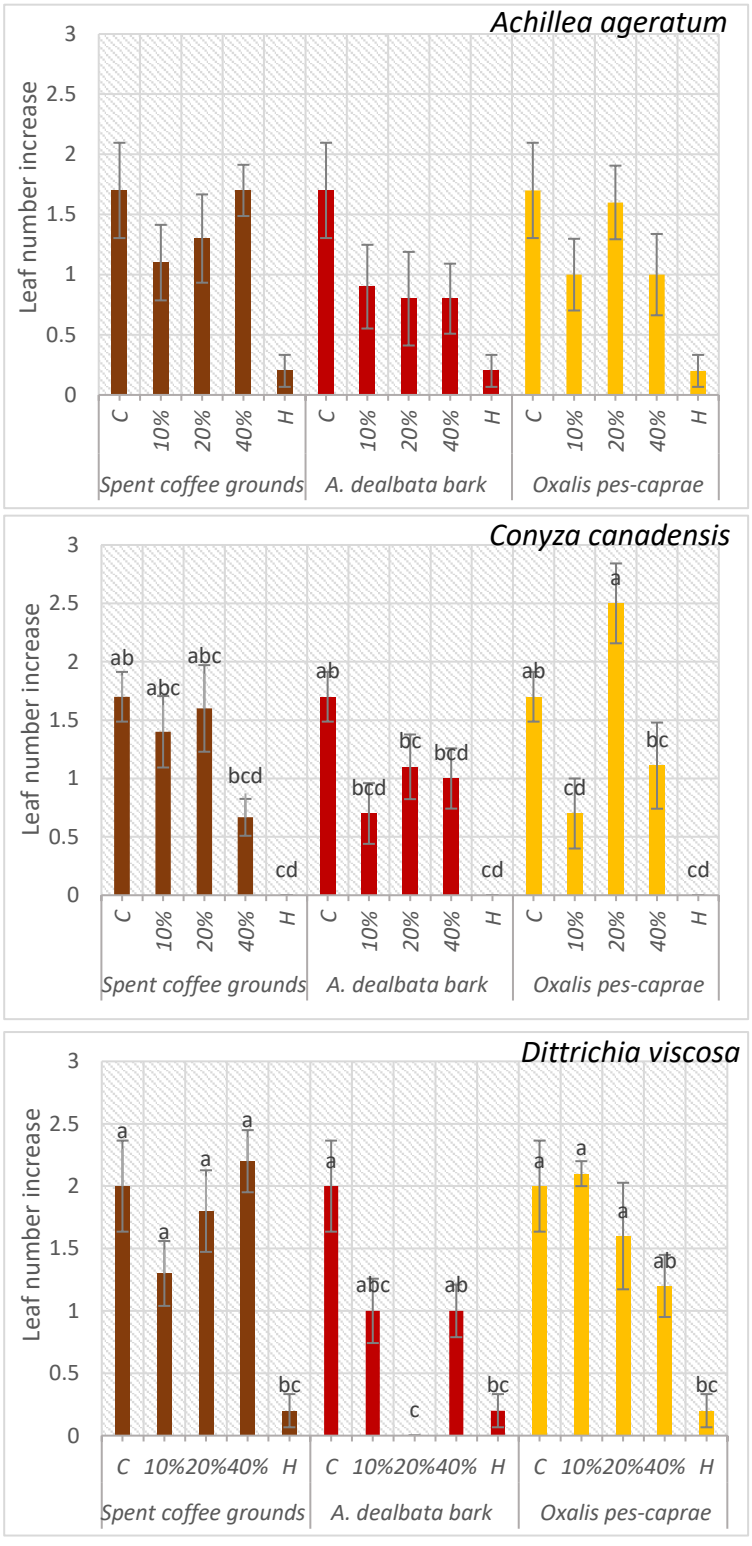

Figure 20. Effect of the interaction between extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) and concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the leaf number increase of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=10. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

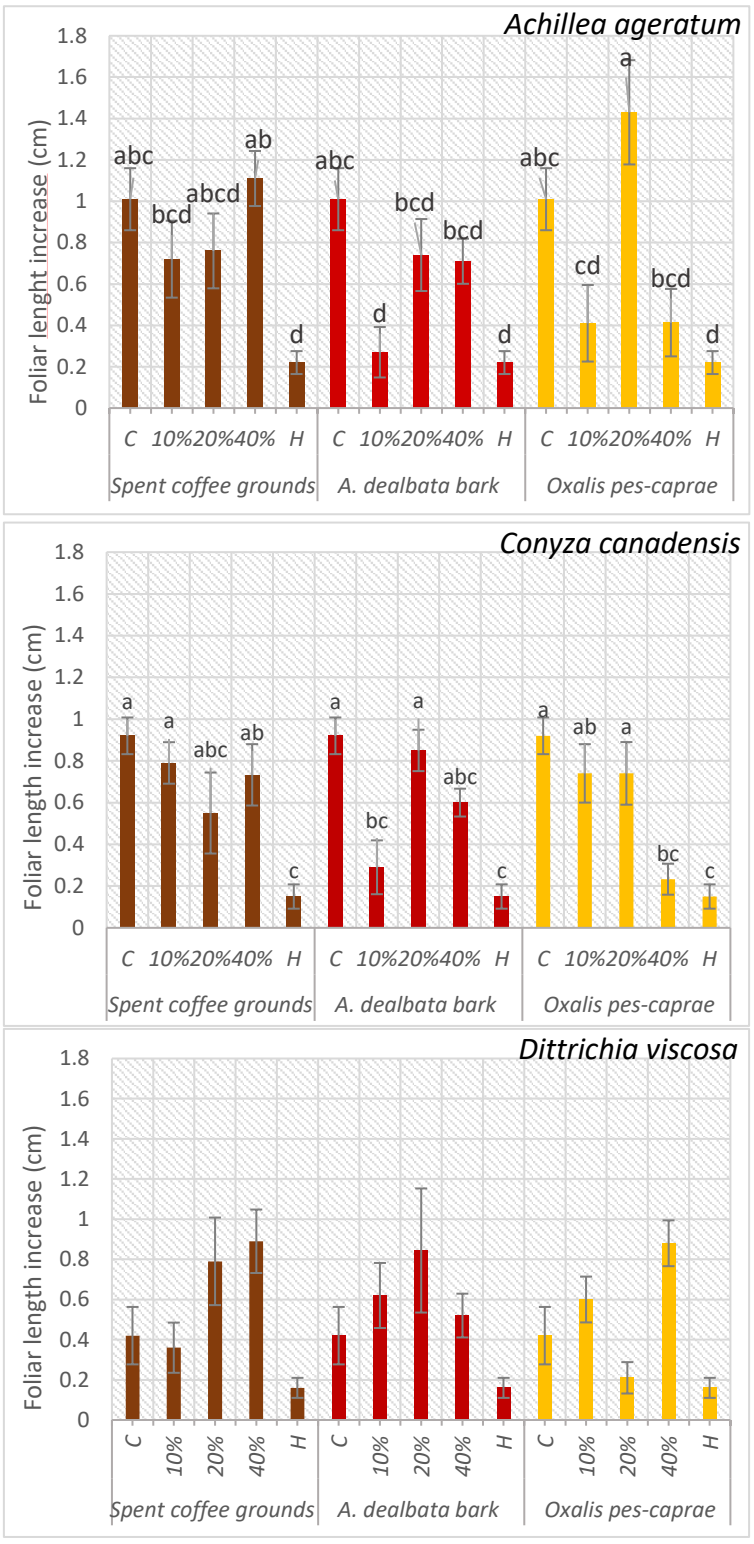

Figure 21. Effect of the interaction between extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) and concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the foliar length increase of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=10. Different letters indicate statistically significant differences (P≤0.05; Linear Models).

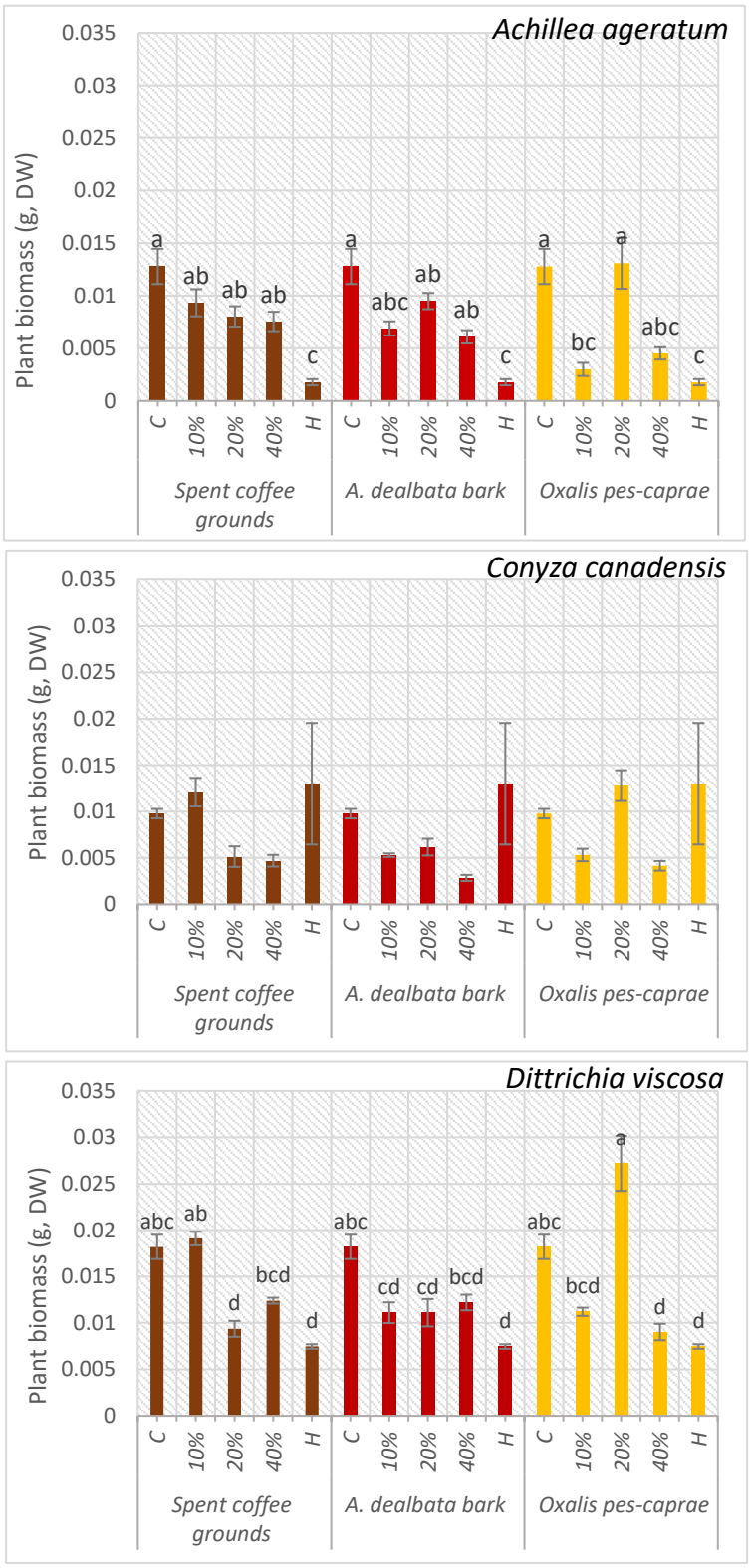

Figure 22. Effect of the interaction between extracts (Spent coffee grounds, *Acacia dealbata* bark and *Oxalis pes-caprae*) and concentrations (distilled water (C), 10 %, 20 %, 40 % and herbicide (H)) on the dry plant biomass of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa* plants. Mean values±SE are shown. n=5. DW= Dry weight. Different letters indicate statistically significant differences (P≤0.05; Generalized Linear Models).

IV. DISCUSSION

1. Preemergence effects of extracts - *in vitro* assays

In general, the results showed that germination and radicle length were severely inhibited with filter paper as a substrate, with an increasing effect as concentrations increased. However, the inhibitory effect was reduced or disappeared in the presence of soil. It was also found some stimulating effects for the radicle length at low concentrations of extracts, mainly in soil. When evaluating the effect of extracts on the stem length, it is evident that the negative effects were not as intense as the ones on the radicle growth. In fact, it was noticed some stimulation in paper, but inhibition was more frequent in soil. These results suggest that soil neutralized or modified the effect of assayed extracts. The fate and effectiveness of bioactive compounds in soil are highly dependent on biotic and abiotic environmental conditions (Inderjit et al., 2011). Different effects found in soil and in filter paper may be related to soil properties. Indeed, previous studies showed that soil properties such as organic matter and cation exchange capacity influence the herbicide effect (Blumhorst et al., 1990; Jursík et al., 2020) and the plant extracts efficacy (Dilipkumar et al., 2012). Soil organic matter may activate, inactivate, or retain herbicides (López-Piñeiro et al., 2013; Bonfleur et al., 2013; Tejada et al., 2017). Soil microbes also play a key role in the deactivation, degradation, or transformation into stimulatory compounds of phytotoxins (Souto et al., 2000; Lankau, 2010; Zhu et al., 2011). For example, the biodegradation of herbicides can be accelerated by increasing the microbiological activity of soils (Gómez et al., 2014). Sunflower leaf extracts not only tend to bind with soil colloids, but also may become diluted in saturated soil, therefore less available (Dilipkumar et al., 2012). Moreover, different soil types have been reported to eliminate phytotoxicity of the plant bioactive compound (+)-catechin (Furubayashi et al., 2007). This compound rapidly disappeared after incubation for four hours due to soil adsorption and transformation reactions (Furubayashi et al., 2007). In the scientific literature related to natural herbicidal research, most of studies conducted bioassays using Petri dishes filled with paper (Leather and Einhellig, 1986, eg. Lorenzo et al., 2016). In the present study, it was demonstrated that soil conditioned the effect of aqueous extracts. This highlights the importance of conducting studies mimicking natural conditions, since there is a necessity to prove the potential bioherbicides's efficacy under field conditions. Nevertheless, it should be highlighted that the tested extracts were more effective in filter paper, which might be relevant as urban weeds are common in pavements with barely any soil (Zimdhal, 2007) and would facilitate weed control once

there is no interference from soil. However, field experiments must be conducted to corroborate this hypothesis.

Furthermore, it was repeatedly identified a tendency for a greater inhibition when extracts' concentrations were higher (20 – 40 %) mostly affecting germination and radicle length. Similar results were found by Chemetova et al. (2019) when observed that the inhibition of seed growth was more relevant with the increase of *A. dealbata* bark extracts concentration. The results obtained in our study with the *Oxalis pes-caprae* extract are also in accordance with those obtained by Travlos et al. (2008), who found that the increase concentrations of the leaves, stems and petioles aqueous extracts of O. *pes-caprae* caused higher growth inhibition of duckweed. In fact, it is known that as concentration increases, the number of individual compounds in extracts also increases exponentially (Crozier & Monteiro, 1990). Distinct reactions may occur because each physiological process has unique responses to certain doses of each specific compound (Cruz-Ortega et al., 1998, Reigosa et al., 1999a). Additionally, the phytotoxic effect of aqueous extracts can result from synergies of several compounds, and consequently, the mixture of compounds could be more effective than their respective individual compound (An et al., 2001; Chon et al., 2003; Reigosa et al., 2007). Other explanation contributing to the reduced germination and radicle length is the seed contamination by fungus occurred in some Petri dishes, mainly in those filled with filter paper. This could be due to the fact that neither seeds nor extracts were sterilized or that extracts might contain sugars that favoured the fungi development (Ismail et al., 2016). On the other hand, it was also registered some stimulatory effects when the extracts concentrations were lower (10 %), mainly when applying spent coffee grounds and *Acacia dealbata* bark extracts. Spent coffee grounds, at low concentrations, were tested as a possible fertilizer on *Lactuca sativa* (Gomes et al., 2014). Positive effects of coffee waste on plant growth have also been found by Kasongo et al. (2013) since it caused an increase of nutrients (N, P, K) in the soil and improved water retention capacity, mainly on low fertility soils. However, several studies showed its potential to impact plant growth probably because of its high content in caffeine, when increasing concentration (Bravo et al., 2012). Batish et al. (2008) showed that caffeine in higher concentrations (2,000 µM) adversely affects the rooting potential of hypocotyl cuttings of *Prunus aureus* L. and Tanti et al. (2016) reported a growth and germination decrease of *Borreria hispida* (L.) K. Schum. seeds at high caffeine concentrations. Both observations were also registered by Mshelmbula et al. (2018): as the caffeine concentration increased, germination and radicle length decreased, and lower concentrations caused an increase in Bambara groundnut germination. Stimulating effects with *A. dealbata* bark solutions were also found on

Lactuca sativa seedlings and *Allium cepa* L. plants by Lorenzo et al. (2016) and (2019), respectively.

It is also important to mention that the most affected pre-emergence parameter was the radicle length. These results agree with previous reports that studied caffeine (Batish et al. 2008; Tanti et al. 2016; Mshelmbula et al.2018) and *Acacia dealbata* (Aguilera et al., 2015a; Aguilera et al., 2015b; Chemetova et al. 2019) effects on germination. Also, other types of plant extracts such as black mustard extract showed the same effect on germination and growth of alfalfa and lentil (Turk & Tawaha 2002; Turk et al., 2003). This outcome might have happened because roots are the first organ to absorb the (toxic) compounds from the environment (Gilbert, 2001). We also registered several visual effects similar to those found by Turk & Tawaha (2002), such as smaller roots, morphological abnormalities (eg. twisted roots) and darker colours mainly in seeds which were treated with *A. dealbata* bark extract (Fig. 23). It is worth mentioning that this parameter was significantly impacted by extracts at 40 % despite of soil interference. This suggests that the studied extracts might still have potential herbicidal activity when directly applied in soil.

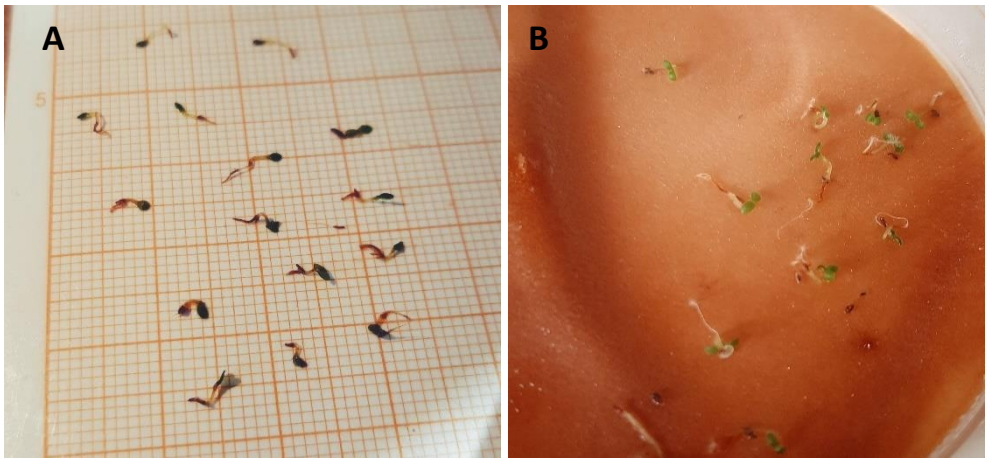

Figure 23. A) Seedlings of *Conyza canadensis* and of B) *Achillea ageratum* plants treated with A. *dealbat*a bark extract with small, twisted, of dark colours or inexistent roots.

2. Post-emergence effects of extracts – pot assays

Results from the post-emergence assays did not show a clear inhibitory pattern as obtained in pre-emergence ones. They also varied in each weed species. However, it was found some reductions on the number of leaves, on the foliar length, and on the dried biomass with *O. pes-caprae* and A. *dealbata* bark extracts at 10 and 40 %. Even though, some chlorosis were recorded on plants before treatments application, extracts

did not increase the area of these discoloured spots. In fact, the pigment content was not negatively affected by the application of treatments, and even some occasional stimulation was registered. This could happen because the extracts were not able to be adsorbed, to dissolve the cuticular fatty acids and, therefore, not capable of penetration (Batish et al. 2007; Hazrati et al., 2017). The non-severely injured weed growth and the no-effect on the content of chlorophylls suggest that the photosynthetic apparatus functioned properly, and the occasional reduction found in plant growth should be related to other factors such as poor soil quality or different plant size when extracts were applied. In fact, the statistical analyses revealed a significant effect of initial plant growth, the CMF0 (Maximum foliar length before treatment application) and NF0 (Leaf number before treatment application), two covariates in the used statistical models. The effect of both covariates indicates a non-homogeneous initial growth probably related to a deficient trays rotation throughout the weeks before extracts application.

3. Overall results

Regarding plant development, the assayed extracts affected more significantly the pre-emergence than post-emergence parameters. Seed germination and seedling emergence are extremely important and vulnerable phases during the plant development (Lamichhane et al., 2019), being the most vulnerable stages to phytotoxic compounds (Larcher, 1995). With maturation, there are gradual changes in plant morphology and anatomy, responsible for the development of physical barriers and chemical defence toward abiotic and biotic stresses (e.g. trichomes and their phenolic compounds, leaf cuticle that thickens as leaves age) (Rankenberg et al., 2021). Consequently, it is expected that well-established mature plants showed less harmful effects by extracts application and the studied extracts may be considered as potential pre-emergence bioherbicides.

Extracts of *Acacia dealbata* bark and *Oxalis pes-caprae* were the most effective according to the objective of this research: search for natural herbicides. For the *A. dealbata* bark extract, the results mainly showed inhibitory evidence, contrary to stimulatory effects found by Lorenzo et al. (2016) and (2019). However, according to some studies, the inhibitory activity of *A. dealbata* bark extract might be related to the presence of a significant amount of tannins (Ruibal Brunet et al., 2003; Barberis et al., 2012 Abilleira et al., 2021). Tannins are phenolic compounds, which have previously been shown to have the ability to disrupt cell membranes' permeability and to alter ions flow and hydraulic activity in the roots, resulting in severe negative impacts on

photosynthesis and respiration rates (González et al., 1995; Seigler 2003). In addition, they affected the germination via inhibition of the process of reserve substances (Einhellig, 2004; Aguilera et al., 2015c). In *O*. *pes-caprae* there is also interesting compounds, such as ester and phenyl cinnamate derivatives, aromatic compounds and phenols found in the leaves and twigs (DellaGreca, Previtera, et al., 2007; DellaGreca et al. 2008; DellaGreca et al. 2010) that have an already reported phytotoxic activity (DellaGreca et al. 2009). Moreover, this plant has a high content in oxalic acid which implies a lower pH value (Vilà et al., 2006), as found in the tested extract. This may also have negatively impacted plant growth since inadequate pH can provoke abiotic stress in terrestrial plants (Pedrol et al. 2006).

In order to find a potential bioherbicide to be applied on a wide range of similar species, weeds belonging to Asteraceae family were used. In fact, the goal of this type of studies is to find a compound or a mixture of compounds, capable of affecting several weed species. However, our results were species-dependent. Different outcomes may indicate actions on distinct physiological processes within each species (Reigosa et al. 1999a). For example, the effect of six phenolic compounds separately and in a mixture on a variety of target species varied with its concentration and the target species (Reigosa et al., 1999b). It is plausible that in natural situations test species have not developed the same strategies to cope with stress and environmental pressures (Weir et al. 2004). Thus, while some plants have detoxification mechanisms to reduce or tolerate negative effects (Weir et al. 2004), others remain helpless. Despite this, there is still the possibility to develop selective bioherbicides that may be effective towards one problematic weed. Even though our tested plants were all from the Asteraceae family, in both pre- and post-emergence assays, the effect of each extract depended on the weed used. Interestingly, *Conyza canadensis*, a weed-resistant herbicide (Heap IM, 2014; Palma-Bautista et al., 2018) and an invasive species that displaces the native flora in different ecological habitats all over the world (Blonde & Aronson, 1999), showed limited early development in soil when treated with *A. dealbata* bark and *O. pes-caprae* biomass extracts. Then, these results may lead to a new promising research line to control this weed since it has high seed production, efficient seed dispersal, flexible life cycle, tolerance to harsh climatic conditions, among others, which make it a problematic and resistant weed (Tozzi et al., 2014). Nevertheless, these results deserve further evaluation.

V. FINAL REMARKS

The present study supports the theory that some plant extracts may have a potential herbicidal effect, which may be due to its high bioactive compounds content. Our results suggest that the tested extracts have a more prominent impact on the pre-emergence physiological process than on well-stablished plants (post-emergence). The lack of remarkable effects after treatments led us to discard the use of any of our aqueous extracts as post-emergence bioherbicides for the tested weeds. Additionally, it seems that soil reduce or neutralize the effect of these extracts, highlighting the need of conducting experimental bioassays as similar as possible to field conditions.

The extracts worth to further study on field and identify its phytotoxic components are the *Acacia dealbata* bark and the *Oxalis pes-caprae* extracts. Both plant species are invasive in Portugal (Marchante et al., 2014; Papini et al., 2017) and by combining the use of waste from management actions to control these invasive species with eco-friendly alternatives to reduce the use of synthetic herbicides, we make the process more sustainable promoting a bio-based circular economy. The inhibitory activities were also concentration and plant species dependent. Thus, our extracts could theoretically be explored as selective preemergence bioherbicides. However, further studies on the germination process and early growth are required to evaluate their real and viable potential under field conditions.

VI. REFERENCES

Abbey L., Abbey J., Leke-Aladekoba A., Iheshiulo E. M. A. & Ijenyo, M. (2019). Biopesticides and Biofertilizers: Types, Production, Benefits, and Utilization. In *Byproducts from Agriculture and Fisheries: Adding Value for Food, Feed, Pharma, and Fuels*, John Wiley & Sons Ltd., NY,USA, 479-500. Doi:10.1002/9781119383956.ch20.

Abilleira F., Varela P., Cancela Á., Álvarez X., Sánchez Á. & Valero E. (2021). Tannins extraction from *Pinus pinaster* and *Acacia dealbata* bark with applications in the industry. Industrial Crops and Products, 164, 113394. Doi: 10.1016/j.indcrop.2021.113394

Abubakar A.R. & Haque M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12, 1-10. Doi: 10.4103/jpbs.JPBS_175_19

Aerts R., Honnay O. & Van Nieuwenhuyse A. (2018). Biodiversity and human health: mechanisms and evidence of the positive health effects of diversity in nature and green spaces. British medical bulletin, 127(1), 5-22. Doi: 10.1093/bmb/ldy021

Aguilera N., Becerra J., Guedes L.M., Villaseñor-Parada C, González L & Hernández V (2015a). Allelopathic effect of the invasive *Acacia dealbata* Link (Fabaceae) on two native plant species in south-central Chile. Gayana. Botanica, 72(2), 231. Doi: 10.4067/S0717-66432015000200007.

Aguilera N., Guedes L.M., Becerra J., Baeza C. & Hernández V. (2015b). Morphological effects at radicle level by direct contact of invasive *Acacia dealbata* Link. Flora-Morphology, Distribution, Functional Ecology of Plants, 215, 54-59. Doi: 10.1016/j.flora.2015.07.007.

Aguilera N., Becerra J., Villaseñor-Parada C., Lorenzo P., González L. & Hernández V. (2015c). Effects and identification of chemical compounds released from the invasive *Acacia dealbata* Link. Chemistry and Ecology, 31(6), 479-493. Doi: 10.1080/02757540.2015.1050004

An M., Pratley J. E. & Haig T. (2001). Phytotoxicity of vulpia residues: III. Biological activity of identified allelochemicals from *Vulpia myuros*. Journal of Chemical Ecology, 27(2), 383-394. Doi: 10.1023/A:1005640708047

Aronson M. F., Lepczyk C. A., Evans K. L., Goddard M. A., Lerman S. B., MacIvor J. S., Nilon C.H. & Vargo T. (2017). Biodiversity in the city: key challenges for urban green space management. Frontiers in Ecology and the Environment, 15(4), 189-196. Doi: 10.1002/fee.1480

Balasubramanian S. & Tyagi R. D. (2017). Biopesticide production from solid wastes. In *Current Developments in Biotechnology and Bioengineering*, 43-58. Elsevier. Doi: 10.1016/B978-0-444-63664-5.00003-4

Balbuena M.S., Tison L., Hahn M-L, Greggers U., Menzel R., Farina W.M. (2015). Effects of sublethal doses of glyphosate on honeybee navigation. Journal of Experimental Biology, 218: 2799-2805. Doi: 10.1242/jeb.117291

Balke N. E. (1985). Effects of allelochemicals on mineral uptake and associated physiological process. In *The Chemistry of Allelopathy*, 161-178. Washington: American Chemical Society. Doi: 10.1021/bk-1985-0268.ch011

Barberis I. M., Mogni V., Oakley L., Alzugaray C., Vesprini J. L. & Prado D. E. (2012). Biología de especies australes: *Schinopsis balansae* Engl. (Anacardiaceae). Kurtziana, 37(2), 59-86.

Barchanska H., Sajdak M., Szczypka K., Swientek A., Tworek M. & Kurek M. (2017). Atrazine, triketone herbicides, and their degradation products in sediment, soil and surface water samples in Poland. Environmental Science and Pollution Research, 24, 644–658. Doi: 10.1007/s11356-016-7798-3

Barton J. & Pretty J. (2010). Urban ecology and human health and wellbeing. In *Urban Ecology*, 202–229. Cambridge University Press. Doi: 10.1017/CBO9780511778483.010

Batish D.R., Singh H.P., Setia N., Kohli R.K., Kaur S., Yadav S.S. (2007). Alternative control of littleseed canary grass using eucalypt oil. Agronomy for sustainable development, 27(3), 171-177. Doi: 10.1051/agro:2007008

Batish D. R., Singh H. P., Kaur M., Kohli R. K. & Yadav S. S. (2008). Caffeine affects adventitious rooting and causes biochemical changes in the hypocotyl cuttings of mung bean (*Phaseolus aureus* Roxb.). Acta physiologiae plantarum, 30(3), 401-405. Doi: 10.1007/s11738-007-0132-4

Beckie H.J., Heap I.M., Smeda R.J. & Hall L.M. (2000). Screening of Herbicide Resistance in Weeds. Weed Technology, 14 (2), 428-445. Doi: 10.1614/0890-037X(2000)014[0428:SFHRIW]2.0.CO;2

Beecham J.E. & Seneff S. (2015). The Possible Link between Autism and Glyphosate Acting as Glycine Mimetic - A Review of Evidence from the Literature with Analysis. Molecular and Genetic Medicine , 9 (4), 1-16. Doi: 10.4172/1747-0862.1000187

Benbrook C.M. (2016). Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe, 28 (3). Doi: 10.1186/s12302-016-0070-0

Ben-Hammouda M., Ghorbal H., Kremer R.J. & Oueslati O. (2001). Allelopathic effects of barley extracts on germination and seedlings growth of bread and durum wheat. Agronomie 21 (1), 65–71. Doi: 10.1051/agro:2001109

Benvenuti S. (2004). Weed dynamics in the Mediterranean urban ecosystem: ecology, biodiversity and management. Weed Research, 44(5), 341-354. Doi: 10.1111/j.1365-3180.2004.00410.x

Benvenuti S., Cioni P.L., Flamini G. & Pardossi A. (2017). Weeds for weed control: Asteraceae essential oils as natural herbicides. Weed Research, 57, 342– 353. Doi: 10.1111/wre.12266

Berdy J. (2005). Bioactive microbial metabolites. Bioactive microbial metabolites. The Journal of antibiotics, 58(1), 1-26. Doi: 10.1038/ja.2005.1

Beuret C.J., Zirulnik F. & Giménez M.S. (2005). Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. Reproductive Toxicology, 19(4), 501-4. DOI: 10.1016/j.reprotox.2004.09.009

Blondel J. & Aronson J. (1999). Biology and wildlife of the Mediterranean region. Oxford University Press, USA.

Blumhorst M. R., Weber J. B. & Swain L. R. (1990). Efficacy of selected herbicides as influenced by soil properties. Weed Technology, 279-283. Available on: http://www.jstor.org/stable/3987073

Bonanomi G., Sicurezza M.G., Caporaso S., Esposito A. & Mazzoleni S. (2006). Phytotoxicity dynamics of decaying plant materials. New Phytologist, 169, 571–578. Doi: 10.1111/j.1469-8137.2005.01611.x

Bonfleur E. J., Kookana R. S., Tornisielo V. L. & Regitano,J. B. (2016). Organomineral interactions and herbicide sorption in Brazilian tropical and subtropical *Oxisols* under no-

tillage. Journal of agricultural and food chemistry, 64(20), 3925-3934. Doi: 10.1021/acs.jafc.5b04616

Bonthoux S., Chollet S., Balat I., Legay N. & Voisin L. (2019a). Improving nature experience in cities: What are people's preferences for vegetated streets?. Journal of environmental management, 230, 335-344. Doi: 10.1016/j.jenvman.2018.09.056

Bonthoux S., Voisina L., Bouché-Pillona S. & Cholletb S. (2019b). More than weeds: Spontaneous vegetation in streets as a neglected element of urban biodiversity. Landscape and Urban Planning, 185, 163-172. Doi: 10.1016/j.landurbplan.2019.02.009

Bordin E. R., Frumi Camargo A., Stefanski F. S., Scapini T., Bonatto C., Zanivan,J., ... & Treichel H. (2020). Current production of bioherbicides: mechanisms of action and technical and scientific challenges to improve food and environmental security. Biocatalysis and Biotransformation, 1-14. Doi: 10.1080/10242422.2020.1833864

Bravo J., Juaniz I., Monente C., Caemmerer B., Kroh L. W., De Peña M. P. & Cid C. (2012). Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. Journal of agricultural and food chemistry, 60(51), 12565-12573. Doi: 10.1021/jf3040594

Brito L.M., Mourão I., Coutinho J. & Smith S. (2013). Composting for management and resource recovery of invasive *Acacia* species. Waste management & research, 31(11), 1125-1132. Doi: 10.1177/ 0734242X13502384.

Brito L.M., Reis M., Mourão I. & Coutinho J. (2015). Use of acacia waste compost as an alternative component for horticultural substrates. Communications in Soil Science and Plant Analysis, 46(14), 1814-1826. Doi: 10.1080/ 00103624.2015.1059843.

Chandler D., Bailey A.S., Tatchell G.M., Davidson G., Greaves J. & Grant W.P. (2011). The development, regulation and use of biopesticides for integrated pest management. Philosophical Transactions of the Royal Society B: Biological Sciences, 366, 1987–1998. Doi: 10.1098/rstb.2010.0390.

Charudattan R. (2001). Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. Biocontrol, 46, 229-260. Doi: 10.1023/A:1011477531101

Chawdhry, M. A., & Sagar, G. R. (1974). Control of *Oxalis latifolia* HBK and *O. pes-caprae* L. by defoliation. Weed Research, 14(5), 293-299. Doi: 10.1111/j.1365-3180.1974.tb01064.x

Chemetova C., Ribeiro H., Fabião A. & Gominho J. (2019). The *Acacia* bark phytotoxic potential: a non-synthetic bio-herbicide. In *III International Symposium on Growing Media, Composting and Substrate Analysis,* 1305, 103-108. Doi: 10.17660/ActaHortic.2021.1305.15

Chen H., Singh H., Bhardwaj N., Bhardwaj S. K. et al. (2020). An exploration on the toxicity mechanisms of phytotoxins and their potential utilities. Critical Reviews in Environmental Science and Technology, 1-41. Doi: 10.1080/10643389.2020.1823172

Chon S. U., Kim Y. M. & Lee J. C. (2003). Herbicidal potential and quantification of causative allelochemicals from several Compositae weeds. Weed research, 43(6), 444-450. Doi: 10.1046/j.0043-1737.2003.00361.x

Clements D. R. & Jones V. L. (2021). Ten Ways That Weed Evolution Defies Human Management Efforts Amidst a Changing Climate. Agronomy, 11(2), 284. Doi: 10.3390/agronomy11020284

Cordeau S., Triolet M., Wayman S., Steinberg C. & Guillemin J. (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. Crop Protection, 87, 44-49. Doi: 10.1016/j.cropro.2016.04.016

Crozier A. & Monteiro A.M. (1990). Chromatography and Analysis. Strategies for the analysis of plant products by HPLC. 3, 5-7.

Cruz S. & Marques dos Santos Cordovil C.S.C. (2015). Espresso coffee residues as a nitrogen amendment for small-scale vegetable production. Journal of the Science of Food and Agriculture, 95 (15), 3059-3066. Doi: 10.1002/jsfa.7325

Cruz-Ortega R., Anaya A. L., Hernández-Bautista B. E. & Laguna-Hernández G. (1998). Effects of allelochemical stress produced by Sicyos deppei on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*. Journal of Chemical Ecology, 24(12), 2039-2057. Doi: 10.1023/A:1020733625727

Czaja K., Góralczyk K., Struciński P., Hernik A., Korcz W. et al. (2015) Biopesticides—towards increased consumer safety in the European Union. Pest Management Science, 71, 3–6. Doi: 10.1002/ps.3829

Damalas C. A. & Koutroubas S. D. (2018). Current Status and Recent Developments in Biopesticide Use. Agriculture, 8, 13. Doi: 10.3390/agriculture8010013.

Das K., Tiwari R.K. & Shrivastava D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. Journal of Medicinal Plants Research, 4, 104-11. Doi: 10.5897/JMPR09.030

Das S.K. & Mondal T. (2014). Mode of action of herbicides and recent trends in development: A reappraisal. International Journal of Agricultural and Soil Science, 2, 27-32. Available on: http://internationalinventjournals.org/journals/IJASS

Davies Z.G., Dallimer M., Fisher J.C., Fulle R.A. (2019). Biodiversity and Health: Implications for Conservation. In *Biodiversity and Health in the Face of Climate Change*. Springer, Cham. Doi: 10.1007/978-3-030-02318-8_12

Davis A.S & Frisvoldb G.B. (2017). Are herbicides a once in a century method of weed control? Pest Management Science, 73 (11), 2209-2220. Doi: 10.1002/ps.4643.

Dayan E.F. & Duke S.O. (2014). Natural Compounds as Next-Generation Herbicides. Plant Physiology, 166, 1090–1105. Doi: 10.1104/pp.114.239061

de Albuquerque M.B., dos Santos R.C., Lima L.M., de Albuquerque Melo Filho P., Nogueira R.J.M.C., Da Câmara C.A.G. et al. (2011). Allelopathy, an alternative tool to improve cropping systems. A review. Agronomy for Sustainable Development, 31(2), 379-395. Doi: 10.1051/agro/2010031

DellaGreca M., Previtera L., Zarrelli A. (2010). A new aromatic component from *Oxalis pes-caprae*. Natural Product Research, 24, 958–961. Doi: 10.1080/14786410902809286

DellaGreca M., Purcaro R., Previtera L., Zarrelli A. (2008). Phenyl cinnamate derivatives from *Oxalis pes-caprae*. Chemistry & Biodiversity, 5, 2408–2414. Doi: 10.1002/cbdv.200890205

DellaGreca M,. Previtera L., Purcaro R., Zarrelli A. (2009). Phytotoxic aromatic constituents of *Oxalis pes-caprae*. Chemistry & Biodiversity. 6, 459–465. Doi: 10.1002/cbdv.200800179

DellaGreca M., Previtera L., Purcaro R., Zarrelli A. (2007). Cinnamic ester derivatives from *Oxalis pes-caprae* (Bermuda Buttercup) #. Journal of natural products, 70(10), 1664-1667. Doi: 10.1021/np0702786

Department for Environment, Food and Rural Affairs. (2015). Amenity pesticides in the United Kingdom. Available on: https://www.gov.uk/government/statistics/pesticide-usage-survey-amenity-pesticides-in-the-uk-2016

Department of Horticulture - College of Agricultural Sciences. Non-selective weed control in non-crop areas. Available on: https://plantscience.psu.edu/research/projects/vegetative-management/publications/other-publications/non-selective-weed-control-in-non-crop-areas

DGAV (2017). Relatório de Vendas de Produtos Fitofarmacêuticos Portugal – 2017. Available on: https://www.agroportal.pt/wp-content/uploads/vendas-2017.pdf

DGAV (2019). Lista de produtos fitofarmacêuticos autorizados para uso não profissional. Available on: https://www.confagri.pt/content/uploads/2019/01/USO-NAO-PROFISSIONAL_31.12.2018.pdf

Did S.U., Akram W., Khan H.A.A., Hussain A., Hafeez F. (2011). Citrus waste-derived essential oils: alternative larvicides for dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). Pakistan Journal of Zoology, 43, 367–372

Dilipkumar M., Adzemi M. A., & Chuah, T. S. (2012). Effects of soil types on phytotoxic activity of pretilachlor in combination with sunflower leaf extracts on barnyardgrass (*Echinochloa crus-galli*). Weed science, 60(1), 126-132. Doi: 10.1614/WS-D-11-00075.1

Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. Official Journal of the European Communities, 309, 71–86.

Donley N. (2019). The USA lags behind other agricultural nations in banning harmful pesticides. Environmental Health, 18 (1). Doi: 10.1186/s12940-019-0488-0

Duke S.O. & Stephen O. (2017). The history and current status of glyphosate. Pest management science, 74(5), 1027-1034. Doi: 10.1002/ps.4652

Duke S.O., Dayan F.E. (2018) Herbicides. In *Encyclopedia of Life Sciences - eLS*. Chichester, UK: John Wiley & Sons, Ltd, 1-9. Doi: 10.1002/9780470015902.a0025264

Duke S.O., Dayan F.E., Romagni J.G, & Rimando A.M. (2000). Natural Products as Sources of Herbicides: Current Status and Future Trends. Journal of Weed Research, 40 (1), 99-111. Doi: 10.1046/j.1365-3180.2000.00161.x

Dunham W. & Trimmer M. (2018). Biological Products Market Around the World – 2018 Spring Meeting & International Symposium (www.bpia.org).

Einhellig F. A., Galindo J. C. G., Molinillo J. M. G. & Cutler H. G. (2004). Mode of allelochemical action of phenolic compounds. In *Allelopathy: chemistry and mode of action of allelochemicals*, 217-238. Doi: 10.1201/9780203492789.ch11

Elmqvist T., Setälä H., Handel S. N., Van Der Ploeg S., Aronson J., Blignaut J. N., Gómez-Baggethun E., Nowak D.J., Kronenberg J. & De Groot, R. (2015). Benefits of restoring ecosystem services in urban areas. Current opinion in environmental sustainability, 14, 101-108. Doi: 10.1016/j.cosust.2015.05.001

El-Wakeel M.A., El-Desoki E.R. & Ahmed, S.ED.A.EG (2019). Bioherbicidal activity of *Eruca sativa* fresh shoot aqueous extract for the management of two annual weeds associating *Pisum sativum* plants. Bulletin of the National Research Centre, 43, 1-7. Doi: 10.1186/s42269-019-0130-x

EPA - Hazardous Materials Advisory Committee (1974). Herbicide Report: Chemistry and Analysis, Environmental Effects, Agricultural and Other Applied Uses. Volume 266006 of U.S. National Technical Information Service. PB, USA, Cornell University.

European Commission (2017). Commission Implementing Regulation (EU) 2017/2324 of 12 December 2017 renewing the approval of the active substance glyphosate in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJ L 333, 10–16.

European Commission (2020a). EU agricultural outlook for markets, income and environment, 2020-2030. European Commission, DG Agriculture and Rural Development, Brussels.

European Commission (2020b). REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL On the experience gained by Member States on the implementation of national targets established in their National Action Plans and on progress in the implementation of Directive 2009/128/EC on the sustainable use of pesticides. COM(2020) 204 final, Brussels.

European Commission. (2019). Organic farming in the EU- A fast growing sector. EU Agricultural Markets Briefs, 13. Available at: https://ec.europa.eu/info/sites/info/files/food-farming-fisheries/farming/documents/market-brief-organic-farming-in-the-eu_mar2019_en.pdf.

Farkhondeh T., Kianmehr M., Kazemi T., Samarghandian S. & Khazdair M. R. (2020). Toxicity effects of *Nerium oleander*, basic and clinical evidence: A comprehensive review. Human & experimental toxicology, 39(6), 773-784. Doi: 10.1177/0960327120901571

Fava F., Totaro G., Diels L., Reis M., Duarte J., Beserra Carioca O., PoggiVaraldo H. M. & Ferreira B. S. (2013). Biowaste biorefinery in Europe: opportunities and research & development needs. New Biotechnology, 32, 100-108. Doi: 10.1016/j.nbt.2013.11.003

Flamini G. (2012). Natural Herbicides as a Safer and More Environmentally Friendly Approach to Weed Control: A Review of the Literature Since 2000. Studies in Natural Products Chemistry (38). 353-396. Doi: 10.1016/B978-0-444-59530-0.00013-7

Fujimoto H., Itoh K., Yamamoto M., Kyozuka J. & Shimamoto K. (1993). Insect resistant rice generated by introduction of a modified δ-endotoxin gene of *Bacillus thuringiensis*. Bio/technology, 11, 1151-1155. Doi: 10.1038/nbt1093-1151.

Fuller R. A. & Gaston K. J. (2009). The scaling of green space coverage in European cities. Biology letters, 5(3), 352-355. Doi: 10.1098/rsbl.2009.0010

Furubayashi A., Hiradate S. & Fujii Y. (2007). Role of Catechol Structure in the Adsorption and Transformation Reactions of l-D opa in Soils. Journal of chemical ecology, 33(2), 239-250. Doi: 10.1007/s10886-006-9218-5

Gadermaier G., Hauser M. & Ferreira F. (2014). Allergens of weed pollen: an overview on recombinant and natural molecules. Methods, 66(1), 55-66. Doi: 10.1016/j.ymeth.2013.06.014

Gaines T. A., Duke S. O., Morran S., Rigon C. A., Tranel P. J., Küpper A. & Dayan F. E. (2020). Mechanisms of evolved herbicide resistance. Journal of Biological Chemistry, 295(30), 10307-10330. Doi: 10.1074/jbc.REV120.013572

Gaston K. J., Avila-Jimenez M.L. & Edmondson J.L. (2013). Managing urban ecosystems for goods and services. Journal of Applied Ecology, 50 (4), 830-840. Doi: 10.1111/1365-2664.12087

German Golf Association (2017). List of permitted plant protection products for golf courses. (In German). Available on: http:// www.golf.de

Gianessi L.P. (2013). The increasing importance of herbicides in worldwide crop production. Pest Management Science, 69, 1099–1105. Doi: 10.1002/ps.3598.

Gilbert S. F. (2001). Developmental biology 6th edition. Sunderland (MA): Sinauer Associates. Available on: https://www.ncbi.nlm.nih.gov/books/NBK9979/

Giles-Corti B., Broomhall M.H., Knuiman M., Collins C., Douglas K., Ng K., Lange A. & Donovan R.J. (2005). Increasing walking: How important is distance to, attractiveness, and size of public open space?, American Journal of Preventive Medicine, 28: 169–176. Doi: 10.1016/j.amepre.2004.10.018

Godard A., De Caro P., Vedrenne E., Mouloungui Z. & Thiebaud-Roux S. (2016). From crops to products for crops: preserving the ecosystem through the use of bio-based molecules. Food Processing Techniques and Technology, 49(4), 652-659. Doi: 10.1051/ocl/2016037

Gomes T., Pereira J. A., Ramalhosa E., Casal S. & Baptista P. (2014). Effect of fresh and composted spent coffee grounds on lettuce growth, photosynthetic pigments and mineral composition. In *VII Congreso Ibérico de Agroingeniería y Ciencias Horticolas*, 1-5.

Gómez, I., Rodríguez-Morgado, B., Parrado, J., García, C., Hernández, T., & Tejada, M. (2014). Behavior of oxyfluorfen in soils amended with different sources of organic matter. Effects on soil biology. Journal of hazardous materials, 273, 207-214. Doi: 10.1016/j.jhazmat.2014.03.051

González L., Souto X. C. & Reigosa M. J. (1995). Allelopathic effects of *Acacia melanoxylon* R. Br. phyllodes during their decomposition. Forest Ecology and Management, 77(1-3), 53-63. Doi: 10.1016/0378-1127(95)03581-T

Government of the Netherlands. (2017). Green deal. Available on: https://www.greendeals.nl/english

Green, J. M. (2014). Current state of herbicides in herbicide-resistant crops. Pest Management Science, 70(9), 1351-1357. Doi: 10.1002/ps.3727

Gruber A., Donaldsson D., Kiely T. & Wu L. (2011). Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates. U.S. Environmental Protection Agency: Washington, DC, USA.

Gupta P. K. (2011). Herbicides and fungicides. In *Reproductive and developmental toxicology,* 657-679. Academic Press. Doi: 10.1016/B978-0-12-382032-7.10039-6

Hahn, D., Sallenave, R., Pornaro, C., & Leinauer, B. (2020). Managing cool-season turfgrass without herbicides: Optimizing maintenance practices to control weeds. Crop Science, 60(5), 2204-2220. Doi: 10.1002/csc2.20175

Hardgrove, S. J., & Livesley, S. J. (2016). Applying spent coffee grounds directly to urban agriculture soils greatly reduces plant growth. Urban forestry & urban greening, 18, 1-8. Doi: 10.1016/j.ufug.2016.02.015

Hazrati H., Saharkhiz M.J., Niakousari M., Moein, M. (2017). Natural herbicide activity of *Satureja hortensis* L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. Ecotoxicology and environmental safety, 142, 423-430. Doi: 10.1016/j.ecoenv.2017.04.041

Heap IM (2014). International survey of herbicide-resistant weeds. Available at http://www.weedscience.org

Hejl A.M. & Koster K.L. (2004). The allelochemical sorgoleone inhibits root H+-ATPase and water uptake. Journal of Chemical Ecology, 30 (11), 2181–2191. Doi: 10.1023/b:joec.0000048782.87862.7f

Helander, M., Pauna, A., Saikkonen, K., & Saloniemi, I. (2019). Glyphosate residues in soil affect crop plant germination and growth. Scientific reports, 9(1), 1-9. Doi: 10.1038/s41598-019-56195-3

Hoagland R.E., Boyette C.D., Weaver M.A. & Abbas H.K. (2007). Bioherbicides: research and risk. Toxin Reviews, 26, 313–342. Doi: 10.1080/15569540701603991

Holm, L. (1978). Some characteristics of weed problems in two worlds. Proceedings of the Western Society of Weed Sciences, 31, 3-12.

Holt, J. S. (2013). Herbicides. Encyclopedia of Biodiversity, 87–95. Doi: 10.1016/b978-0-12-384719-5.00070-8

Imatomi M., Novaes P., Ferraz-Machado Miranda M. & Juliano S. (2015). Phytotoxic effects of aqueous leaf extracts of four Myrtaceae species on three weeds. Acta Scientarum, 37, 241–248. Doi: 10.4025/actasciagron.v37i2.19079

Inderjit, Wardle D. A., Karban R. & Callaway R. M. (2011). The ecosystem and evolutionary contexts of allelopathy. Trends in ecology & evolution, 26(12), 655-662. Doi: 10.1016/j.tree.2011.08.003

Irshad A., Cheema Z.A. (2004). Effect of sorghum extract on management of barnyard grass in rice crop. Allelopathy Journal, 14, 205–212.

Ismail, A. M., Mohamed, E. A., Marghany, M. R., Abdel-Motaal, F. F., Abdel-Farid, I. B., & El-Sayed, M. A. (2016). Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. Journal of the Saudi Society of Agricultural Sciences, 15(2), 112-117.

James T., Rahman A., Trolove M. & Frith H. (2002). Efficacy of a certified organic herbicide based on pine essence. New Zealand Plant Protection, 55, 207-212. Doi: 10.30843/nzpp.2002.55.3891

Jayasumana C., Gunatilake S. & Senanayake P. (2014). Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits Behind the Epidemic of Chronic Kidney Disease of Unknown Etiology in Sri Lanka? International Journal of Environmental Research and Public health, 11(2), 2125–2147. Doi: 10.3390/ijerph110202125

Jelassi A., Ayeb-Zakhama A. El, Nejma A. Ben, Chaari A., Harzallah-Skhiri F. & Jannet H. Ben (2016). Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species. Industrial Crops and Products, 83, 339–345. Doi: 10.1016/j.indcrop.2016.01.020

Jursík M., Kočárek M., Kolářová M. & Tichý L. (2020). Effect of different soil and weather conditions on efficacy, selectivity and dissipation of herbicides in sunflower. Plant, Soil and Environment, 66(9), 468-476. Doi: 10.17221/223/2020-PSE

Kaab S.B., Rebey I.B., Hanafi M., Hammi K.M., Smaoui A., Fauconnier M.L., De Clerck C., Jijakli M.H. & Ksouri R. (2020). Screening of Tunisian plant extracts for herbicidal activity and formulation of a bioherbicide based on *Cynara cardunculus.* South African Journal of Botany 128*,* 67-76. Doi: 10.1016/j.sajb.2019.10.018

Kanthal S., Baishya A. & Ghosh A. (2020). 16 - Glyphosate Prohibition and its Effects on Conservation Agriculture. Agrobios Newsletter, 18 (11), 29-30.

Kapoor D., Rinzim, Tiwari A., Amit Sehgal A., Landi M. et al. (2019). Exploiting the allelopathic potential of aqueous leaf extracts of *Artemisia absinthium* and *Psidium guajava*

against *Parthenium hysterophorus*, a Widespread Weed in India. Plants, 8, 552–565. Doi: 10.3390/plants8120552.

Kasongo R. K., Verdoodt A., Kanyankogote P., Baert G. & Van Ranst E. (2013). Response of Italian ryegrass (*Lolium multiflorum* Lam.) to coffee waste application on a humid tropical sandy soil. Soil use and management, 29(1), 22-29. Doi: 10.1111/sum.12006

Keken Z., Sedoník J., Kušta T., Andrášik R. & Bíl M. (2019). Roadside vegetation influences clustering of ungulate vehicle collisions. Transportation research part D: transport and environment, 73, 381-390. Doi: 10.1016/j.trd.2019.07.013

Kristoffersen P., Rask A.M., Grundy A., Franzen I., Kempenaar C., Raisio J., et al. (2008). A review of pesticide policies and regulations for urban amenity areas in seven European countries. Weed Research, 48, 201-214. Doi: 10.1111/j.1365-3180.2008.00619.x

Kudsk P. & Mathiassen S.K. (2019). Pesticide regulation in the European Union and the glyphosate controversy. Weed Science 68, 214–222. Doi: 10.1017/wsc.2019.59

Kumar S. & Singh A. (2015). Biopesticides: Present Status and the Future Prospects. Journal of Fertilizers & Pesticides, 6, e129. Doi: 10.4172/2471-2728.1000e129.

Kumar V., Brainard D.C. & Bellinder R.R. (2009). Suppression of powell amaranth (*Amaranthus powellii*) by buckwheat residues: role of allelopathy. Weed Science, 57 (1), 66-73. Doi: 10.1614/WS-08-028.1

Kuo F. E. & Sullivan W. C. (2001). Aggression and violence in the inner city: Effects of environment via mental fatigue. Environment and behavior, 33(4), 543-571. Doi: 10.1177/00139160121973124

Lamichhane J. R., Messéan A., & Ricci P. (2019). Research and innovation priorities as defined by the Ecophyto plan to address current crop protection transformation challenges in France. Advances in agronomy, 154, 81-152. Doi: 10.1016/bs.agron.2018.11.003

Lankau R.A. (2010). Soil microbial communities alter allelopathic competition between *Alliaria petiolata* and a native species. Biological Invasions 12, 2059e2068. Doi: 10.1007/s10530-009-9608-z

Larcher W. (1995). Physiological plant ecology. Springer-Verlag: Berlin.

Leather G. R. & Einhellig, F. A. (1986). Bioassays in the study of allelopathy. In The Science of Allelopathy; Wiley: New York

Lee, I., Choi, H., Bang, K. S., Kim, S., Song, M., & Lee, B. (2017). Effects of forest therapy on depressive symptoms among adults: A systematic review. International journal of environmental research and public health, 14(3), 321. Doi: 10.3390/ijerph14030321

Leifa F., Pandey A. & Soccol C. R. (2000). Solid state cultivation—an efficient method to use toxic agro-industrial residues. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 40(3), 187-197. Doi: 10.1002/1521-4028(200007)40:3<187::AID-JOBM187>3.0.CO;2-Q

Li Z.R, Amist N. & Bai L.Y. (2019). Allelopathy in sustainable weeds management. Allelopathy Journal, 48 (2), 109-138. Doi: 10.26651/2019-48-2-1249

Li, H., Feng, Y., Li, X., & Zeng, D. (2018). Analytical confirmation of various herbicides in drinking water resources in sugarcane production regions of Guangxi, China. Bulletin of environmental contamination and toxicology, 100(6), 815-820. Doi: 10.1007/s00128-018-2324-6

Liu X., Cao A., Yan D., Ouyang C., Wang Q., Li Y. (2021). Overview of mechanisms and uses of biopesticides. International Journal of Pest Management, 67, 65-72. Doi: 10.1080/09670874.2019.1664789.

Lonhienne T., Garcia M. D., Pierens G., Mobli M., Nouwens A. & Guddat L. W. (2018). Structural insights into the mechanism of inhibition of AHAS by herbicides. Proceedings of the National Academy of Sciences, 115(9), E1945-E1954. Doi: 10.1073/pnas.1714392115

López-Piñeiro A., Peña D., Albarrán A., Becerra D. & Sánchez-Llerena J. (2013). Sorption, leaching and persistence of metribuzin in Mediterranean soils amended with olive mill waste of different degrees of organic matter maturity. Journal of environmental management, 122, 76-84. doi: 10.1016/j.jenvman.2013.03.006

Lorenzo P. & Rodríguez-Echeverría S. (2015). Cambios provocados en el suelo por la invasión de acacias australianas. Revista Ecosistemas, 24(1), 59-66. Doi: 10.7818/ECOS.2015.24-1.10

Lorenzo P., Palomera-Pérez A., Reigosa M.J. & González L. (2011). Allelopathic interference of invasive *Acacia dealbata* Link on the physiological parameters of native understory species. Plant Ecology, 212 (3), 403–412. Doi: 10.1007/s11258-010-9831-9

Lorenzo P., Pazos-Malvido E., González L. & Reigosa M. J. (2008). Allelopathic interference of invasive *Acacia dealbata*: physiological effects. Allelopathy Journal, 22(2), 64-76.

Lorenzo P., Reboredo-Durán J., Múñoz L., González L., Freitas H. & Rodríguez-Echeverría S. (2016). Inconsistency in the detection of phytotoxic effects: a test with *Acacia dealbata* extracts using two different methods. Phytochemistry Letters, 15, 190-198. doi.org/10.1016/j.phytol.2015.11.001

Lorenzo P., Souza-Alonso P., Guisande-Collazob A & Freitas H. (2019). Influence of *Acacia dealbata* Link bark extracts on the growth of *Allium cepa* L. plants under high salinity conditions. Journal of the Science of Food and Agriculture, 99, 4072–4081. Doi: 10.1002/jsfa.9637

Macias F., Molinillo J.M.G., Galindo J.C.G., Varela R.M., Simonet A.M. & Castellano D. (2001). The use of allelopathic studies in the search for natural herbicides. Journal of Crop Production 4, 237-55. Doi: 10.1300/J144v04n02_08

Macías, F. A., Mejías, F. J., & Molinillo, J. M. (2019). Recent advances in allelopathy for weed control: from knowledge to applications. Pest management science, 75(9), 2413-2436. Doi: 10.1002/ps.5355

Maes, J., Zulian, G., Günther, S., Thijssen, M., & Raynal, J. (2019). Enhancing resilience of urban ecosystems through green infrastructure (EnRoute). Inception Report. Doi: 10.2760/602928

Marble, S. C., Koeser, A. K., Hasing, G., McClean, D., & Chandler, A. (2017). Efficacy and estimated annual cost of common weed control methods in landscape planting beds. HortTechnology, 27(2), 199-211. Doi: 10.21273/HORTTECH03609-16

Marchante H., Morais M., Freitas H., Marchante E. (2014) Guia prático para a identificação de Plantas Invasoras em Portugal. Coimbra. Imprensa da Universidade de Coimbra. 207 pp.

Marisa C. Gaspar, Diogo A. Fonseca, Manuel J. Antunes, Christian Frigerio, Nelson G. M. Gomes, M. Vieira, Armanda E. Santos, Maria T. Cruz, Maria D. Cotrim & Maria G. Campos (2018). Polyphenolic characterisation and bioactivity of an *Oxalis pes-caprae* L. leaf extract, Natural Product Research, 32:6, 732-738. Doi: 10.1080/14786419.2017.1335728

McDonald R. I., Mansur A. V., Ascensão F., Crossman K., Elmqvist T., Gonzalez A., ... & Ziter, C. (2019). Research gaps in knowledge of the impact of urban growth on biodiversity. Nature Sustainability, 3, 16-24. Doi: 10.1038/s41893-019-0436-6

McLeod, R. (2018). Annual Costs of Weeds in Australia. eSYS Development Pty Limited. Published by the Centre for Invasive Species Solutions, Canberra, Australia.

Medina-Pastor P. & Triacchini G. (2020). The 2018 European Union report on pesticide residues in food. EFSA Journal, 18(4). Doi: 10.2903/j.efsa.2020.6057

Melander B., Holst N., Grundy A. C., Kempenaar C., Riemens M. M., Verschwele A. & Hansson D. (2009). Weed occurrence on pavements in five North European towns. Weed Research, 49(5), 516-525. Doi: 10.1111/j.1365-3180.2009.00713.x

Mesnage R., Defarge N., Spiroux de Vendômois J. & Séralini G.E. (2015). Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food and Chemical Toxicology, 84, 133-53. doi: 10.1016/j.fct.2015.08.012.

Moiteiro C., Joao M., Curto O.M., Mohamed N., Bailen M., Martínez-Díaz R. & González-Coloma A. (2006). Biovalorization of friedelane triterpenes derived from cork processing industry byproducts. Journal of Agricultural and Food Chemistry, 54:3566–3571. Doi: 10.1021/jf0531151

Mshelmbula B. P., Akomolafe G. F., Thomas T. L., Okogbaa J. I. & Thomas T. H. (2018). Effects of Caffeine on the Seed Germination of Bambara Groundnut (*Vigna subterranean* (L.) Verdc). Nigerian Journal of Basic and Applied Sciences, 26(1), 128-131. Doi: 10.4314/njbas.v26i1.14

Mussatto S. I., Carneiro L. M., Silva J. P., Roberto I. C. & Teixeira J. A. (2011). A study on chemical constituents and sugars extraction from spent coffee grounds. Carbohydrate polymers, 83(2), 368-374. Doi: 10.1016/j.carbpol.2010.07.063

Narwal S.S. (2010). Allelopathy in ecological sustainable organic agriculture. Allelopathy Journal, 25(1), 51-72. Doi: 10.1007/1-4020-4280-9_24

Nascimento G. G., Locatelli J., Freitas P. C. & Silva G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian journal of microbiology, 31(4), 247-256. Doi: 10.1590/S1517-83822000000400003

Ndolo D., Njuguna E., Adetunji C. O., Harbor C., Rowe A., Den Breeyen A., ... & Hospet R. (2019). Research and development of biopesticides: Challenges and prospects. Outlooks on Pest Management, 30(6), 267-276. Doi: 10.1564/v30_dec_08

Nilon C. H., Aronson M. F., Cilliers S. S., Dobbs C., Frazee L. J., Goddard M. A., O'Neill K.M., Roberts D., Stander E.K., Werner P., Winter M. & Yocom, K. P. (2017). Planning for the future of urban biodiversity: a global review of city-scale initiatives. BioScience, 67(4), 332-342. Doi:10.1093/biosci/bix012

Omezzine F., Ladhari A., Rinez A. & Haouala, R. (2011). Potent herbicidal activity of *Inula crithmoides.* L.. Scientia Horticulturae, 130, 853–861. Doi: 10.1016/j.scienta.2011.08.013

ONU (2015). Guia sobre Desenvolvimento Sustentável 17 objetivos para transformar o nosso mundo

Paganelli A., Gnazzo V., Acosta H., López S.L. & Carrasco A.E. (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology, 23(10), 1586-95. Doi: 10.1021/tx1001749.

Pallett, K. (2016). Can we expect new herbicides with novel modes of action in the foreseeable future?. Outlooks on Pest Management, 27(1), 39-43. Doi: 10.1564/v27_feb_09

Palma-Bautista C., Tahmasebi B. K., Fernández-Moreno P. T., Rojano-Delgado A. M., Alcántara de la Cruz R. & De Prado R. (2018). First case of *Conyza canadensis* from Hungary with multiple resistance to glyphosate and flazasulfuron. Agronomy, 8(8), 157. Doi: 10.3390/agronomy8080157

Panouillé M., Ralet M. C., Bonnin E., Thibault J. F. (2007). Recovery and reuse of trimmings and pulps from fruit and vegetable processing. In: *Handbook of waste management and co-product recovery in food processing,* 417-47. Cambridge: Woodhead Publishing Limited. Doi: 10.1533/9781845692520.4.417

Papini, A., Signorini, M. A., Foggi, B., Della Giovampaola, E., Ongaro, L., Vivona, L., ... & Bruschi, P. (2017). History vs. legend: Retracing invasion and spread of *Oxalis pes-caprae* L. in Europe and the Mediterranean area. *PloS one*, *12*(12), e0190237. Doi: 10.1371/journal.pone.0190237

Paspatis, E. A. (1985). Chemical, cultural and biological control of *Oxalis pes-caprae* in vineyards in Greece. In *Weed Control on Vine and Soft Fruits*, 27-29. CRC Press.

Pedrol N, González L, Reigosa MJ (2006). Allelopathy and abiotic stress. In: *Allelopathy: a physiological process with ecological implications,* 171–209. Springer, The Netherlands.

Prophecy Market Insights, 2020. Global Bioherbicides Market, By Mode of Application (Seed Treatment, Soil Application, Foliar, and Post-Harvest), By Source (Microbial, Biochemical, and Others (Plant Phytotoxic Residues and Other Botanical Extracts)), By Formulation (Granular, Liquid, and Others (Pellets, Dust, and Powder Form)), By Application (Agricultural Crops, Cereals & Grains, Oilseeds & Pulses, Fruits & Vegetables, Non-Agricultural Crops, Turf & Ornamentals, and Plantation Crops), and By Region (North America, Europe, Asia Pacific, Latin America, and the Middle East & Africa) - Trends, Analysis and Forecast till 2029"

Pujol D., Liu C., Gominho J., Olivella M. À., Fiol N., Villaescusa I. & Pereira H. (2013). The chemical composition of exhausted coffee waste. Industrial Crops and Products, 50, 423-429. Doi: 10.1016/j.indcrop.2013.07.056

Quercus (2016). Glifosato: o herbicida que contamina Portugal. Quercus Ambiente, 77, 9-12.

Radhakrishnanv R., Alqarawi A.A.& Abd_Allahv E.F. (2018). Bioherbicides: Current knowledge on weed control mechanism. Ecotoxicology and Environmental Safety, 158, 131-138. Doi: 10.1016/j.ecoenv.2018.04.018.

Ramírez-García R., Gohil N. & Singh V. (2019). Recent advances, challenges, and opportunities in bioremediation of hazardous materials. In *Phytomanagement of Polluted Sites*, 517-568. Elsevier. Doi: 10.1016/B978-0-12-813912-7.00021-1

Rankenberg T., Geldhof B., van Veen H., Holsteens K., Van de Poel B. & Sasidharan R. (2021). Age-dependent abiotic stress resilience in plants. Trends in Plant Science, 20(20), 1-14. Doi: 10.1016/j.tplants.2020.12.016

Rastandeh A., Brown D.K. & Pedersen Zari M. (2017). Biodiversity conservation in urban environments: a review on the importance of spatial patterning of landscapes. In *Ecocity World Summitt*. Melbourne, Australia.

Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Communities, 309, 1–50.

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EECText with EEA relevance. Official Journal of the European Communities, 70, 1–16.

Reigosa M. J. & Pazos-Malvido E. (2007). Phytotoxic effects of 21 plant secondary metabolites on *Arabidopsis thaliana* germination and root growth. Journal of chemical ecology, 33(7), 1456-1466. Doi: 10.1007/s10886-007-9318-x

Reigosa M.J., Sánchez-Moreiras A.M. & González L. (1999a). Ecophysiological approach in allelopathy. Critical Reviews in Plant Sciences 18, 577–608. doi:10.1016/S0735-2689(99)00392-5

Reigosa M.J., Souto X.C., Gonzalez L. (1999b). Effect of phenolic compounds on the germination of six weeds species. Plant Growth Regulation 28, 83–88. doi:10.1023/A:1006269716762

Richardson D.M. & Rejmanek M. (2011). Trees and shrubs as invasive alien species – a global review. Diversity and distributions, 17(5), 788-809. Doi: 10.1111/j.1472-4642.2011.00782.x

Ritchie H. & Roser M. (2020) - "Urbanization". Our World in Data. Available on: https://ourworldindata.org/urbanization

Ritchie H., & Roser M. (2013). Land use. Our World in Data. Available on: https://ourworldindata.org/land-use

Romano R.M., Romano M.A., Bernardi M.M., Furtado P.V., Oliveira C.A. (2010). Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology, 84(4), 309-17. Doi: 10.1007/s00204-009-0494-z

Ruibal Brunet I. J., Dubed Echevarría M., Martínez Luzardo F., Noa Romero E., Vargas Guerra L. M. & Santana Romero J. L. (2003). Inhibición de lareplicación del virus de inmunodeficiencia humana por extractos de taninos de *Pinus caribaea* Morelet. Revista Cubana de farmácia, 37(2).

Ruiz-Avila R.J. & Klemm V.V. (1996). Management of *Hydrocotyle ranunculoides* L.f., an aquatic invasive weed of urban waterways in Western Australia. Hydrobiologia 340, 187–190. Doi: 10.1007/BF00012753

Ruth M. & Coelho D. (2007). Understanding and managing the complexity of urban systems under climate change. Climate Policy, 7(4), 317–336.oi 10.1080/14693062.2007.9685659

Saini R. & Singh S. (2019). Use of Natural Products for Weed Management in High-Value Crops: An Overview. American Journal of Agricultural Research, 4 (25), 1-13. Doi: 10.20944/preprints201810.0737.v1

Sánchez-Moreiras A. M., Weiss O. A. & Reigosa-Roger M. J. (2003). Allelopathic evidence in the Poaceae. The Botanical Review, 69(3), 300-319. Doi: 10.1663/0006-8101(2003)069[0300:AEITP]2.0.CO;2

Santana-Méridas O., González-Coloma A. & Sánchez-Vioque R. (2012). Agricultural residues as a source of bioactive natural products. Phytochemistry reviews, 11(4), 447-466. Doi: 10.1007/s11101-012-9266-0

Sbai H., Saad I., Ghezal N., Greca M. Della & Haouala R. (2016). Bioactive compounds isolated from *Petroselinum crispum* L. leaves using bioguided fractionation. Industrial Crops and Products, 89, 207–214. Doi: 10.1016/j.indcrop.2016.05.020

Scavo A., Pandino G., Restuccia A. & Mauromicale G. (2020). Leaf extracts of cultivated cardoon as potential bioherbicide. Scientia Horticulturae, 261, 109024. Doi: 10.1016/j.scienta.2019.109024

Schmidt O., Padel S. & Levidow L. (2012). The bio-economy concept and knowledge base in a public goods and farmer perspective. Bio-based and applied economics, 1(1), 47-63. Doi: 10.13128/BAE-10770

Seigler D.S. (2003). Phytochemistry of Acacia-sensu lato. Biochemical systematics and ecology, 31(8), 845-873. Doi: 10.1016/S0305-1978(03)00082-6

Seto K. C., Guneralp B. & Hutyra L. R. (2012). Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. Proceedings of the National Academy of Sciences, 109, 16083-16088. Doi: 10.1073/pnas.1211658109

Shanahan D. F., Franco L., Lin B. B., Gaston K. J. & Fuller R. A. (2016). The benefits of natural environments for physical activity. Sports Medicine, 46(7), 989-995. Doi:10.1007/s40279-016-0502-4

Sherwani S. I., Arif I. A. & Khan H. A. (2015). Modes of Action of Different Classes of Herbicides. In: *Herbicides, physiology of action, and safety,* 165-186. London: IntechOpen; https://doi.org/10.5772/61779.

Shoemaker M., Hamilton B., Dairkee S. H., Cohen I. & Campbell M. J. (2005). In vitro anticancer activity of twelve Chinese medicinal herbs. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 19(7), 649-651. Doi: 10.1002/ptr.1702

Sikorski Ł., Baciak M., Bęś A. & Adomas B. (2019). The effects of glyphosate-based herbicide formulations on *Lemna minor*, a non-target species. Aquatic Toxicology, 209, 70-80. Doi: 10.1016/j.aquatox.2019.01.021

Silvério F.O., De Alvarenga E.S., Moreno S.C. & Picanço M.C. (2009). Synthesis and insecticidal activity of new pyrethroids. Pest Management Science, 65, 900–905. Doi: 10.1002/ps.1771.

Sims D. A., & Gamon J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote sensing of environment, 81(2-3), 337-354. Doi: 10.1016/S0034-4257(02)00010-X

Singh M., Sharma S.D. (2008). Benefits of triazine herbicides and other weed control technology in citrus management. *In The Triazine Herbicides – 50 Years Revolutionizing Agriculture,* 199–209. San Diego: Elsevier. Doi: 10.1016/B978-044451167-6.50019-2

Soltys D., Krasuska U., Bogatek R. & Gniazdowska A. (2013). Allelochemicals as bioherbicides—present and perspectives. In Herbicides—current research and case studies in use, 517–542. IntechOpen. Doi:10.5772/56743

Souto X.C., Chiapusio G. & Pellissier F. (2000). Relationship between phenolics and soil microorganisms in spruce forest: singnificance for natural regeneration. Journal of Chemical Ecology 26, 2015–2023. doi:10.1023/A:1005551912405

Storup K., Mattfolk K., Voinea D., Jakobsen B., Bain M., Reverté Casas M., & Oliveira P. (2016). Combating Food Waste: An Opportunity for the EU to Improve the Resource-Efficiency of the Food Supply Chain. European Court of Auditors, European Union: Luxembourg.

Tanti A., Bhattacharyya P. N., Sandilya S. P. & Dutta P. (2016). Allelopathic potential of caffeine as growth and germination inhibitor to popular tea weed, *Borreria hispida* L. Current Life Sciences, 2(4), 114-117.

Tejada, M., & Benítez, C. (2017). Flazasulfuron behavior in a soil amended with different organic wastes. Applied Soil Ecology, 117, 81-87. Doi: 10.1016/j.apsoil.2017.05.009

Thirumurugan D., Cholarajan A., Raja S.S.S. & Vijayakumar R. (2018). An Introductory Chapter: Secondary Metabolites. In *Secondary Metabolites - Sources and Applications,* 1-21. Doi: 10.5772/intechopen.79766

Thogmartin W. E., Wiederholt R., Oberhauser K., Drum R. G., Diffendorfer J. E., Altizer S., Taylor O.R., Pleasants J., Semmens D., Semmens B., Erickson R., Libby K. & Lopez-Hoffman L. (2017). Monarch butterfly population decline in North America: identifying the threatening processes. Royal Society open science, 4(9), 170760. Doi: 10.1098/rsos.170760

Tozzi E., Beckie H., Weiss R., Gonzalez-Andujar J. L., Storkey J., Cici S. Z. H. & Van Acker R. C. (2014). Seed germination response to temperature for a range of international populations of *Conyza canadensis*. Weed Research, 54(2), 178-185. Doi: 10.1111/wre.12065

Travlos I. S., Paspatis E. & Psomadeli E. (2008). Allelopathic potential of *Oxalis pes-caprae* tissues and root exudates as a tool for integrated weed management. Journal of agronomy. 7: 202-205. Doi: 10.3923/ja.2008.202.205

Turk M A. & Tawaha A. R. M. (2002). Inhibitory effects of aqueous extracts of black mustard on germination and growth of lentil. Pakistan Journal of Agronomy, 1(1), 28-30. Doi: 10.3923/ja.2002.28.30

Turk M. A., Shatnawi M. K. & Tawaha A. M. (2003). Inhibitory effects of aqueous extracts of black mustard on germination and growth of alfalfa. Weed Biology and Management, 3(1), 37-40. Doi: 10.1046/j.1445-6664.2003.00079.x

UN (2018). The World's cities in 2018 - Data Booklet (ST/ESA/ SER.A/417). Department of Economic and Social Affairs, Population Division, World Urbanization Prospects, 1-34.

USDA-NASS, USDA National Agricultural Statistics Service: Quick stats. USDA (2017). Available on: https://quickstats.nass.usda.gov/

Van Bruggen A. H., He M. M., Shin K., Mai V., Jeong K. C., Finckh M. R. & Morris Jr, J. G. (2018). Environmental and health effects of the herbicide glyphosate. Science of the Total Environment, 616, 255-268. Doi: 10.1016/j.scitotenv.2017.10.309

van Wilgen B. W., Fill J. M., Baard J., Cheney C., Forsyth A. T. & Kraaij T. (2016). Historical costs and projected future scenarios for the management of invasive alien plants in protected areas in the Cape Floristic Region. Biological Conservation, 200, 168-177. Doi: 10.1016/j.biocon.2016.06.008

Vardanega R., Prado J. M. & Meireles M. A. A. (2015). Adding value to agri-food residues by means of supercritical technology. The Journal of Supercritical Fluids, 96, 217-227. Doi: 10.1016/j.supflu.2014.09.029

Vats S. (2015) Herbicides: History, Classification and Genetic Manipulation of Plants for Herbicide Resistance. In *Sustainable Agriculture Reviews. Sustainable Agriculture Reviews*. Springer, Cham. Doi: 10.1007/978-3-319-09132-7_3

Vilà M., M. Tessier C. M. Suehs, G. Brundu, L. Carta, A. Galanidis, P. Lambdon et al. (2006). Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. Journal of Biogeography 33: 853–861. Doi: 10.1111/j.1365-2699.2005.01430.x

Ward B.G., Henzell R. F., Holland P. T. & Spiers A.G. (1999). Non-spray methods to control invasive weeds in urban areas. Proceedings of 52nd New Zealand Plant Protection Conference: 1-5.

Weir T.L., Park S-W. & Vivanco J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. Current Opinion in Plant Biology 7, 472–479. Doi: 10.1016/j.pbi.2004.05.007

Werrie P.-Y., Durenne B., Delaplace P. & Fauconnier M.-L. (2020). Phytotoxicity of Essential Oils: Opportunities and Constraints for the Development of Biopesticides. A Review. Foods, 9, 1291. Doi: 10.3390/foods9091291

Westbrooks, R.G. (1998). Invasive Plants, Changing the Landscape of America: Fact Book. Federal Interagency Committee for the Management of Noxious and Exotic Weeds,109. Washington, D.C.

Wilms W., Woźniak-Karczewska M., Syguda A., Niemczak M., Ławniczak Ł., Pernak J., Rogers R. D. & Chrzanowski Ł. (2020). Herbicidal Ionic Liquids: A Promising Future for Old

Herbicides? Review on Synthesis, Toxicity, Biodegradation, and Efficacy Studies. Journal of agricultural and food chemistry, 68(39), 10456–10488. Doi: 10.1021/acs.jafc.0c02894

Yamane K., Kono M., Fukunaga T., Iwai K., Sekine R., Watanabe Y. & Iijima M. (2014). Field evaluation of coffee grounds application for crop growth enhancement, weed control, and soil improvement. Plant Production Science, 17(1), 93-102. Doi: 10.1626/pps.17.93

Zhao J., Pacenka S., Wu J., Richards B. K., Steenhuis T., Simpson K. & Hay A. G. (2018). Detection of glyphosate residues in companion animal feeds. Environmental pollution, 243, 1113-1118. Doi: 10.1016/j.envpol.2018.08.100

Zhu M., Wang L., Wang Y., Zhou J., Ding J., Li W., Xin Y., Fan S., Wang Z. & Wang Y. (2018). Biointeractions of herbicide atrazine with human serum albumin: UV-Vis, fluorescence and circular dichroism approaches. International journal of environmental research and public health, 15(1), 116. Doi: 10.3390/ijerph15010116

Zhu X., Zhang J. & Ma K. (2011). Soil biota reduce allelopathic effects of the invasive Eupatorium adenophorum. Plos One 6, e25393. doi.org/10.1371/journal.pone.0025393

Zimdahl, R. L. (2007). Fundamentals of Weed Science. 3th Edition. Academic press.

Zulet A., Gil-Monreal M., Villamor J. G., Zabalza A., van der Hoorn R. A. & Royuela M. (2013). Proteolytic pathways induced by herbicides that inhibit amino acid biosynthesis. PLoS One, 8(9), e73847. Doi: 10.1371/journal.pone.0073847

APPENDIX

TABLE 1. Generalized Linear Models (GLMs) for the effects of each extract (Tr), concentration (C) and the interaction between them on the germination in different substrates (soil and paper) of *Achillea ageratum*, *Conyza canadensis* and *Dittrichia viscosa*. Linear Mixed Models (LMMs) for the effects of each extract (Tr), concentration (C) and the interaction between them on the radicle length and stem length of *Achillea ageratum*, *Conyza canadensis* and *Dittrichia viscosa* in different substrates (soil and paper). Results correspond to degrees of freedom (Df) or numerator degrees of freedom (Num Df), residual degrees of freedom (Resid Df) or denominator degrees of freedom (Dem Df), residual deviance (Resid deviance) or F values and Pr(>Chi) values or P values. Values in bold indicate significance at P≤0.05 level.

Species	Variable	Substrate	Model	Error family	Link function	Factor	Df / Num DF	Deviance	Resid Df/Dem Df	Resid deviance /F	Pr(>Chi) / *P*
Achillea ageratum	Germination	Soil	GLM	Poisson	Log	Tr	2	5.049	72	217.385	0.080
						C	4	190.196	68	27.189	**<0.001**
						TrxC	8	4.882	60	22.307	0.770
		Paper	GLM	Poisson	Log	Tr	2	11.668	72	387.82	**0.003**
						C	4	311.445	68	76.380	**<0.001**
						TrxC	8	38.915	60	37.460	**<0.001**
	Radicle length	Soil and paper	LMM	-	-	Tr	2	-	2013	22.931	**<0.001**
						C	4	-	2013	88.309	**<0.001**
						TrxC	8	-	2013	26.698	**<0.001**
	Stem length	Soil	LMM	-	-	Tr	2	-	1031	12.865	**<0.001**
						C	4	-	1031	92.051	**<0.001**
						TrxC	8	-	1031	3.199	**0.005**
		Paper	LMM	-	-	Tr	2	-	885	37.903	**<0.001**
						C	4	-	885	27.249	**<0.001**
						TrxC	8	-	885	6.205	**<0.001**

Species	Variable	Substrate	Model	Error family	Link function	Factor	Df / Num DF	Deviance	Resid Df/Dem Df	Resid deviance /F	Pr(>Chi) / *P*
Conyza canadensis	Germination	Soil	GLM	Poisson	Log	Tr	2	1.181	72	282.729	0.554
						C	4	203.573	68	79.156	**<0.001**
						TrxC	8	5.571	60	73.585	0.695
		Paper	GLM	Poisson	Log	Tr	2	65.88	72	536.20	**<0.001**
						C	4	395.33	68	140.87	**<0.001**
						TrxC	8	109.09	60	31.790	**<0.001**
	Radicle length	Soil	LMM	-	-	Tr	2	-	1012	62.400	**<0.001**
						C	4	-	1012	50.261	**<0.001**
						TrxC	8	-	1012	15.551	**<0.001**
		Paper	LMM	-	-	Tr	2	-	769	74.272	**<0.001**
						C	3	-	769	14.512	**<0.001**
						TrxC	6	-	769	59.256	**<0.001**
	Stem length	Soil	LMM	-	-	Tr	2	-	1012	96.576	**<0.001**
						C	4	-	1012	74.640	**<0.001**
						TrxC	8	-	771	16.019	**<0.001**
		Paper	LMM	-	-	Tr	2	-	771	83.588	**<0.001**
						C	3	-	771	241.137	**<0.001**
						TrxC	6	-	771	28.091	**<0.001**
Dittrichia viscosa	Germination	Soil	GLM	Poisson	Log	Tr	2	0.112	72	85.121	0.946
						C	4	33.533	68	51.589	**<0.001**
						TrxC	8	4.388	60	47.201	0.821

Species	Variable	Substrate	Model	Error family	Link function	Factor	Df / Num DF	Deviance	Resid Df/Dem Df	Resid deviance /F	Pr(>Chi) / *P*
		Paper	GLM	Poisson	Log	Tr	2	2.720	71	170.855	0.159
						C	4	130.214	67	40.642	**<0.001**
						TrxC	8	7.679	59	32.963	0.488
	Radicle length	Soil	LMM	-	.	Tr	2	-	599	19.543	**<0.001**
						C	4	-	599	126.029	**<0.001**
						TrxC	8	-	599	7.582	**<0.001**
		Paper	LMM	-	-	Tr	2	-	261	11.027	**<0.001**
						C	3	-	261	79.416	**<0.001**
						TrxC	6	-	261	6.767	**<0.001**
	Stem length	Soil	LMM	-	-	Tr	2	-	601	3.349	**0.036**
						C	4	-	601	16.705	**<0.001**
						TrxC	8	-	601	1.736	0.087
		Paper	LMM	-	-	Tr	2	-	269	1.731	0.179
						C	3	-	269	4.535	**0.004**
						TrxC	6	-	269	2.830	**0.011**

TABLE 2. Generalized Linear Models (GLMs) for the effects of each extract (Tr), concentration (C) and the interaction between them on leaf number increase, dry biomass and mortality of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa*. Linear Models (LMs) for the effects of each extract (Tr), concentration (C) and the interaction between them on the foliar length increase of *Achillea ageratum, Conyza canadensis* and *Dittrichia viscosa*. Results correspond to degrees of freedom (Df) or numerator degrees of freedom (Num Df), residual degrees of freedom (Resid Df) or Mean square (Mean sq), residual deviance (Resid deviance) or F values and Pr(>Chi) values or P values. Values in bold indicate significance at P≤0.05 level and italic refers to analysed covariables: NF0 (Leaf number before treatment application), CMF0 (Maximum foliar length before treatment application), INF (Leaf number increase), ICF (Foliar length increase).

Species	Variable	Model	Error family	Link function	Factor	Df / Num DF	Deviance/Sum sq	Resid Df/Mean sq	Resid deviance /F	Pr(>Chi) / *P*
Achillea ageratum	Leaf number increase	GLM	QuasiPoisson	Log	Tr	2	1.344	144	81.010	0.221
					C	4	19.401	140	61.609	**<0.001**
					TrxC	8	2.406	132	59.203	0.713
					NF0	*1*	*0.112*	*146*	*82.354*	*0.616*
	Foliar length increase	LM			Tr	2	0.824	0.412	1.818	0.166
					C	4	9.15	2.288	10.095	**<0.001**
					TrxC	8	5.909	0.737	3.26	**0.002**
					CMF0	*1*	*4.733*	*4.733*	*20.888*	***<0.001***
	Dry biomass	GLM	Gamma	identity	Tr	2	0.909	68	37.324	0.110
					C	4	20.11	64	17.214	**< 0.001**
					TrxC	8	4.335	56	12.879	**0.007**
					INF	*1*	*2.423*	*71*	*42.945*	***0.001***
					ICF	*1*	*4.712*	*70*	*38.233*	***<0.001***
	Mortality	GLM	Binomial	cloglog	Tr	2	2.238	146	9.763	0.327
					C	4	3.484	142	6.279	0.480
					TrxC	8	0	134	6.279	1
	Chlorophyll a	LM			Tr	2	0.118	0.059	0.496	0.612
					C	4	1.946	0.486	4.074	**0.006**
					TrxC	8	0.474	0.059	0.496	0.853

Species	Variable	Model	Error family	Link function	Factor	Df / Num DF	Deviance/Sum sq	Resid Df/Mean sq	Resid deviance /F	Pr(>Chi) / *P*
	Chlorophyll b	LM			Tr	2	0.073	0.037	0.517	0.599
					C	4	0.412	0.103	1.455	0.229
					TrxC	8	0.392	0.049	0.693	0.696
	Carotenoids	LM			Tr	2	0.038	0.019	0.446	0.643
					C	4	0.039	0.010	0.232	0.919
					TrxC	8	0.244	0.031	0.717	0.676
Conyza canadensis	Leaf number increase	GLM	QuasiPoisson	Log	Tr	2	1.137	144	74.516	0.109
					C	4	33.008	140	41.508	**<0.001**
					TrxC	8	7.09	132	34.418	**0.001**
					NF0	*1*	*0.047*	*146*	*75.653*	***0.668***
	Foliar length increase	LM			Tr	2	0.138	0.069	0.603	0.549
					C	4	10.195	2.549	22.271	**<0.001**
					TrxC	8	3.438	0.430	3.755	**0.001**
					CMF0	*1*	*0.005*	*0.005*	*0.042*	*0.839*
	Dry biomass	GLM	Gamma	identity	Tr	2	0.948	68	44.133	0.503
					C	4	9.879	64	34.254	**0.006**
					TrxC	8	3.998	56	30.256	0.671
					ICF	*1*	*2.297*	*70*	*45.081*	*0.068*
	Mortality	GLM	Binomial	cloglog	Tr	2	0	145	< 0.001	1
					C	4	0	141	< 0.001	1
					TrxC	8	0	133	< 0.001	1
	Chlorophyll a	LM			Tr	2	0.925	0.463	0.616	0.544
					C	4	10.609	2.652	3.534	**0.012**

Species	Variable	Model	Error family	Link function	Factor	Df / Num DF	Deviance/Sum sq	Resid Df/Mean sq	Resid deviance /F	Pr(>Chi) / *P*
					TrxC	8	9.808	1.226	1.634	0.136
	Chlorophyll b	LM			Tr	2	0.340	0.170	0.913	0.407
					C	4	2.450	0.613	3.287	**0.017**
					TrxC	8	3.121	0.390	2.093	0.052
	Carotenoids	GLM	Gamma	identity	Tr	2	0.480	68	54.227	0.608
					C	4	10.119	64	44.108	**<0.001**
					TrxC	8	6.473	56	37.635	0.099
Dittrichia viscosa	Leaf number increase	GLM	QuasiPoisson	Log	Tr	2	5.941	146	74.283	**<0.001**
					C	4	28.59	142	45.693	**<0.001**
					TrxC	8	9.279	134	36.414	**<0.001**
					NF0	*1*	*1.577*	*148*	*80.224*	***0.014***
	Foliar length increase	LM			Tr	2	0.067	0.033	0.168	0.846
					C	4	8.057	2.014	10.139	**<0.001**
					TrxC	8	1.012	0.126	0.637	0.746
					CMF0	*1*	*2.319*	*2.319*	*11.671*	***0.001***
	Dry biomass	GLM	Gamma	identity	Tr	2	0.436	71	13.267	**0.022**
					C	4	4.823	67	8.444	**<0.001**
					TrxC	8	4.677	59	3.767	**<0.001**
					INF	*1*	*1.708*	*73*	*13.703*	***<0.001***
	Mortality	GLM	Binomial	cloglog	Tr	2	2.211	147	9.804	0.331
					C	4	3.302	143	6.502	0.509
					TrxC	8	0	135	6.502	1
	Chlorophyll a	LM			Tr	2	4.108	2.054	5.757	**0.005**

Species	Variable	Model	Error family	Link function	Factor	Df / Num DF	Deviance/Sum sq	Resid Df/Mean sq	Resid deviance /F	Pr(>Chi) / *P*
					C	4	8.973	2.243	6.287	**<0.001**
					TrxC	8	8.231	1.029	2.884	**0.009**
	Chlorophyll b	LM			Tr	2	0.906	0.453	5.419	**0.007**
					C	4	1.183	0.296	3.54	**0.012**
					TrxC	8	1.881	0.235	2.814	**0.010**
	Carotenoids	LM			Tr	2	1.231	0.616	6.361	**0.003**
					C	4	0.988	0.247	2.552	**0.048**
					TrxC	8	2.084	0.260	2.692	**0.013**