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Progress in the development of new biological herbicides from phytotoxic plant extracts applied *in vitro*

Avances en el desarrollo de nuevos herbicidas biológicos a partir de extractos vegetales fitotóxicos aplicados *in vitro*

Luis Cruz-Ortiz[1]
Mariana Flores Méndez[2]

[1]Universidad Autónoma de Chiapas (Mexico). Email: cruzortizluisangel@gmail.com
orcid: https://orcid.org/0000-0001-5013-3985

[2]Universidad Autónoma de Chiapas (Mexico). Email: marianafm44@gmail.com
orcid: https://orcid.org/0000-0002-4744-9556

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Abstract

Agriculture is affected by the appearance of weeds that reduce crop production. These weeds are controlled with agricultural chemicals that unbalance the environment and human health. For this reason, the need arises to extract the bioactive compounds from them, to produce and apply powerful bioherbicides, which inhibit the spread of weeds that disturb the crops. Recently, the use of biological plant extracts has been reported, where it is shown that they have phytotoxic bioactive properties that alter cell structure. The objective of this literature review is to report the progress in the development of new biological herbicides, from phytotoxic plant extracts applied in vitro. This review describes the compounds with phytotoxic capacity in plants and their phytotoxic biological activity, such as polyphenols, tannins, alkaloids, quinones, saponins antagonists, and glycosides, to mention some, through the use of the most common experimental methods that include the extraction, determination, and quantification of the metabolites. In addition, the response variables that were considered to evaluate the effect of these biological active substances are mentioned, which include the percentage of inhibition of germination, growth, and root length in plants.

Keywords: allelopathy; secondary metabolites; vigor; bioherbicide; phenol; germination; biological solution.

Resumen

La agricultura es afectada por la aparición de malezas que reducen la producción de los cultivos, estas se controlan con químicos agrícolas que desequilibran el ambiente y la salud humana. Recientemente se ha reportado el empleo de extractos biológicos de plantas, donde se demuestra que poseen propiedades bioactivas fitotóxicas que alteran la estructura celular, por lo que surge la necesidad de extraer los compuestos bioactivos de estos, con el fin de producir y aplicar bioherbicidas potentes, que inhiban la propagación de malezas que perturban los sembradíos. El objetivo de esta revisión bibliográfica fue reportar los avances en el desarrollo de nuevos herbicidas biológicos, a partir de extractos vegetales fitotóxicos aplicados in vitro.

La presente revisión describe los compuestos con capacidad fitotóxica en las plantas y su actividad biológica fitotóxica, como los polifenoles, taninos, alcaloides, quinonas, saponinas antagónicas y los glucósidos, por mencionar algunos, mediante los métodos experimentales más usados, que incluyen la extracción, la determinación y la cuantificación de los metabolitos. Además, se hace mención de las variables respuestas que se consideraron para evaluar el efecto de estas sustancias activas biológicas, donde se incluyen el porcentaje de inhibición de la germinación, el crecimiento y la longitud radical en plantas.

1. Introduction

Agriculture is one of the main activities in the world, which guarantees the food of the population and the economic sustenance of the producers. However, one of the frequent problems is the appearance of weeds that displace the crops. They are controlled mechanically, manually, or with heavy machinery. Uncontrolled weeds can generate losses of 10 % to 12 % in the crops, foliar application of fast-acting herbicides limits the development and spread of weeds (Armellina; Bezic; Brevedan, 2008; Alvarado; Carrera-Maridueña; Yance-Carvajal, 2016), however, the inappropriate and excessive use of agricultural chemicals causes an environmental imbalance, impoverishing soils, contaminating aquifers, decreasing the bee population in the form of pollination, and jeopardizing human health.

This agricultural practice is becoming less sustainable, as market demands increase. Moreover, plants become more pesticide-resistant, forcing growers to adopt new alternatives (Ordeñana, 2013; Ramdas; Biwek; Davie; Ozgur; Alferez, 2019; Beckie, 2020), such as the use of biological components, which are a new scientific trend, of many, that are environmentally friendly (Stanley; Shah; Jain; Bhatt; Sushil, 2015; Reichert *et al.*, 2019).

In plants there are bioactive substances that alter the cellular structure (phytotoxic) with desirable characteristics: they are biodegradable and of little or no toxicity in mammals (Cordeau; Triolet; Wayman; Steinberg; Guillemin, 2016; Morra; Popova; Boydstonb, 2018; Gonzalez; Flies; Navarrete; Lopez; Troncoso, 2019). Bioactive compounds extracted from plant organs (leaves, roots, flowers, stems, and seeds) have phytotoxic power, which makes them candidates for bioherbicides. Reports such as the use of methanolic extracts of radish (*Raphanus sativus)* and kohlrabi (*Brassica napobrassica*) show negative effects on germination and strength in the crops of lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*), and rice (*Oryza sativa*) (Díaz-Mota *et al.*, 2017).

Other authors such as Flores, Chávez, and Leal (2015) reported that acetonic, dichloromethane, and methanolic extracts of purple mad grass flower (*Astragalus mollissimus*) disfavor the germination of the seeds of Johnson grass or Aleppo sorghum (*Sorghum halepense*) and reed or cane of Castile (*Arundo donax)*. The extract obtained from the bark of the Canelo tree (*Drimys winteri*) delayed germination, growth of the stem, and radicle of bindweed or lesser bindweed (*Convolvulus arvensis*), spikelet or yellow foxtail (*Setaria pumila*), wild carrot (*Daucus carota*) and common chicory (*Cichorium intybus*) (Zapata; Vargas; Medina, 2011).

Likewise, phytotoxic properties have been found in extracts of apple pepper (*Capsicum pubescens R. and P.*) (Gualteros; Triviño; Salazar, 2017), kohlrabi (*Brassica campestris subsp. rapa*), darnel ryegrass (*Lolium temulentum* L.), clove oil (*Eugenia caryophyllus*) (Copping; Duke, 2007), myrtle tree (*Blepharocalyx salicifolius*), guava (*Myrcia multiflora*), Myrcia splendens, (*Myrcia tomentosa*), fatty acids obtained from (*Achillea gypsicola*), and Achillea biebersteinii Afan (Zamorano; Fuentes, 2005; Kordali *et al.,* 2009; Imatomi; Novaes, Miranda; Gualtieri, 2015). Therefore, the objective of this literature review is to describe the progress in the development of new biological herbicides from phytotoxic plant extracts applied in vitro.

2. Methodology

In the present work, an exhaustive search of scientific journals was carried out, through the review of articles, theses, and scientific notes (digital databases Science Direct, Springer Link, SciELO, Scopus, digital books, and websites), with the keywords "biocide, bioherbicide, phenols, germination, biological solutions". By analyzing the impact information, the documents that adhered to the research topic were considered. The selection criteria for the articles were framed within the publication term in an interval from 2004 to 2020.

3. Results

The search for information in the digital databases yielded around 12,750 results. Therefore, to facilitate the search a date range of 2004-2020 was entered in the digital databases. All the appropriate results considering the subject were selected. Finally, 64 documents were selected and used to build the review article.

3.1. Compounds with phytotoxic capacity in plants

The need to protect the environment and human health from chemicals has resulted in the interest in resorting to plants with biocidal potential. Some research has shown the versatility of plant extractions in the control of fungi, bacteria, weeds, and pests. Plants have the ability to synthesize molecules related to different defense mechanisms, including phenolic acids, flavonoids, alkaloids, and quinones, which are found in virtually all plant tissues (Lottina-Hensen; King; Aguilar; Hernandez, 2006; Cheng; Cheng, 2015). Many of these bioactive substances demonstrate bioherbicidal properties (White; Hernandez; Urra; Leyva, 2007; Batish; Singh; Kohli; Kaur, 2008).

Phenols are one of the active compounds in plants with antioxidant and biocidal characteristics, they influence several physiologic processes, mainly in membrane permeability, enzymatic activity, ion balance, photosynthesis, water-plant ratio, respiration, and protein and chlorophyll synthesis (Yan *et al.,* 2014; Ribeiro; Feitoza; Lima; Carvalho, 2015; Zhi-Qiang *et al.,* 2015; Lim; Basri; Ee; Omar, 2017). Polyphenols, tannins, alkaloids, quinones, saponins, antagonists, and glycosides can cause oxidative stress, where the main damages occur in the hydrophobic tails of mitochondrial membranes by altering the redox state of the cell and lipid peroxidation, where the content of phospholipids and the production of ATP decreases and, furthermore, the transport of metabolites and ions or the pumping of protons and evolution of oxygen in chloroplasts is blocked, and this favors the collapse of cells (Blanco, 2006; Jaramillo; Jaramillo; D'Armas; Troccoli; Rojas, 2016; Radwan; Alghamdi; Kenawy, 2019).

Phenolic, ferulic, p-coumaric, and vanillic acids reduce biomass by reducing the concentration of chlorophyll (Patterson, 1981). Vanillic acid interferes with mitochondrial metabolism by reducing Ca^{+2} (Yang; Ing-Feng; Lee; Zhou, 2002). Flavonoid glycosides such as naringenin, 2',4,4'-trihydroxychalcone and phloridzin strongly stimulate enzymes of the AIA oxidase type, involved in the degradation of auxins, which are responsible for activating ethylene which, mainly, is a primordial hormone for the start of germinal sprouts of seeds. Catechins inhibit the DNA polymerase and topoisomerase enzyme by intervening in DNA replication and reducing cytoplasm (Santos *et al.,* 2019).

3.2. Techniques used for the extraction of plant phytotoxic compounds

There are different processes of extraction of phytotoxic molecules, in different plant organs (roots, leaves, shoots, stems, flowers, and fruits), these are usually crushed and are in contact with solvents. The techniques used are: Soxhlet, where solvents are used (ethanolic, hexane, petroleum ether or others, depending on polarity), steam dragging, infusion, distillation, ultrasound, microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction. High-resolution chromatography coupled to mass spectrophotometry and nuclear magnetic resonance characterize the metabolites found in plant extracts (HPLC-DAD-MS) (Barreto, 2009; Rojas, 2009; Sources; Aranda, 2013; Flowers; Castañeda; Montiel; Hernandez, 2014; Barrera, 2015; Fuentes; Aranda, 2013; Mena *et al.*, 2015).

3.3. Identification and quantification of compounds extracted from plants

The identification and quantification of metabolites is important to be certain of the active molecule and its phytotoxic activity. Indolizidine alkaloids, called *swainsonine of Astragalus mollissimus Torr.*, have been identified, which inhibit the lysosomal alpha-Golgi enzymes, as well as the a-mannosidase and a-mannosidase II enzymes, and reduce the activity of DNA and RNA, protein biosynthesis and membrane permeability, destabilizing their cellular metabolism. Alkaloids function as allelopathic substances, a property that some plants possess, and that gives them a natural herbicide potential (Flores *et al.*, 2015).

Likewise, Díaz-Mota et al. (2017) reported that *Dieffenbachia amoena, Brassica napobrassica*, and *Raphanus Sativus* contain terpenoids (glycoalkaloids) that decrease the percentage of germ buds and the growth of seedlings. Concentrations of monoterpene hydrocarbons [a-pinene (32.80 %), γ-terpinene (19.50 %) and p-cymene (15.37 %)] were found in *Eucalyptus grandis*, and oxygenated monoterpenes [citronellal (29.31 %), geraniol (27.63 %) and β-citronellol (14.88 %)] (Aragao *et al.,* 2015). Crude methanolic extractions of *C. cardunculus* displayed the presence of syringic acid (0.108 mg/g), p-coumaric acid (0.487 mg/g), myricitrin (0.755 mg/g), quercetin (0.383 mg/g) and naringenin (0.359 mg/g) (Kaab *et al.*, 2020). The extractions of *Nauplius graveolens* displayed flavonoid contents (5.51 mg/g) and alkaloids (4.67 mg/g), while *Reichardia tingitana* and *Picris asplenioides* contained tannins (12.54 and 13.69 mg/g), saponins (7.13 and 5.27 mg/g) and phenolic acid (8.17 and 8.77 mg/g) respectively, while *Urospermum picroides* included phenols (9.3 1 mg/g) in its cells (El-Amier; Abbas; Dowood, 2015).

3.4. Response variables obtained from phytotoxic extracts applied to weeds

To assess the phytotoxic potential of molecules extracted from plants, bioassays were performed both "*in vitro*" and "*in vivo*". Many studies present the seeds or plants models in different concentrations of plant extracts in their methodology, and it is, therefore, necessary to expose the seeds, germinated sprouts or explants to contact the bioactive components. The variables used to determine the phytotoxic capacity are the percentage of germinated or non-germinated seeds, the strength of the plant (root length and length of the aerial part of the seeds that germinated) and the fresh weight. The length of the root is measured at the base of the neck of the root tip, and the length of the aerial part is calculated according to the length of coleoptile for monocots and the length of hypocotyl for dicotyledons, expressed as a percentage of growth reduction (Kadioglu; Yanar, 2004; Da Silva *et al.,* 2013; Afrin; Pramanik; Saidur; Awal, 2016; Dafaallah; Ahmed, 2019).

3.5. Plant extracts with weed germination inhibitory potential

The phytotoxicity of biological extracts depends on the extraction methodology (aqueous, ethanolic, methanolic) of the vegetative material (dehydrated, fresh, and frozen), the stage of the plant, species, non-vegetable organ (root, stem, leaf, and flower), the harvest date and the abiotic conditions of the means. Moreover, it should be noted that weed types have different tolerances to phytotoxins (El-Amier *et al.,* 2015; Flores *et al.*, 2015; Díaz-Mota *et al.,* 2017) (see Table 1).

Table 1. *Inhibition of weed germination with phytotoxic plant extracts*

Model plant	Extract Evaluated	Concentration	Weeds	% of ungerminated seeds	Reference
Astragalus mollissimus Torr.	Aqueous	1-3.5 % (v/v)	*Sorghum halepense*	100	Flores *et al.,* 2015
	Methanol	2-3.5 %(v/v)	*Sorghum halepense*	100	
	Methanol	2-3.5 % (v/v)	*Arundo donax*	100	
	Dichloromethane	1-2 % (v/v)	*Sorghum halepense*	98	
	Dichloromethane	1-2 % (v/v)	*Arundo donax*	98	
	Acetone	2-3.5 % (v/v)	*Lactuca virosa*	90	
	Acetone	2-3.5 % (v/v)	*Sorghum halepense*	90	
Brassica napobrassica	Methanol	5 % (v/v)	*Echinochloa crus-galli*	99.3	Díaz-Mota *et al.,* 2017
	Methanol	5 % (v/v)	*Oryza sativa*	99.6	
Nerium oleander L.	Methanol	20 % (v/v)	*Chenopodium album L.*	96.2	
Dieffenbachia amoena W. Bull	Methanol	20 % (v/v)	*Echinochloa crus-galli*	95.6	
Raphanus sativus L.	Methanol	20 % (v/v)	*Echinochloacrus-galli*	95.6	
Eucalyptus citriodora	Steam distillation	0.01 % (v/v)	*Lactuca sativa L.*	87	Aragáo *et al.,* 2015
Eucalyptus grandis		0.01 % (v/v)	*Lactuca sativa L.*	82	
Mentha piperita L.	Aqueous	20 % (w/v)	*Chenopodium album L.*	100	Isik; Mennan; Cam; Tursun; Arsla, 2016
Mentha piperita L.	Aqueous	10 % (w/v)	*Chenopodium album L.*	100	
Coriandrum sativum L.	Aqueous	20 % (w/v)	*Chenopodium album L.*	100	
Thymus vulgaris L.	Aqueous	20 % (w/v)	*Chenopodium album L.*	100	
Cynara cardunculus	Methanolic	0.75 % (w/v)	*Trifolium Incarnatum L.*	87.57	Kaab *et al.,* 2020
Datura stramonium L.	Aqueous	5 % (w/v)	*Parthenium hysterophorus L.*	95	Safdar; Tanveer; Khaliq; Ali; Burgos, 2016
Rumex dentatus L.	Aqueous	5 % (w/v)	*Parthenium hysterophorus L.*	95	
Achyranthes aspera L.	Aqueous	5 % (w/v)	*Parthenium hysterophorus L.*	95	

Model plant	Extract Evaluated	Concentration	Weeds	% of ungerminated seeds	Reference
Reichardia tingitana	Ethanolic	40 % (w/v)	*Echinochloa crus-galli*	77.08	El-Amier *et al.*, 2015
	Aqueous	40 % (w/v)	*Echinochloa crus-galli*	62.5	
Tamarix mannifera Lactuca virosa	Methanol	40 % (w/v)	*Pyrola minor*	100	El-Mergawi; Al-Humaid, 2019
	Methanol	40 % (w/v)	*Pyrola minor*	95	
	Methanol	40 % (w/v)	*Portulaca oleracea*	94	
Ocimum tenuiflorum	Methanol-p	10 % (w/v)	*Phleum pratense L.*	75	Islam; Kato-Noguchi, 2014
Eucalyptus erythrocorys L.	Ethanolic	2.5 % (v/v)	*Sinapis arvensis*	100	Ghnaya *et al.*, 2015
	Ethanolic	3.5 % (v/v)	*Sinapis arvensis*	100	
	Aqueous	2.5 % (v/v)	*Phalaris canariensis*	100	
	Aqueous	3.0 %(v/v)	*Phalaris canariensis*	100	
	Ethanolic	2.5 % (v/v)	*Phalaris canariensis*	100	
	Ethanolic	3.0 % (v/v)	*Phalaris canariensis*	100	
Calotropis procera L.	Aqueous	10 % (v/v)	*Triticum aestivum L.*	60	Radwan *et al.*, 2019
Cortaderia speciosa	Ethanolic	0.065 % (v/v)	*Solanum lycopersicum*	50	Bravetti; Carpinella; Palacios, 2020

Source: own elaboration.

3.6. Plant extracts with inhibition of weed growth

The seeds that manage to germinate after the application of phytotoxic solutions are not completely exempt but are affected in their growth, root length, and weight. Much of the research has been documented, for example, Santos *et al.* (2019) reduced root length by 50 % of *Lactuca sativa* L. with the use of extracts from two plants, Solanum muricatum Ait. and Solanum betaceum. Both had a mitodepressive effect, where the condensation of the nuclei of L. sativa was observed, associated with DNA fragmentation, and cytoplasmic shrinkage.

Also, Xuan, Toyama, Fukuta, Khanh, and Tawata (2009) isolated 2,4-DTBP from cogongrass (*Imperata cylindrica* L.), inhibiting 78 to 95 % the growth of the roots and sprouts of Beggarticks (*Bidens pilosa* L.), white lead tree [*Leucaena leucocephala* (Lam.)] and free-range grasses (Rany Das; Iwasaki; Suenaga; Kato-Noguchi, 2019), using concentrations of 300 mg on a dry basis of Crescentia alata Kunth, achieving a 100 % inhibition of the growth of the sprouts and roots of watercress, lettuce, Italian ryegrass, and Timothy. Murari, Kumar, and Pragati (2014) mentioned that the seed germination and radicle length of *Vigna sinensis* L. decreased when using *Calotropis gigantea* L. leaf extract. It is worth mentioning that decreasing its length also decreases weight, a loss documented by many researchers (Watanabe *et al.*, 2014; Ma *et al.*, 2018; Ximenez; Santin; Ignoate; Souza; Pastorini, 2019). Javaid, Shafique, Kanwal, and Shafique (2010) observed another factor, "yellowing of parthenium seedlings (*Parthenium hysterophorus* L.) using mango flavonoids (*Mangifera indica* L.)".

4. Conclusions

Advances in the study of new biological herbicides based on plant extracts promise a lot in the agricultural sector due to the ability they have to inhibit the germination, growth, and elongation of the radicle of weeds. The phytotoxic capacity of plant extracts is the best alternative to supplant agrochemicals. This is all at a low cost and also reduces the level of environmental pollution.

Most of the research only reports concentration with an inhibitory effect, so it is necessary to find out the effect of plant extracts on physicochemical, molecular, structural aspects, and action mechanism, the toxicological effect and thus also determine which are the biomolecules that determine phytotoxic bioactivity.

Moreover, the importance of plant extracts has been observed, where thanks to the defense mechanism of plants, phytotoxic metabolites are developed, which many researchers have extracted and evaluated against different plant models, proposing them as possible candidates for biological herbicides. It is necessary to know more in detail about the phytotoxic molecules, and the spatial distribution since this information would enable scientists to predict which part of the plant cell is affected. Moreover, it would be possible to determine which type of carrier or facilitator would be suitable to transport bioactive agents from the outside to the cell interior, maximizing their toxic effect. It is suggested to make consortia of different molecules that interact in different areas of the cells, to know if they are linked by weak interaction or by no interactions at all, which may increase or decrease their biocidal capacity. Furthermore, the optimal storage conditions, production efficiency, cost-benefit, and stability of biological solutions must be considered.

A recommendation resulting from this exhaustive bibliographic review is to classify the plants that have the greatest phytotoxic potential, and also to study the combinations between the best plant extracts.

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