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Review

Allelopathic research in Brazil

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ABSTRACT

In this article, we review allelopathy studies conducted in Brazil or involving plant species that occur in the country. Conceptions and misconceptions associated with allelopathy, as well as some international criteria to be applied in allelopathic research, are presented and discussed. We observed a sharp increase in the number of papers on this subject conducted in Brazil between 1991 and 2010. However, most studies are conducted under laboratory conditions, lack a clear hypothesis or a solid justification, and typically make use of target species that do not co-exist with the donor species under natural conditions. We also found that most studies do not take the additional steps in order to purify and identify the bioactive molecules. We recommend that further studies be conducted in order to explore the potential of plant biodiversity in Brazil. Such studies could lead to the development of new molecular structures (allelochemicals) that could be used in the control of pests and weeds, thereby reducing the use of the harmful synthetic herbicides that are currently being widely employed.

Key words: allelopathy, allelochemicals, competition, interference, phytotoxicity, weed control

Introduction

The International Allelopathy Society defines allelopathy as "any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agriculture and biological systems" (IAS, 1996). The first recorded use of the word "allelopathy" was by Theophrastus (ca. 300 B.C.), a disciple of Aristotle, who observed that, apart from affecting the development of invader plants, chickpea plants (*Cicer arietinum* L.) did not enrich the soil as other plants did. Since then, numerous studies have described plant interactions characterised as allelopathic (Inderjit & Keating 1999; Fujii & Hiradate 2007; Macías *et al.* 2004; Reigosa *et al.* 2006; Blum 2011). Over time, new definitions have been proposed. According to Inderjit & Callaway (2003), allelopathy is the negative effect that one plant can have on another by releasing chemical compounds into the environment. In addition to a certain adaptation of the term for plant-plant interactions, this definition restricts the concept to negative effects of plants on other plants. This is in contrast to the explanation given by Ferreira (2004), who defined allelopathy as the positive or negative influence that secondary metabolites produced by a plant and shed into the environment have on the growth of other plants (Ferreira, 2004). Here, positive effects can also be considered allelopathic. Within this context, the secondary metabolites involved in allelopathic interactions have been designated allelochemicals.

Despite its tarnished history (Reigosa *et al.* 1999), the scientific study of allelopathy has flourished in recent years. This is due to the fact that, although there has yet to be a true experimental separation of allelopathy from other forms of plant interactions, there is considerable evidence of phenomena that only can be explained in terms of accumulation of allelochemicals in the field. In addition, many authors have found interesting and promising effects of plant residues in the field, as well as of weed-crop interactions that are probably due to intense production and release of such bioactive compounds.

We searched the Scientific Electronic Library Online (SciELO) database (www.scielo.br), using the search terms "allelopathy"" and "allelochemicals", as well as browsing the archives of the *Allelopathy Journal*, limiting our searches to articles published between 1991 and 2010 and involving plant species found in Brazil. Table 1 shows the characteristics of the studies selected. It is noteworthy that

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Table 1. Selected studies employing the terms "allelopathy" and "allelochemicals", published between 1991 and 2010 in journals listed in the Thomson-Reuters Journal Citation Reports.

	1991-1995	1996-2000	2001-2005	2006-2010
Allelopathy or alelopatia world	295	379	646	905
Allelopathy or alelopatia Brazil	3	11	26	85
Allelochemical(s) or aleloquimico(s) World	299	376	525	901
Allelochemical(s) or aleloquimico(s) Brazil	1	11	24	88
Percentage	0,67%	2,91%	4,27%	9,58%

the proportion of papers coming from Brazilian authors is rapidly climbing, indicating that the scientific production of Brazil is gaining greater international exposure. More papers dealing with allelopathy could have been added to our selection had we sought papers related to phytotoxicity, the modes of action of allelochemicals or even the genetic aspects of allelochemical production and release.

This literature review focuses on allelopathy studies conducted in Brazil. This theme has been addressed in earlier reviews (Ferreira *et al.* 1992; Rodrigues *et al.* 1999), as well as in a more recent review of experimental procedures used in laboratory-based allelopathy studies (Souza-Filho 2010). Only the interactions between plants were considered in this review, regardless of whether the effects were negative or positive.

Conceptions and misconceptions associated with allelopathic studies

Although they are two different concepts, "allelopathy" can be confused with "competition" (Ferreira, 2004). Competition can be viewed as a type of interaction between organisms which involves the removal of an element from the environment, such as water, light or minerals, by an organism, which in some way affects the growth of neighbours sharing the same habitat. Allelopathy, on the other hand, is a kind of interaction between organisms that involves the production and release of substances into the environment by one organism, which affects the growth of other, nearby organisms (Dakshini *et al.* 1999). Naturally, in affecting the growth of other organisms, the species that produces and releases such molecules into the environment can benefit in the competition for resources, so that such processes (competition and allelopathy) can occasionally be considered complementary or collaborative (Inderjit & Callaway, 2003).

Allelopathic studies can also be confused with phytotoxicity studies. However, differences in the procedures employed in the extraction of active compounds distinguish one from the other. An allelopathy study is one that is conducted with chemical substances extracted from plant tissue using natural methods such as leaching, exudation and release through the deterioration of plant matter, or even vaporisation; or with allelochemicals extracted under laboratory conditions that replicate a natural process, such as aqueous extraction from plant tissue that has been crushed or decomposed, simulating to some extent the action of rain and dew on parts of the plants in nature. The water to be used for solubilisation of substances can be slightly acidified or alkalinised in order to resemble more closely the pH of the substrate on which the species occur. In contrast, a phytotoxicity study is one that is carried out with substances extracted from plant tissue through any non-natural chemical or physico-chemical procedure, for example, through the use of organic solvents, such as hexane, dichloromethane, and methanol, or by using technologies to extract bioactive compounds from plants, such as ultrasonic, Soxhlet, and high-pressure extraction methods, with the aim of maximising the solubility of chemical substances. Because of these similarities, field and laboratory studies must comply with established criteria and follow basic procedures, as described below, in order to be considered allelopathy studies.

Secondary metabolites and chemical interactions

All plants produce secondary metabolites at various levels of concentration, diversity and composition in a variety of plant tissues (Bonner & Varner 1965; Harbone 1993; Hadacek 2002; Taiz & Zeiger 2010). Although such metabolites can belong to any one of a great number of classes, they generally belong to one of three major categories, specifically terpenes, phenolic compounds and alkaloids (Taiz & Zeiger 2010; Gleason 2012). The production of secondary metabolites by plants is determined by the genetic characteristics of the species producing them and by the environmental conditions in which the plants are found (Hadacek 2002). Variables such as temperature, humidity and light intensity, added to the effects of the biota and the physicochemical structure of the soil, can affect not only the production of metabolites but also the chemical structure and degree of activity of substances released into the environment.

It is of note that the chemical interactions between plants are almost never limited to one compound, but rather to mixtures, often complex ones, of various substances (Gonzalez & Reigosa, 2001). These mixtures can have synergistic or antagonistic effects, which can be modified by the chemical, physical, and biotic properties of the soil (Einhellig 1999). The toxicity of substances and the degree of interaction between organisms depend also on the stage of growth of the donor and recipient species alike (Rice 1984; González & Reigosa 2001).

Many allelopathy studies are carried out to detect direct effects of allelochemicals produced by one (donor) plant on the growth of a target plant. However, quite often the allelochemical under study has an indirect effect on the organism. For example, Grove *et al.* (2012) showed that the abundance of ectomycorrhizal fungi is lower on Douglas-fir seedlings grown in forest soils invaded by *Cytisus scoparius* than on those grown in un-invaded forest soils . Because mycorrhizae help root systems absorb soil nutrients more efficiently, the authors concluded that allelochemicals produced by *C. scoparius* affect the growth of the conifer by reducing the quantity of ectomycorrhizae associated with its roots.

Allelopathic studies can involve concepts and approaches in various fields such as agronomy, forestry, ecology, physiology, anatomy, plant systematics, cellular biology, molecular biology, and molecular chemistry. Consequently, applying the appropriate criteria in allelopathy studies can require a diversified team of scientists, as well as the equipment and infrastructure required in order to conduct experiments in the laboratory, in the greenhouse, and in the field (Ferreira & Aquila, 2000).

Modes of action of allelochemicals

The extent of allelopathic effects has not been fully demonstrated. Nevertheless, there is evidence that some secondary metabolites, apart from helping the producer plant avoid the effects of insects, fungi, herbivores, etc., can be useful as natural bioherbicides. The increasing need for alternative herbicides is another factor currently driving allelopathy research. Therefore, there are two possible objects of interest: allelochemicals acting as synthetic herbicides do (i.e., with a concrete mode of action); and allelochemicals acting subtly, with multiple modes of action, probably quite dependent on the ecological and physiological stage of the recipient plant. We will examine both, because even these mild, subtle effects can be of agro-ecological interest (Reigosa & Carballeira 1992; Reigosa *et al.* 1999).

To date, there have been few studies of the ecological role of many secondary metabolites (Field *et al.* 2006; Duke *et al.* 2010; Martínez-Peñalver *et al.* 2012), although many of them can be considered to play pivotal roles in the life of the plant, improving its fitness as defense substances or attracting beneficial organisms. Many secondary metabolites, despite playing a primary role in defending the producer plant against pathogens or herbivores, can be considered to play secondary roles in plant-plant interactions, by which they nevertheless enhance the competitive potential of the producer (Reigosa *et al.* 1999). The number of studies investigating the modes of action of secondary metabolites is on the rise, which can be mainly attributed to two factors: first, many weeds have evolved resistance to herbicides, and there is some hope that new sites of action, or new modes of action that could alleviate resistance, will be discovered; second, there is hope that, with appropriate management, allelopathic crops will be able to protect themselves from weeds and other pests. This latter approach would be quite good from the ecological and economic point of view, minimising the release of synthetic non-degradable molecules (Dayan *et al.* 2009).

Under normal circumstances, many different molecules are simultaneously released into the environment. Certain potent molecules have been identified as possibly being responsible for real allelopathic relationships in the field. Good examples are juglone (released by *Juglans* spp.); sorgoleone (produced by sorghum); avenacin (a triterpene glycoside produced by *Avena fatua* and potentially responsible for its invasive capacity); momilactone B (released by rice plants), coumarin (released by *Arctostaphylos* spp.); non-protein amino acids (such as mimosine and tyrosine, produced by *Festuca rubra* or *Leucaena leucocephala*); glucosinolates (produced in great quantities by Brassicaceae); and cyanogenic glycosides. Although none of those molecules are potent enough to act individually, they can have significant effects when acting in concert. Most phenolic compounds, for example, are released simultaneously and continuously, perhaps acting synergistically (Reigosa *et al.*, 1999).

To summarize the whole picture of how secondary metabolites can act as allelochemicals, it is worth to mention that these bioactive substances can present different types of effects on plants (Reigosa *et al.* 1999; Field *et al.* 2006; Lotina-Hensen *et al.* 2006). Some allelochemicals have subtle and varied effects on the recipient plant. For example, benzoxazolinone (a quite common hydroxamic acid that is released by several cereals) can simultaneously affect processes such as the cell cycle of the root meristems and the production of reactive oxygen species (and therefore the oxidative stress cycle), as well as membrane permeability, water balance, and osmotic regulation (Baerson *et al.* 2005; Hussain *et al.* 2011; Sánchez-Moreiras & Reigosa 2005; Sánchez-Moreiras *et al.* 2005, 2008, 2009, 2010). This multi-functional activity is quite difficult to investigate, typically requiring the use of numerous high-throughput techniques. These types of allelochemicals are probably best suited for ecologically accepted control of weeds and other pests, only improving the balance between the crop (that produces and releases the allelochemicals) and the target organisms.

There are, of course, some examples of secondary metabolites that have a definite and unique mode of action. In fact, several molecules of natural origin (most from micro-organisms but some also synthesised by higher plants) have been commercialised, either as mixtures of natural compounds or as molecules synthetically modified to enhance their selectivity and capacity of action (Copping & Duke 2007; Duke *et al.* 2010).

Allelopathy in natural and cultivated systems

Allelopathic research can be divided into two main categories: one following concepts and ecological-based

approaches, corresponding to studies on phenomena occurring in natural ecosystems (allelopathy *sensu stricto*); and the other following commercial and economic criteria and interests, corresponding to studies based on interactions between cultivated species that do not naturally occur in the same habitat (applied allelopathy). In the first category, preliminary or complementary laboratory studies should replicate, under controlled conditions, the expected effects of rainfall or dew on the leaching of substances supposed to occur in the natural environment. Indeed, the species under study should co-exist in the same habitat, and such studies should follow these and other criteria (see below) to be considered "true" (*sensu stricto*) allelopathic studies. In the second category, the purpose is directed towards understanding how cultivated species interact with each other and to modify such interactions in some way. Those studies may use different kinds of solvents to extract the bioactive compounds involved in such interactions, and the species under study do not naturally co-exist in the same habitat but do co-exist due to commercial interests, irrespective of whether they are native or exotic. From these two categories, a third approach can emerge, which is to look for and identify bioactive molecules that could potentially be used to develop new herbicides and plant growth regulators (Vyvyan, 2002).

Criteria for allelopathy studies

Plants produce and release various bioactive substances into the environment. Such substances have been frequently shown, under laboratory conditions, to stimulate, neutralise or inhibit biological processes in other individuals. However, it can be quite complex and difficult to prove that such released substances are involved in chemical interactions between plants in nature. The same can be said about competition for resources between plants. Plants require resources such as energy, water and nutrients. However, under field conditions, it is not always possible to demonstrate that competition is taking place (Blum 2011). Nevertheless, although competition for resources is a widely accepted concept in the literature, as is that of chemical interactions between plants and insects, between plants and micro-organisms, and between micro-organisms (Harbone 1993), the same cannot be said about of allelopathic interactions (Blum 2011). Perhaps the difficulties in accepting this line of investigation have resulted from the following (Blum 2011):

- The definition of the term 'allelopathy' has been changed several times.
- The first allelopathic bioassays have not been clearly stated.
- The scepticism of opponents to this kind of research.
- The high level of rigour required to demonstrate that such plant interactions are allelopathic.

The challenge, therefore, lies perhaps in setting real and applicable criteria that could be used as evidence of allelopathic interaction between plants. In this context, the following criteria have typically been recommended and adopted (Blum 2011):

- Research studies must show clear patterns of stimulus or inhibition that donor plants exert over the development of recipient plants.
- Such patterns cannot be explained only by physico-chemical modifications to the environment, by the absorption and use of substances as sources of nutrients or energy, mycorrhizal transfer or grafting, or by biotic factors such as competition for resources, herbivory or acquired disease.
- Allelopathic plants or their waste products must produce and release organic substances into the environment, which are capable of stimulating or inhibiting the functioning of other, nearby plants.
- The recipient plants must be in contact and interact with organic substances produced directly or indirectly by the donor plants (whether modified or not by the environment).
- Such organic substances must be in appropriate concentrations and the exposure time must be sufficient to modify the functioning of the recipient plant.

Although some of these criteria can be met in research conducted under laboratory conditions, or even in the field, observing and demonstrating all of these criteria in nature can be a major challenge for researchers seeking to carry out allelopathy studies. Very few studies have successfully "completed the cycle" or, in other words, have shown the production of a specific metabolite by the allelopathic (donor) plant, its journey through the environment (soil, water, or atmosphere), its arrival at the target, and its influence on the affected (recipient) plant.

Laboratory studies

In studies conducted under controlled conditions, allelopathic activity has frequently been demonstrated. However, as mentioned above, demonstrating that plant tissues from a specific plant produce bioactive substances that affect the functions of the target species in a laboratory experiment cannot be considered proof of allelopathic interaction; for that, it should also be shown that the allelopathic effects are exerted under natural conditions. Such laboratory studies, at most, show only that the donor plant produces bioactive compounds and only suggest that, under natural conditions, those compounds may have some kind of effect on the growth of neighbouring species.

In the literature, there are many experiments purported to be allelopathic that make use of organic solvents, procedures and equipment to optimise the extraction of bioactive substances produced by plant tissues. These procedures maximise the release of substances from plant tissues and can strengthen their biological effects on the development of other plants; consequently, such studies do not in fact represent interaction between neighbouring species in the

field and cannot be used as a proof of allelopathic interaction taking place under natural conditions (Inderjit & Weston 2000).

Preliminary studies focused on identifying and elucidating allelopathic interactions should follow a set of criteria for their results to be considered indicative of the existence of allelopathic interaction in the field. Within this context, Inderjit & Weston (2000) established individual components or criteria that should be observed if a study is to be clearly recognized as allelopathic research. According to those authors, bioassays conducted in the laboratory (or even in the field, under controlled conditions) must at least comply with the following criteria (with adaptations):

- The extracts to be tested must be aqueous, reproducing the effects of rainfall, dew or other forms of aqueous solubilisation occurring naturally in the environment. Because extraction by organic solvents such as dichloromethane or methanol do not occur in nature, such extraction does not replicate a natural process of compound solubilisation.
- The substrate used in experiments must be representative of the place where the plants co-exist or at least of the region where they occur. Experiments using filter paper, vermiculite or other types of substrates exclude the biotic and abiotic effects of the substrate on the bioactivity of the substances.
- Sensitive species must be avoided, because their use can result in an overestimation of allelopathic effects. The sensitivity of exotic or cultivated species to bioactive extract can be completely different from that displayed by native species or by those that occur naturally near the donor plant.
- Species to be studied must co-exist within their natural ecosystems. Although the use of cultivated or exotic plants as target species can help describe the effects of bioactive substances, or to quantify their activity, they are not representative of what can occur between neighbouring plants in nature.
- More than one extract concentration should be tested, and it is recommended that a dose-response test be set up with at least three levels of concentration. Thus, it can be established what levels of allelochemical concentrations are active in the environment. In addition, the concentrations must be compatible with what is expected to occur under natural conditions.
- In studies in which the bioactive compounds are purified and identified, their biological activity must be tested individually and as a mixture (crude extract), to determine whether the biological effects are synergistic or individualised. In fact, the effects observed under laboratory conditions should be also evaluated under natural conditions in order to determine the real allelopathic potential of the crude extracts and of the purified compounds.

Each bioassay must be designed to evaluate the allelopathic interactions between species after careful consideration of their growth habits, the biotic and abiotic characteristics of the area, and ecophysiological factors, with reference to key issues, to determine the relevance of a specific laboratory test (Inderjit & Weston, 2000). As with all these criteria, experiments conducted in the laboratory at least serve to predict or postulate allelopathic interactions likely to occur in the field but cannot be used to confirm that the species being studied truly affect the functioning of neighbouring species and, consequently, the dynamics of the local vegetation.

Allelopathy studies carried out in Brazil

There have been several reviews of allelopathy studies conducted in Brazil (Ferreira *et al.* 1992; Rodrigues *et al.* 1999; Ferreira & Aquila 2000). More recently, Souza-Filho *et al.* (2010) carried out a review of experimental procedures used in laboratory-based allelopathy studies. In the last two decades, there has been a proliferation of studies on the allelopathic properties of species that are native to, introduced to or cultivated in Brazil. Our review of the SciELO database showed a clear increase in the number of publications including the terms "allelopathy" or "allelochemicals" (Figure 1). That increase might reflect the growing interest not only in allelopathic interactions in natural ecosystems and agro-ecosystems but also in the products that can be derived from allelochemicals, such as natural herbicides and plant growth regulators. Although the increase likely represents an improvement in this type of research in Brazil, it also generates the need to evaluate the way in which such studies have been conducted. This implies the importance of establishing appropriate criteria and procedures so that data produced are consistent and the study will be recognised for its value as allelopathy research. Hence, it is important to assess whether studies self-designated as allelopathic meet the criteria set forth in the international literature, as discussed above, which allow them to be considered to have made a real contribution to allelopathy.

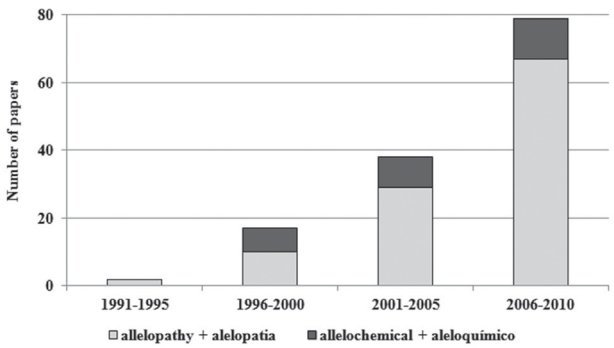

Figure 1. Number of articles, published between 1991 and 2010 and included in the Scientific Electronic Library Online database (www.scielo.br), citing terms associated with allelopathy studies.

We based our review on the main Brazilian periodicals indexed for the SciELO database and on articles published in the *Allelopathy Journal*, combining articles in which the terms "allelopathy" or "allelochemicals" were used. In the *Allelopathy Journal*, only studies by Brazilian authors or conducted in Brazil were considered. We focused on studies involving native Brazilian species, although a few studies involving exotics (occurring in Brazil) were also included. We selected 115 articles, all published between 2000 and 2012, involving a total of 105 species, the majority of which (n = 92) were native to Brazilian biomes. For each article, we evaluated the type of substrate used, the extract concentrations employed, the species tested, as well as other parameters. On the basis of these results, informative and comparative tables were drawn up to analyse the results. The raw data and information emerging from them are fully described in Appendix 1 of this review.

Although we have to consider the possibility that many studies conducted under field conditions were not been identified in this review, the results obtained here allow us to conclude that the majority of allelopathy research has been conducted under laboratory conditions or at least under controlled conditions. Although such studies are certainly important in order to isolate variables and identify the true factors involved in plant-plant interactions, there is a need for complementary studies describing the allelopathic properties of plants under natural conditions.

This review also shows that most studies have investigated the allelopathic properties of leaves, followed by those investigating that of other plant parts, such as stems and, to a lesser degree, reproductive structures (Table 2). Most have also made use of two or more plant structures. The preference for leaves might reflect the fact that it is certainly easier to collect leaves than to collect roots, for example, as well as that leaves represent a large part of the litter produced by the vegetation—biomass that directly impacts seedling growth and recruitment in various ways.

Table 2 also shows that more than 70% of these studies have made use of filter paper, and less than 20% have made use of soil as substrate (Table 2). Filter paper has been routinely used in laboratory studies because of its practicality, simplicity and because it is, in theory, an inert substrate. However, for allelopathy studies aimed at recognising and quantifying chemical interactions between plants that co-exist in the field, the substrate to be used is fundamental, as it may change the properties and form of action of the allelochemicals involved (Inderjit 2001). In our review, we noticed that some studies were carried out on filter paper and in soil, thus determining the ways in which the substrate can influence the allelopathic activity. Other authors have performed comparative analyses of sterilised and non-sterilised soil. Those studies are particularly interesting because they permit to separate the effects of the soil *per se* (physical structure, chemical composition, pH, etc) from the effects of the soil biota on the activity of the allelochemicals (Kaur *et al*. 2009). It has become clear that a true allelopathic experiment must, at some point, make use of soil, specifically the same soil in which the species involved in the supposed interaction grow, as substrate.

A little more than half of the studies selected made use of water to solubilise bioactive substances, and less than 33% made use of methanol or ethanol as solvent (Table 2). This is a positive feature, since the use of water as solvent is a procedure that approximates what is presumed to take place under natural conditions, such as the leaching of leaves or litter during a rainfall event, or solubilisation of bioactive molecules in wet soil or aquatic environments (Ferreira 2004). Some studies have initially used aqueous extracts for solubilisation of the bioactive substances, then performing procedures involving the use of organic solvents and sophisticated equipment to optimise the extraction and purification of the molecules. The latter procedures cannot be considered strictly allelopathy because such laboratory methods do not reflect processes that occur in nature. In either case, the bioactivity of the extracts should be determined by bioassay.

Among the studies reviewed, the most common measure used to refer the concentrations employed in allelopathic experiments is the percentage (Table 2). In these studies, the weight-volume relationship (the weight or mass of the plant matter per volume of solvent), is the most commonly cited rationale, and the majority of the bioassays are conducted at concentrations of 1-5% (Table 2). As a general rule, the solutions obtained from plant tissues are filtered (typically through filter paper) and diluted to obtain solutions designed to establish dose-response effects. In some cases, when researchers progressed to the purification stage, they used parts per million (ppm) or similar measures (e.g., mg/ml).

Although it may be difficult to determine which concentrations are closest to those occurring naturally in the field, good sense dictates that the use of solutions that are highly concen-

Table 2. Plant tissues, substrates, solvents and concentrations used in allelopathic studies carried out in Brazil. Data based on articles included in the Scientific Electronic Library Online database and in the *Allelopathy Journal*.

Plant parts	n	%	Substrate	n	%	Solvent	n	%	Concentration	n	%
Leaves	101	66.0	Filter paper	100	72.5	Water	83	54.6	ppm or mg/ml (< 1%)	20	15.4
Bark, branches, roots	32	20.9	Soil	25	18.1	Methanol, ethanol	50	32.9	1-10%	70	53.9
Fruits, seeds, seedlings	20	13.1	Sand, coconut fibre, vermiculite	13	9.4	Other organic solvents	19	12.5	≥ 10%	40	30.8
Total	153	100		138	100		152	100		130	100

trated in allelopathy bioassays do not represent what can be observed in nature. Solutions that are highly concentrated can also generate osmotic effects in the bioassay, possibly leading to confusion with the supposed effects of allelochemicals (Wardle *et al.* 1992). For example, it has been indicated that seed germination is quite sensitive to solutions over 100 mOsmol (mmol kg^{-1}), suggesting that extracts of similar or higher osmolarity can affect seed germination irrespective of any allelopathic property (Leather & Einhellig, 1988). In fact, crude extracts are usually rich in sugars, amino acids and other substances with osmotic potential. Extracts at 3-4% (w/v) would be equivalent to −0.2 MPa of osmotic pressure. Values more negative than that will probably have osmotic effects on the extract solutions (Astarita *et al.*, 1996: Oliveira *et al.* 2004a).

It should be borne in mind that extremely high concentrations are likely to generate some biological effects by the mere fact that the target plants are subjected to high doses of bioactive substances, which would be phytotoxic rather than allelopathic. Therefore, it is recommended that the extracts be prepared at concentrations that are more comparable to those to which the plants under study are subjected in the natural environment. It would be ideal to determine the concentrations of allelochemicals in the soil, or at least which quantity of plant biomass is produced per unit of soil or covered area, for example. This information would serve as a parameter to establish the concentrations to be used in allelopathy studies under controlled conditions.

The majority of studies reviewed have made use of exotic and or cultivated species as the target species to describe the effects of extracts on plants (Table 3). The most common species used in allelopathy was *Lactuca sativa* (lettuce), followed by *Sesamum indicum* (sesame). It appears that these and other species have been chosen because they present fast germination as well as rapid, uniform initial growth, which is certainly desirable when experiments are designed to compare various treatments. In addition, such experiments conducted in Brazil have apparently been modelled on studies carried out in countries where the use of such species is common. However, despite the ease which cultivated species may bring to allelopathic studies, in the majority of cases they represent species which have been introduced and consequently do not present a history of co-existence with the (native) Brazilian species.

The most common physiological parameters used for identifying allelopathic effects are the germination percentage (or rate) and the initial (seedling) growth of the target species (Table 4). Some studies have also described effects of extracts on the morphology of the target species, the effects on the root growth and differentiation being the most frequently cited. Fewer than 15% of the studies evaluated here described some effect on the shoot parts of the target plants; This might be because the allelopathic activity is more pronounced in the roots, or because less attention has been paid to the effects on the shoot parts of target plants.

In most studies, the effects of the plant extracts have been described as inhibitory of the physiological process under investigation (Table 4). This pattern is also observed in the international literature. These results suggest that allelochemicals present in plant tissues are predominantly inhibitory of physiological processes. However, this highlights the importance of screening for substances that promote germination or the initial growth of plant species. In the present review, less than 8% of the studies selected identified plant extracts or substances that stimulate physiological processes.

A little more than 20% of the allelopathy studies progressed toward the identification of the bioactive compounds or at least toward the identification of the main classes of substances (such as phenolic compounds and alkaloids) present in the bioactive fractions of the plant tested (Table 5).

Based on the present review, we can state that none of the allelopathy studies conducted to date in Brazil have adequately identified the allelochemicals involved in allelopathic interactions. In fact, the majority of the studies reviewed did not describe any kind of procedure carried out to identify allelochemicals or even the classes of compounds present in the bioactive plant extracts (Table 5).

Table 3. Target species used with the greatest frequency in allelopathic studies carried out in Brazil. Data based on articles included in the Scientific Electronic Library Online database and in the *Allelopathy Journal*.

Target species		n	%
Lactuca sativa		80	41.7
Sesamum indicum		22	11.5
Allium cepa		11	5.73
Senna obtusifolia		6	3.13
Mimosa pudica		7	3.65
Lycopersicon esculentum		7	3.65
Raphanus sativus		8	4.17
Others (with fewer than 5 mentions)		51	26.6
	Total	192	100

Table 4. Physiological effects most commonly reported in allelopathy studies carried out in Brazil. Data based on articles included in the Scientific Electronic Library Online database and in the *Allelopathy Journal*.

Effects on germination		n	%	Effects on growth	n	%	Various effects	n	%
Reduction of germination percentage or rate		92	82.9	Growth reduction	78	85.7	Necrosis/root darkening	24	47.1
Promotion of germination percentage or rate		5	4.50	Growth promotion	7	7.69	Root differentiation interference	20	39.2
No effects		14	12.6	No effects	6	6.59	Effects on shoot parts	7	13.7
	Total	111	100		91	100		51	100

Table 5. Studies having identified the bioactive compounds or at least the classes of compounds present in plant extracts that show allelopathic properties. Data based on articles included in the Scientific Electronic Library Online database and in the *Allelopathy Journal*.

Identification of allelochemicals		n	%
Purification/identification of classes of compounds in active fractions		25	21.4
No purification/identification		92	78.6
	Total	117	100

Final considerations

There were many purely qualitative studies that were excluded from this review on the basis of the criteria established for a study to be classified as allelopathic research. In most cases, those studies lacked a clear working hypothesis or justification. Another issue was the use of target plants that are cultivated species, which are sometimes useful for comparative analysis but do not naturally co-exist with the donor species. Consequently, studies using such species yield very little information on the allelopathic processes that might occur under natural conditions. There is little or no correspondence between laboratory and field studies in respect to interactions between plant species. In addition, there is a lack of research on the purification and identification of bioactive molecules and their *in situ* effects. Furthermore, the effects that soil micro-organisms and mycorrhizal fungi have on allelopathic activity constitute an open question in Brazilian research. Additional research is also needed to explore the allelopathic potential of the Brazilian flora in order to develop new molecular structures to be used in the control of pests and invasive weeds, thus reducing the damage caused by the harmful synthetic herbicides currently in use. Moreover, there is a need for genetic and molecular studies of allelopathic plants, in order to increase their protection against competitors, as well to identify allelopathic genes for the transgenic improvement of crop plants.

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Appendix 1. Species, plant part, substrate, target species, effects, substance used, cited in literature for Brazilian studies on allelopathy. Between 2000 and 2012.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Acacia bahiensis	N	Leaves and fruits	L	Filter paper and sand	Water and ethanol	20%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Scopoletin	Oliveira *et al.* 2005
Acosmium subelegans	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Adiantopsis radiata	N	Leaves	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Lactuca sativa* and *Allium cepa*	No effect	Reduced	Root necrosis	Not recorded	Peres *et al.* 2004
Adiantum serratodentatum	N	Leaves	L	Sterilised soil	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Lactuca sativa* and *Allium cepa*	No effect	Reduced	Root necrosis	Not recorded	Peres *et al.* 2004
Adiantum tetraphyllum	N	Leaves	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Lactuca sativa* and *Allium cepa*	No effect	Reduced	Root necrosis	Not recorded	Peres *et al.* 2004
Albizia blanchetii	N	Leaves	L	Filter paper and sand	Water and ethanol	20%	*Lactuca sativa*	Germination inhibition; decreased germination speed	Reduced	Not stated	Not recorded	Oliveira *et al.* 2005
Aloe arborescens	E	Leaves from each season	L	Filter paper	Chloroform and ethanol	1%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Increase in root diameter and absence of root hairs	Not recorded	Murakami *et al.* 2009
Amburana cearensis	N	Seeds	L	Filter paper	Water and ethanol	0.1, 0.5, 1.0, and 1.5%	*Lactuca sativa* and *Raphanus sativus*	Decreased or inhibited germination	Reduced	Necrosis, oxidation and root darkening	Not recorded	Felix *et al.* 2007
Anadenanthera colubrina	N	Leaves and fruits	L	Filter paper and sand	Water and ethanol	20%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Oliveira *et al.* 2005
Anadenanthera falcata	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Anadenanthera falcata	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination percentage	Reduced	Not stated	Not recorded	Gatti *et al.* 2007
Andira humilis	N	Stem and leaves	L	Filter paper	Water	4, 8, 12, and 16%	*Lactuca sativa* and *Raphanus sativus*	Dose-dependent decrease in germination percentage and speed	Reduced	Necrosis in parts of seeds	Not recorded	Periotto *et al.* 2004
Annona coriacea	N	Stem and leaves	L	Filter paper	Methanol	1%	*Lactuca sativa*	no effect	Reduced	Not stated	Flavonoids, steroids, and triterpenoids	Formagio *et al.* 2010
Annona crassiflora	N	Stem and leaves	L	Filter paper	Methanol	1%	*Lactuca sativa*	no effect	Reduced	Not stated	Flavonoids, steroids, tannins, and triterpenoids	Formagio *et al.* 2010
Annona dioica	N	Stem and leaves	L	Filter paper	Chloroform and ethanol	1%	*Lactuca sativa*	Decreased germination speed	Reduced	Not stated	Alkaloids, flavonoids, steroids, tannins, and triterpenoids	Formagio *et al.* 2010
Annona glabra	N	Leaves	L	Filter paper	Hexane and ethyl acetate fractioned by other organic solvents	10%; 39.5-1000 ppm	*Lactuca sativa*, *Echinochloa crus-galli*, *Euphorbia heterophylla*, and *Ipomoea grandifolia*	Only ethyl acetate extract decreased lettuce seed germination; no effect with other extracts	Ethyl acetate extract decreased growth of all target species	Extracts inhibited or promoted hypocotyl growth depending on concentration and type of extract	Alkaloids, flavonoids, steroids, tannins, and triterpenoids	Matsumoto *et al.* 2010
Annona sylvatica	N	Stem and leaves	L	Filter paper	Methanol	1%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Flavonoids, steroids, tannins, and triterpenoids	Formagio *et al.* 2010

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Aristolochia esperanzae	N	Leaves, stem, and roots	L	Filter paper and coconut fibre	Water	Germination: 100, 75, 50, and 25% (crude extract); growth: 100 and 50% (crude extract, 33%)	*Lactuca sativa* and *Raphanus sativus*	Decreased germination percentage and speed	Reduced	Root necrosis	Not recorded	Gatti *et al.* 2004
Aspidosperma tomentosum	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Aspidosperma tomentosum	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	No effect on *Lactuca sativa* seeds, decreased germination speed for *Sesanum indicum*	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Avena spp. (various genotypes)	E	Seedlings	L	Filter paper and vermiculite	Water	Seedlings tests not carried out; scopoletin, 10^{-4} and 10^{-5} M	*Triticum aestivum* and *Lolium multiflorum*	Inhibition dependent on oat genotype	Reduced	Not stated	Scopoletin	Jacobi & Fleck, 2000
Byrsonima coccolobifolia	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Byrsonima verbacifolia	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not recorded	Not stated	Not recorded	Silva *et al.* 2006
Campomanesia adamantinum	N	Leaves	L	Soil	Water	1 and 3%	*Sesamum indicum*	Not stated	Reduced	Fewer rootlets	Not recorded	Souza *et al.* 2007
Caryocar brasiliense	N	Leaves	L	Soil	Water	1 and 3%	*Sesamum indicum*	Not stated	Reduced	Fewer rootlets	Not recorded	Souza *et al.* 2007
Caryocar brasiliense	N	Leaves	L	Filter paper	Crude extract (methanol); fractions: hexane, dichloromethane, ethyl ethanoate, ethyl ethanoate-methanol, and methanol	0-200 ppm	*Panicum maximum*	Not stated	Roots reduced	Not stated	Not recorded	Moreira *et al.* 2008
Casearia sylvestris	N	Leaves	L	Filter paper	Water	10, 30, 50, 70, 90, and 100% (crude extract = 10% w/v)	*Brassica oleracea*, *Lactuca sativa*, and *Lycopersicon esculentum*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Capobiando *et al.* 2009
Casearia sylvestris	N	Leaves	L		Aqueous extract and ethanol for bioassays; ethanol, hexane, chloroform, and ethyl ethanoate for analysis	30% for bioassays	*Allium cepa*, *Glycine max*, *Lactuca sativa*, and *Brassica oleracea*	Decreased germination percentage	Reduced root growth	Chromosome disruption	Alkaloids, coumarins, flavonoids, tannins, steroids, and triterpenes	Sousa *et al.* 2007
Cecropia pachystachia	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination speed	Reduced	Thicker roots, increased number of root hairs	Not recorded	Maraschin-Silva & Aquila, 2006
Chloroleucon tortum	N	Leaves and flowers	L	Filter paper and sand	Water and ethanol	20%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Oliveira *et al.* 2005
Coffea arábica	E	Bark	L	Soil	Water	5, 10, 15, and 20%	*Amaranthus viridis*	Increased seed germination	Improved	Increased number of leaves	Not recorded	Santos *et al.* 2002

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Copaifera langsdorffii	N	Leaves and bark	L	Soil	Water and alkaloid fraction	Crude extract: 10%; alkaloid fraction: 62.5, 125, and 250 mg/ml	*Bidens pilosa*	Decreased germinability, speed and synchronism; increased mean germination time and coefficient of variation	Not stated	Not stated	Alkaloids	Santana *et al.* 2006
Copaifera langsdorffii	N	Leaves and fruits	L	Filter paper and sand	Water and ethanol	20%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Oliveira *et al.* 2005
Cynodon dactylon	E	Leaves, stems, rhizome, and root	L	Filter paper	Water	100 g/L (10%)	*Oryza sativa*, *Zea mays*, and *Triticum aestivum*	Decreased germination or no effect depending on plant part used and target species	Growth promotion or no effect depending on plant part used and target species.	Not stated	Not recorded	Novo *et al.* 2009
Crinum americanum	E	Leaves, roots, and sheaths	L	Filter paper	Water	12.5, 25, 50, 75, and 100%	*Lactuca sativa*, *Sesamum indicum*, *Raphanus sativus*, *Echinochloa crus-galli*, *Ipomoea grandifolia*, and *Bidens pilosa*	Decreased germination percentage and increased mean germination time	Reduced	Brownish, weak roots, spots on cotyledons	Not recorded	Ribeiro *et al.* 2009
Davilla elliptica	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Dicranopteris flexuosa	N	Frond (leaves)	L	Filter paper and washed sand	Crude extracts: ethanol; fractions: hexane, ethyl acetate, and ethanol-water	0, 250, 500, and 1000 mg L^{-1}	*Lactuca sativa*, *Lycopersicon esculentum*, *Allium cepa*, and *Triticum aestivum*	Decreased germination speed	Growth promotion or no effect depending on plant part used and target species.	Decreased number of leaves and biomass	Terpenes and phenols	Silva *et al.* 2011
Dicranopteris flexuosa	N	Frond (leaves)	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Allium cepa*	Decreased germination speed	No effect	Not stated	Not recorded	Muller *et al.* 2007
Didymopanax vilosum	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and rate	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Diospyros hispida	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Dodonaea viscosa	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Root browning	Not recorded	Maraschin-Silva & Aquila, 2005
Duguetia furfuracea	N	Leaves and stem	L	Filter paper	Methanol	1%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Alkaloids, coumarins, flavonoids, tannins, steroids and triterpenes.	Formagio *et al.* 2010
Erythrina velutina	N	Bark	L	Not recorded	Crude extracts: ethanol; fractions: hexane, ethyl acetate, and ethanol-water	0.6, 0.4, 0.3, 0.2, 0.1, 0.05, and 0.25 mg	*Lactuca sativa*	Increased germination depending on concentration and fraction used	Reduced, but depending on fraction	Not stated	Not recorded	Centenaro *et al.* 2009
Erythroxylum argentinum	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	No effect	Reduced	Root browning	Flavonoids	Maraschin-Silva & Aquila 2006
Eugenia dysenterica	N	Leaves	L	Filter paper and soil	Water	1 and 3%	*Sesamum indicum* and *Raphanus sativus*	No effect	Reduced	Root browning, decreased number of branches and root hairs, changed gravitropism	Not recorded	Pina *et al.* 2009

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Eugenia dysenterica	N	Leaves	L	Filter paper	Water	1, 2, 3, 4, and 5%	*Lactuca sativa*	Decreased germination speed	Reduced	Necrosis and root browning, fewer root hairs	Not recorded	Giotto *et al.* 2007
Eugenia dysenterica	N	Leaves	L	Soil	Water	1 and 3%	*Sesamum indicum*	Not stated	Reduced	Not stated	Not recorded	Souza *et al.* 2007
Euterpe edulis	N	Fruits	L	Filter paper	Crude extracts: ethanol; fractions: hexane, ethyl acetate, and ethanol-water	0.8, 0.4, 0.2, and 0.1 mg ml^{-1}	*Lactuca sativa*	Decreased germination speed	Reduced	Not stated	Not recorded	Lima *et al.* 2011
Gleichenella pectinata	N	Frond (leaves)	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Allium cepa*	Decreased germination speed	Root reduction and larger coleoptile	Not stated	Not recorded	Muller *et al.* 2007
Raphanus raphanistrum	E	Leaves and pseudocarp	L	Filter paper	Water	1, 2 and 4%	*Lactuca sativa*	Decreased germination	Reduced root and shoot growth	Not stated	Not recorded	Wandscheer *et al.* 2011
Ilex paraguariensis	N	Leaves and fruits	L	Filter paper	Water	Crude extract: 20%; treatments: 100-200 mg/ml	*Lactuca sativa*	Decreased germination speed and percentage, in some cases	Reduced	Hypocotyl-root axis withering, root tip hypertrophic, chlorosis in cotyledons	Not recorded	Aquila, 2000
Joanesia princeps	N	Seeds	L	Filter paper	Water	10, 30, 50, 70, 90, and 100% (crude extract = 10% w/v)	*Brassica oleracea*, *Lactuca sativa*, and *Lycopersicon esculentum*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Capobiando *et al.* 2009
Kielmeyera coriacea	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Kielmeyera variabilis	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Lonchocarpus muelhbergianus	N	Leaflets and galls	L	Filter paper	Water	5%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Root necrosis and anatomical disorders in root system	Not recorded	Oliveira *et al.* 2008
Luehea divaricata	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Increased germination	Reduced	No effects	Tannins	Maraschin-Silva & Aquila 2006
Machaerium scleroxylon	N	Leaves	L	Filter paper and sand	Water and ethanol	20%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Oliveira *et al.* 2005
Machaerium villosum	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Miconia albicans	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Mimosa caesalpiniaefolia	N	Leaves	L	Autoclaved sand	Water	1:8 (12.5%), 1:16 (6.25%), and 1:32 (3.125%)	*Tabebuia alba*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Piña-Rodrigues *et al.* 2001
Myrcia guianensis	N	Leaves	L	Filter paper	Crude extracts: hexane, dichloromethane, and ethyl acetate	Crude extract: 1%; essential oil: 1 ppm for germination and 5, 10, 15, and 20 ppm for growth; pure substance: 15-60 ppm.	*Mimosa pudica* and *Senna obtusifolia*	Increased or decreased seed germination depending on extract, fraction and target plant	Reduced	Not stated	Protocatechuic acid and gallic acid	Souza Filho *et al.* 2006

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Myrsine guianensis	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination speed	Reduced	No effect	Tannins	Maraschin-Silva & Aquila 2006
Ocotea odorifera	N	Leaves, bark, and root bark	L	Filter paper	Water	1:10 (p/v) = 10%	*Sorghum bicolor*	Not stated	Reduced	Chlorophyll production inhibited and decreased respiratory function of root cells	Not recorded	Carmo *et al.* 2007
Ocotea odorifera	N	Leaves, branches, and roots	L	Filter paper and coconut fibre	Water	Crude extract: 1:3 (w/v), filtrated and diluted to 25, 50, 75, and 100%	*Lactuca sativa* and *Raphanus sativus*	Decreased germination percentage and speed	Stimulated in coconut fibre and reduced to no effect in filter paper	Not stated	Not recorded	Gatti *et al.* 2008
Ocotea puberula	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	No effect	Reduced	Browning and swelling of roots	Saponins	Maraschin-Silva & Aquila 2006
Oryza sativa	E	Glumes and exocarp	L	Soil	Water	5, 10, 15, and 20%	*Amaranthus viridis*	Decreased germinability but increased rate of seedling sprouting at 10%; above that concentration, there was a decrease	No effect	Chlorosis and necrosis of leaves	Not recorded	Santos *et al.* 2002
Ouratea spectabilis	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol; fractions: hexane, chloroform, ethyl acetate and n-butanol	Soil, 1:1 and 1:5, filter paper, 1% of ethanol extract and 1, 0.5, 0.2, 0.1, and 0.05% of fractions	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Paspalum maritimum	N	Leaves and rhizome	L	Filter paper	Methanol-water	0.5, 1.5, and 3.0%	*Mimosa pudica*, *Senna obtusifolia*, *Pueraria phaseoloides*, and *Brachiaria brizantha*	Inhibition	Reduced root system.	Not stated	Not recorded	Souza Filho, 2006
Peltophorum dubium	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination speed	Reduced	Root browning	Not recorded	Maraschin-Silva & Aquila, 2006
Phytolacca dioica	N	Leaves	L	Filter paper	Water	1, 2, 4, and 8%	*Lycopersicon esculentum* and *Bidens pilosa*	Decreased germination percentage and speed	Reduced	Root necrosis, tap root reduced and altered	Flavonoids	Borella & Pastorini, 2009
Pinus taeda	E	Acicular needles (green, dry, and rotted)	L	Filter paper	Water	25, 50, 75, and 100%	*Avena strigosa*	Green needle extract increased the mean germination time but reduced germinability	Extract of green leaf reduced growth	Not stated	Not recorded	Sartor *et al.* 2009
Piptocarpha rotundifolia	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Pityrogramma calomelanos	N	Frond (Leaves)	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Lactuca sativa* and *Allium cepa*	No effect	Reduced	Root necrosis	Not recorded	Peres *et al.* 2004
Pouteria ramiflora	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol; fractions: hexane, chloroform, ethyl acetate and n-butanol	Soil, 1:1 and 1:5, filter paper, 1% of ethanol extract and 1, 0.5, 0.2, 0.1, and 0.05% of fractions	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Pouteria torta	N	Leaves (young and mature)	L	Filter paper	Water	1, 5, and 10%	*Lactuca sativa*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Nascimento *et al.* 2007
Psychotria leiocarpa	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination speed	Reduced	Root browning and fragile seedlings	Not recorded	Maraschin-Silva & Aquila, 2006
Pteris denticulata	N	Frond (leaves)	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Lactuca sativa* and *Allium cepa*	No effect	Reduced	Root necrosis	Not recorded	Peres *et al.* 2004
Pterodon emarginatus	N	Stem	L	Filter paper and sand	Crude extract: methanol; fractions: Hexane, dichloromethane, chloroform, and ethyl acetate	25, 50, 100, 150, 200, 300, and 400 ppm	*Panicum maximum*	Decreased germination	Reduced	Not stated	Aliphatic compounds, phytol, oleic acid, palmitic acid, 1,2,4-trimethylbenzene, isopropylbenzene, keto-isomers	Hernandez-Terones *et al.* 2007
Pueraria phaseoloides	E	Leaves and branches	L	Filter paper	Methanol: water; fractions: hexane, dichloromethane, and ethyl acetate	0.5-4 ppm	*Mimosa pudica*, *Senna obtusifolia*, *Senna occidentalis*, *Urena lobata*	Dichloromethane and ethyl acetate fractions reduced germination	Pure substances reduced growth	Not stated	Isoflavones and Methyl benzoate	Arruda *et al.* 2005
Pyrostegia venusta	N	Leaves	L	Filter paper	Hexane, ethyl acetate, and methanol	0, 1.25, 2.5, 3.75, and 5 mg L^{-1}	*Cucumis sativus*	Not stated	Reduced	Increased number of branches roots	Not recorded	Silva *et al.* 2011 b
Qualea grandiflora	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol; fractions: hexane, chloroform, ethyl acetate and n-butanol	Soil, 1:1 and 1:5, filter paper, 1% of ethanol extract and 1, 0.5, 0.2, 0.1, and 0.05% of fractions	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Qualea parviflora	N	Leaves	L	Soil	Water	1 and 3%	*Sesamum indicum*	Not stated	Reduced	Not stated	Not recorded	Souza *et al.* 2007
Rapanea guianensis	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Raphanus raphanistrum	E	Leaves and roots	L	Filter paper	Water	5 and 10%	*Lactuca sativa* and *Solanum lycopersicon*	Decreased germination percentage and speed	Reduced root growth, no effect on shoot.	Not stated	Not recorded	Wandscheer & Pastorini, 2008
Sapindus saponaria	N	Leaves (young and mature)	L	Filter paper	Water	0, 2.5, 5, 7.5, and 10%	*Echinochloa crus-galli* and *Ipomoea grandifolia*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Grisi *et al.* 2012
Sapium glandulatum	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination speed	Reduced	Swollen roots and brown seedlings	Not recorded	Maraschin-Silva & Aquila, 2006
Schefflera vinosa	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Senna occidentalis	E	Pool of leaves, fruits, and flowers	L	Filter paper	Hexane, ethyl acetate, and ethanol-water	0.025%, 0.05%, and 0.1%	*Lactuca sativa*, *Lycopersicon esculentum*, *Allium cepa*, and *Triticum aestivum*	Decreased germinability and germination speed, depending on species and fraction used	Reduced or increased depending on species and fraction used.	Weak roots or no root hairs in some species	Terpenes (hexane fraction), phenols and alkaloids (ethyl acetate fraction).	Cândido *et al.* 2010
Senna rugosa	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Siparuna guianensis	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Solanum lycocarpum	N	Leaves and fruits	L	Soil	Leaf powder: water	Leaf: 3%; fruit: 0.5 and 1%	*Sesamum indicum*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Aires *et al.* 2005
Solanum lycocarpum	N	Leaves	L	Filter paper	Water	1, 2, 3, 4, and 5%	*Sesamum indicum*	Decreased germination speed	Reduced	Root necrosis, no root hairs, no formation of branches or roots, inversion of gravitropism.	Not recorded	Oliveira *et al.* 2004
Solanum lycocarpum	N	Fruit	L	Filter paper	Water	1, 2, 3, 4, and 5%	*Sesamum indicum*	Decreased germination percentage and speed	Reduced	Root necrosis, no root hairs, no formation of branches or roots	Not recorded	Oliveira *et al.* 2004
Solanum palinacanthum	N	Leaves	L	Filter paper and soil (autoclaved or not)	Water	1, 2, 3, 4, and 5%	*Sesamum indicum*	Decreased germinability and germination speed in soil; increased inhibition in autoclaved soil	Growth reduction similar for the three substrate.	Inversion of gravitropism, formation of branched roots, reduction of root hairs	Not recorded	Oliveira & Campos, 2006
Sorghum bicolor	E	Leaves, stems, and roots	L	Filter paper	Water	3%	*Glycine max*	No effect; decreased germinability in some species	Reduction of root	Not stated	Not recorded	Correia *et al.* 2005
Sorocea bonplandi	N	Leaves	L	Filter paper	Water	2 and 4%	*Lactuca sativa*	Decreased germination speed	Reduced	Root browning	Not recorded	Maraschin-Silva & Aquila, 2006
Sticherus penniger	N	Frond (leaves)	L	Filter paper	Ethanol	0, 250, 500, and 1000 mg L^{-1}	*Allium cepa*	Decreased germination speed	No effect	Not stated	Not recorded	Muller *et al.* 2007
Stryphnodendron polyphyllum	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Stryphnodendron adstringens	N	Leaves	L	Filter paper, sterilised soil, and non-sterilised soil	Leaf powder: water; crude extract: ethanol; fractions: hexane, chloroform, ethyl acetate and n-butanol	Soil, 1:1 and 1:5, filter paper, 1% of ethanol extract, and 1, 0.5, 0.2, 0.1, and 0.05% of fractions	*Lactuca sativa*, *Zea mays*, *Phaseolus vulgaris*, and *Bidens pilosus*	Decreased germination percentage and speed	Not stated	Not stated	Terpenes	Silva *et al.* 2006
Styrax ferrugineus	N	Leaves	L	Filter paper, sterilised soil, and non-sterilised soil	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Syzygium aromaticum	E	Floral buds	L	Filter paper and a soil-sand mix	Ethanol for the crude extract, water for the tests.	Crude extract: 7.75, 31, and 62 mg/ml; pure substance (eugenol, 4-allyl-2-methoxyphenol): 1.23 and 6.2 mg/ml	*Lycopersicon esculentum*, *Impatiens balsmina*, *Raphanus sativus*, *Crotalaria spectabilis*, *Triticum aestivum*, *Lactuca sativa*, *Zolium multiflorum*, *Zea mays*, *Arnica lanceolata*, *Rumex obitusifolius*, and *Sinapis alba*	Decreased germinability in some species	Not significant	Not stated	Soluble phenols and eugenol	Mazzafera, 2003
Tachigali myrmecophyla	N	Leaves	L	Filter paper	Crude extract: methanol-water; fractions: hexane, dichloromethane, chloroform and ethyl acetate	Crude extract: 0.5 and 1%; pure substance: 0.5, 1, 1.5, and 2%.	*Mimosa pudica* and *Senna obtusifolia*	Inhibition	Reduced	Not stated	4,5-dihydroblumenol A	Lôbo *et al.* 2008

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Appendix 1. Continuation.

Species	Identity	Plant part(s) studied	Type of study	Substrate	Solvent	Concentration	Target species	Effects on germination	Effects on growth	Morpho-physiological effect	Substance	Reference
Virola surinamensis	N	Leaves	L	Filter paper	Hexane, methanol-water, and chloroform	Leaf: 3%; fruit: 0.5 and 1%	*Mimosa pudica*, *Senna obtusifolia*, and *Senna occidentalis*	Weak inhibition of germination	Reduced	Not stated	Neolignans: surinamensine and viroline	Borges *et al.* 2007
Vochysia tucanorum	N	Leaves	L	Sterilised soil, non-sterilised soil, and filter paper	Leaf powder: water; crude extract: ethanol	Soil, 1:1 and 1:5; filter paper, 1% of ethanol extract	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Not stated	Not recorded	Silva *et al.* 2006
Xylopia aromatica	N	Leaves	L	Filter paper	Water	10%	*Lactuca sativa* and *Sesamum indicum*	Decreased germination speed	Not stated	Not stated	Not recorded	Gatti *et al.* 2007
Ziziphus joazeiro	N	Seeds	L	Filter paper	Water	0, 25, 50, 75 and 100%	*Lactuca sativa*	Decreased germination percentage and speed	Not stated	Root necrosis, no epicotyl, negative root gravitropism, abnormally swollen seeds	Not recorded	Coelho *et al.* 2011
Baccharis trimera Less. (DC.)	N	Not stated	L	Organic substrate	Not recorded	5 µl, 10 µl, 15 µl, 20 µl, and control.	*Vigna unguiculata*	No effect	Not stated	Not stated	Not recorded	Xavier *et al.* 2012
Caesalpinia ferrea Mart.	N	Leaves, bark, and mature legume	X	Filter paper	Water	2.5%, 5%, 7.5%, 10%, and control.	*Lactuca sativa*	Decreased germination percentage and speed	Decreased of root and shoot growth	Withered or necrotic roots; no branches or roots	Not recorded	Oliveira *et al.* 2012 a
Erythrina velutina Wild.	N	Seeds, flowers, and dry epicarp	L	Filter paper	Water	10% and control	*Lactuca sativa*	Decreased germination percentage and speed	Decreased of root growth	Root necrosis and negative root gravitropism	Not recorded	Oliveira *et al.* 2012 b
Sapindus saponaria L.	N	Leaves (young and mature)	L	Filter paper	Water	0, 2.5, 5, 7.5, and 10%	*Echinochloa crus-galli* and *Ipomoea grandifolia*	Decreased germination percentage and speed	Reduced	Not stated	Not recorded	Grisi *et al.* 2012
Calopogonium mucunoides,	N	Leaves and stem	L	Filter paper	Hexane, dichloromethane, ethyl acetate, and methanol	2% (crude extracts), 5 mg L^{-1} (pure substance), and control	*Cassia tora*, *Mimosa pudica* and *Cassia occidentalis*	Decreased germination speed	Not evaluated	Not stated	Atropine, cinchonidine, pilocarpine, quinine, many organic acids, genistein, rutin, naringin, campherol, quercetin, nerolidol, terpineol, linalool, geraniol, t-anethole	Santos *et al.* 2012
Piper mikanianum	N	Leaves	L	Filter paper	Water	2%, 4%, 8%, and control	*Raphanus sativus*	Decreased germination percentage and speed	Decreased root growth and increased hypocotyl size	Not stated	Not recorded	Borella *et al.* 2012
Pyrostegia venusta	N	Leaves	L	Filter paper	Hexane, ethyl acetate, and methanol	1.25, 2.5, 3.75, and 5 mg ml^{-1}	*Cucumis sativus*	Not stated	Decreased root and hypocotyl growth.	Inhibited branch and root growth	Not recorded	Silva *et al.* 2011
Gomphrena globosa	N	Shoots	X	Filter paper	Water	2.5%, 5%, 10.0%, and control	*Bidens pilosa* and *Lactuca sativa*	Inhibition	No effect	Not stated	Not recorded	Alves *et al.* 2011
Tabernaemontana catharinensis	N	Leaves	X	Filter paper	Water	2.5%, 5%, 10.0%, and control	*Bidens pilosa* and *Lactuca sativa*	Decreased germination speed	Decreased weight	Not stated	Not recorded	Alves *et al.* 2011

N – native; E – exotic; L – laboratory; X – field.