Current Applied Science and Technology Vol. 22 No. 4 (July-August 2022)

Review article

Assessment of Phytotoxins Using Different Technologies

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Received: 4 May 2021, Revised: 18 September 2021, Accepted: 22 November 2021

DOI: 10.55003/cast.2022.04.22.010

Abstract

Keywords

bioassay;
biopesticide;
chromatography;
detection;
identification;
phytotoxin

Nowadays, phytotoxins as natural compounds extracted from different plants are widely used as growth regulators or growth inhibitors for other plants or microorganisms. Furthermore, phytotoxin study may lead to the synthesis of new products with various biological activities. Therefore, the detection and identification of these compounds is very important in the study of their effects on living organisms and the environment. Different methods have been used for the identification and characterization of phytotoxins. This study is devoted to reviewing various methods for the extraction, purification, detection, and identification of phytotoxins from different plant species. The most common methods for detecting plant toxins include a variety of bioassays (plant, cell, and enzyme assay) and the application of a wide range of chemical and analytical methods (especially chromatographic techniques). Both types of methods will be discussed in more details. Moreover, applications of these methods in the study of phytotoxin interaction from recent studies are exemplified to aid understanding f these interactions. It can be concluded that bioassay and analytical methods are the two fundamental techniques for phytotoxin analysis. In addition, new advanced techniques that can enable better understanding of phytotoxins and their functions and which are based on biochemistry, robotics, biosensors, nanotechnology, and enzyme-based methods will be developed in the near future.

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1. Introduction

In the world around us, in addition to the enormous quantity of chemical pesticides applied deliberately into the environment by humans, there are also various toxins produced and released by some living organisms. Various plants and some microorganisms can produce different toxic substances with toxicity potential for other living organisms. These substances are called phytotoxins. The most important types of these toxins are phytotoxins (produced by plants), mycotoxins (produced by fungi), and bacterial toxins (produced by bacteria) [1]. Nowadays, the application of phytotoxins as one of the most important alternatives to agricultural pesticides has attracted a great deal of attention in agroecosystem management, especially in organic production systems. Plant toxins can play an essential role as biopesticides in reducing the environmental pollution caused by agrochemicals [2-4]. Plant toxin production appears to be a defensive strategy for control of herbivores, fungi, and bacteria. Many plants, such as cassava (*Manihot esculenta*), can produce cyanogenic glucosides as a defensive phytotoxin against different animals [5]. Besides their antioxidant and therapeutic effects, plant toxins and secondary metabolites produced by plants can function as bioherbicides in weed management practices as they can inhibit the germination and growth of weeds [6-8]. To date, a number of the antifungal, antimicrobial, and antibacterial effects of these compounds have been reported by various researchers [9-13]. Phytotoxins include various secondary metabolites and biochemical constituents produced by different plant parts such as leaves, stems, roots, flowers, seeds, fruits, and so on [14-17]. The most essential phytotoxins identified are phenolic compounds [18, 19], quinones [20], terpenoids [21], alkaloids [22], glycosides [23], and flavonoids [24], The disruption of photosystems I and II, and the inhibition of chlorophyll synthesis, mitosis, and amino acid synthesis are the main modes of action associated with phytotoxins [25].

The identification, extraction, and detection of phytotoxins are usually complex processes that are very costly and require specialized equipment. However, there are also bioassay methods that can effectively detect plant toxins at low cost and are simple procedures. This article is devoted to reviewing the identification and characterization, extraction, purification, and detection of phytotoxins and secondary metabolites produced by plants.

2. Identification and Classification of Toxins

Based on production source of toxins, these compounds can be classified as plant toxins (phytotoxins), microbial toxins, fungal toxins (mycotoxins), algal toxins, and animal toxins. However, the synthetic toxins made by humans (pesticides) are also an important class of toxins. The biological activity of phytotoxins is an important factor in the classification of these compounds. Phytotoxins are a group of plant secondary metabolites that include alkaloids, terpenes, and phenolics [26, 27]. Figure 1 presents a main classification scheme for phytotoxin compounds. Understanding the environmental behavior of phytotoxins requires an appropriate identification and characterization of these compounds in the agroecosystem. More than 200000 phytotoxins and secondary metabolites have been estimated in plants [28]. Despite the enormous number of phytotoxins and secondary metabolites produced in plants, these toxins' comprehensive identification and characterization have been studied in limited numbers. Mehmood *et al*. [29] identified the different types of phenolic compounds such as quercitin, coumaric acid, ferulic acid, benzoic acid, cinnamic acid, caffeic acid, syringic acid, gallic acid, and chlorogenic acid in *Brassica napus*. Günthardt *et al*. [30] conducted a comprehensive study and identified 1,586 plant phytotoxins, of which more than 60% belonged to the alkaloid and terpene classes. Rios *et al*. [31] identified four different phytotoxins, including the flavone linarin, the triterpene lupenone, the

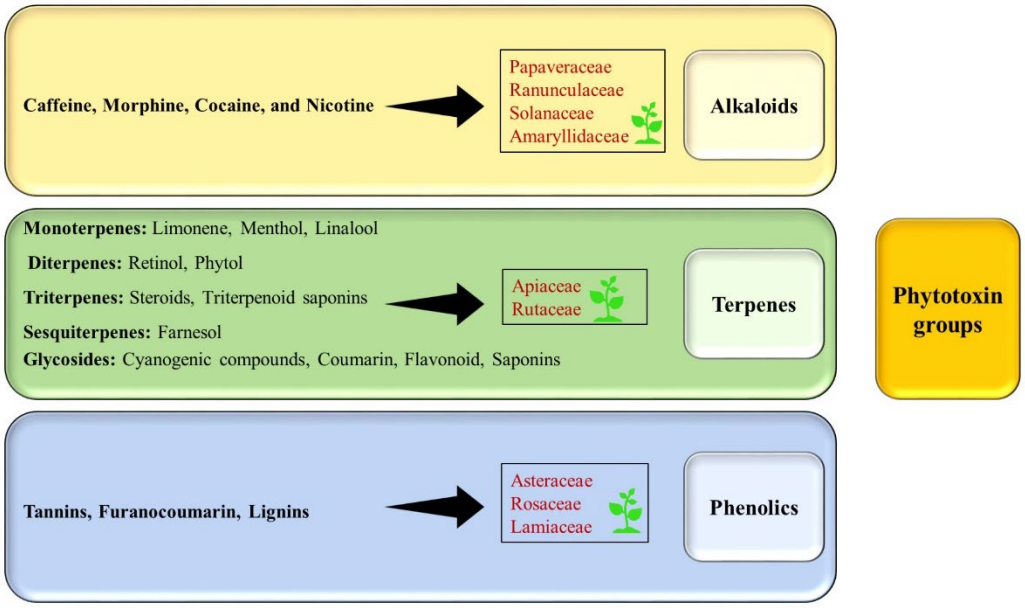

Figure 1. The main classification for phytotoxin compounds

tocopherol (vitamin E), and a new natural alkaloid (affineine) from *Zanthoxylum affine* plant and the use of the new natural alkaloid as a bioherbicide. The toxicity level of the toxins produced by plants varies greatly depending on the plant species. Generally, plant phytotoxins belong to the Leguminosae family, including chickpea, lentils, beans, and peas, which are protease inhibitor compounds. However, the Solanaceae plants can produce glycoalkaloid compounds, and cyanogenic glycosides cna be considered the main phytotoxins generated by the Rosaceae plants family [32]. These toxins may only have a repulsive role, or they may possess a range of toxicities to different organisms. *Pteridium aquilinum* produces a carcinogenic compound that is highly toxic for humans and animals [33]. Estrogenic isoflavones are produced by *Trifolium pretense* [34], and *Solanum tuberosum* produces a highly toxic glycoalkaloid [35].

3. Analysis, Extraction, and Purification of Plant Material

Through the production of phytotoxins, plants can affect the biological and physiological activities, growth and cell division, and other vital activities of receiver organisms. However, this process depends on the nature of the produced toxin. These toxins can pose a risk to human and animal health and safety. Therefore, the appearance of these compounds in the environment and in foods should be considered. For this reason, an understanding of the analysis, extraction, and purification of phytotoxins in different media is very important. Generally, the extraction and purification of phytotoxic compounds from various media can be performed using different procedures. Exhaustive extraction is one of the commonly used methods for the extraction of phytotoxins. In this method, all the chemical compounds and phytotoxins in the plant are extracted using the appropriate solvents. However, additional treatments are used to isolate specific compounds [36]. Stavropoulou *et al*. [37] successfully used the exhaustive extraction method to evaluate phytotoxins in different plant species. These methods are usually very time-consuming and require large amounts of organic

solvents, which can cause structural changes or degradation of desired toxic compounds [38]. When extracting phytotoxins and plant extracts, all requirements and limitations should be considered, and an efficient and reliable extraction protocol selected. Therefore, the extraction protocol directly affects the efficiency, quantity, and quality of toxins extracted from the plant materials.

Furthermore, the extraction conditions must be adjusted to prevent any alteration in the phytotoxin biological activity and chemical structure [39]. It is desirable to choose an extraction method that minimizes the costs, time, organic solvents, and energy consumption. Plant toxins contain various lipophilic and hydrophilic low molecular weight compounds, and generally, the isolation and extraction of these compounds from plant material is a complex and multi-step process. For example, phytotoxin extraction from hydroponic tomato was successfully performed using ethyl acetate and diethyl ether from concentrated and acidified solution [40]. Choosing an appropriate solvent for phytotoxins extraction in different plants depends on the toxin chemical structure and nature. Lim *et al*. [41] found that the application of methanol as extracting solvent was more effective than other solvents such as dichloromethane, hexane, and ethyl acetate when extracting phytotoxins from *Mikania micrantha*.

4. Detection of Phytotoxins

In general, many plant species and microorganisms are available in nature. The presence of an unlimited number of phytotoxins in the environment is not unexpected. However, only a small number of these phytotoxins have been identified and characterized. This situation indicates the importance of selecting and optimizing standard methods for studying phytotoxins. In general, most plant species are valuable sources of secondary compounds and phytotoxins. Identification and extraction of these compounds require the use of specific methods. Choosing an appropriate method for detecting and identifying plant biological active compounds is very important in phytotoxin research. There are many different methods for the detection and quantification of phytotoxins. Bioassays and analytical methods are two basic and widely used methods for identifying and quantifying phytotoxins [42-44]. In bioassay methods, depending on the plant species, the phytotoxin content of seeds, buds, leaves, roots, and even protoplasts can be targeted. However, the whole plant bioassay is also one of the most common methods [29, 45].

On the other hand, analytical methods offer accurate identification and detection. However, instrumental analysis methods are costly and time-consuming and require different chemicals and high purity solvents. High-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC) are the standard methods for the detection of phytotoxins. Coupling mass spectrometry (MS) with some of these analytical methods can enhance the accuracy of detecting and identifying compounds on a molecular basis [43]. Figure 2 presents a schematic representation for phytotoxins detection.

4.1 Bioassay methods

The biological assay or bioassay is based on the mode of action of a secondary metabolite, phytotoxin, or any compound as it exerts its toxicity on a target organism. Plant bioassay (seed, leaf, root, whole plant, etc.), enzyme bioassay, and cell bioassay are the most commonly used methods for detecting and quantifying phytotoxins. Depending on the type of phytotoxins involved, different plant functions may be affected. Phytotoxins may inhibit seed germination, root, leaf, shoot or pollen growth, mitochondrial or membrane efficiency, the fixation of carbon dioxide, and they may interfere with stomatal closure and opening, and water conductivity [46-47]. These are the main

plant

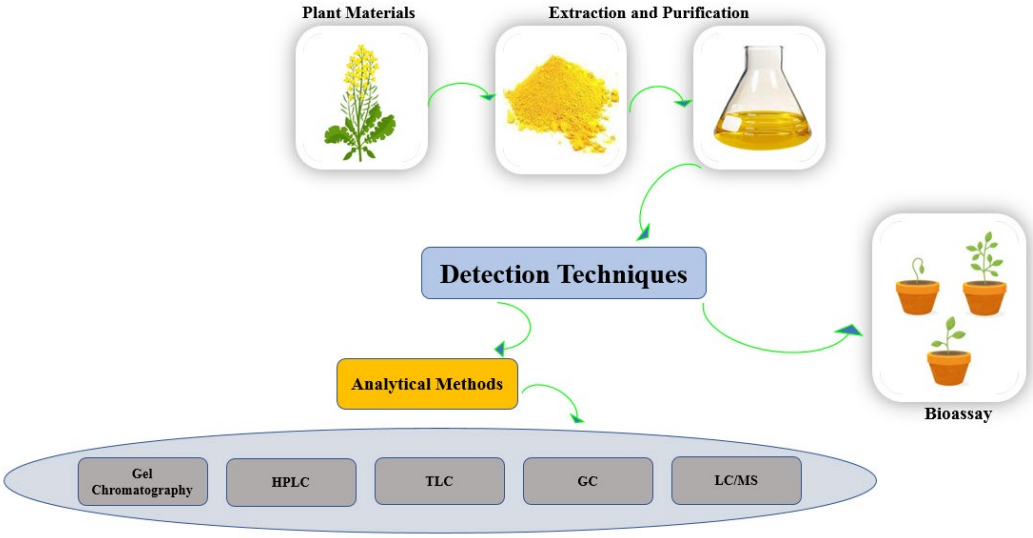

Figure 2. Schematic representation of phytotoxins detection

physiological responses to phytotoxins and methods based on them are considered fast, simple, and sensitive ways to detect unknown amounts of phytotoxins in the environment. At the same time, certified standards are not required for the detection of phytotoxins. Bioassays is a commonly used technique in ecotoxicology research to detect and determine phytotoxins or bioavailable phytotoxic residues of pesticides [48]. One of the main disadvantages of using bioassays in phytotoxin studies is their weakness in identifying phytotoxic compounds [49]. Therefore, an analytical method is used to identify the phytotoxin compound along with a bioassay in such a situation. The combination of bioassay and analytical methods can be used to identify phytotoxins and evaluate their toxicity on target plants. Okada *et al*. [16] assessed the phytotoxic effects of kiwifruit leaf extracts on different plant species and isolated an epicatechin compound using bioassay and chromatography. Scognamiglio *et al*. [50] conducted a bioassay assessment of the phytotoxic extracts and phytotoxins from the Mediterranean plant species *Triticum durum*, *Triticum ovatum*, and *Avena fatua*. However, they used an NMR-based metabolomics technique for the identification of the phytotoxins.

4.1.1 Plant bioassay

Generally, different steps are performed during the bioassay process. In the first step, the targeted plant parts (root, leaf, shoot, flower, etc.) should be separated from the donor plant. After washing, depending on the plant species and tissue, they are dried over a specified time. Then, the plant parts are powdered to fine-grained powder and involved in successive extraction processes with appropriate organic solvents. The shaking and filtration of the samples and evaporation of the filtrate should continue until the final extract is obtained. Then, the toxicity effects of the final extract on the target plants are evaluated. In plant bioassay, the response of different parts of a sensitive plant to known or unknown concentrations of secondary metabolites or phytotoxins can be considered. Seed bioassays used to assess phytotoxin inhibition effect on seed germination and are usually

conducted under petri dish conditions. Seeds used in phytotoxin bioassay studies should usually have quick germination and be readily available [51].

Suzuki *et al*. [52] conducted a petri dish bioassay to assess the phytotoxic activity of Chinese violet *Asystasia gangetica* (L.) and two phytotoxic substances found in this plant. Besides the petri dish bioassay, there has recently appeared a method named the sandwich technique used for evaluating phytotoxic effects on seed germination [53]. In this method, the extracted metabolites or phytotoxins are placed between agar layers on an appropriate culture [54, 55]. Tefera [56] found that the leaf and flower extracts of Congress grass (*Parthenium hysterophorus*) had a significant adverse effect on the seed germination of the test plant (*Eragrostis tef*). The seed germination of radish and wheat was affected by phytotoxins extracted from *Drimys brasiliensis* [57]. Another bioassay method called the equal compartment agar method is based on the cultivation of both the donor and receiver species using agar as substrate [58]. In this method, the production of phytotoxins in donor plants and their effects on receiver plants can be measured simultaneously.

The leaf bioassay is based on evaluating the toxic effects of phytotoxins extracted from a specific plant on growth parameters and leaf biomass of a sensitive target plant. This method is much easier than other plant bioassays [59, 60]. Labruzzo *et al*. [61] identified the phytotoxic compounds of *Artemisia arborescens* using a systematic bioassay method and isolated two lignans, sesamin and ashantin. Islam *et al*. [62] used a whole plant bioassay method to evaluate phytotoxic potential and identify phytotoxic compounds in *Rumex maritimus*. They found the phytotoxic effects on cress seedling growth, rapeseed, barnyard grass, and foxtail fescue. Chen *et al*. [63] isolated nine different terpenoids that were active phytotoxins from *Ligularia cymbulifera* using a root plant bioassay and UHPLC-MS technique. Boonmee *et al*. [64] conducted bioassay experiments to determine the effects of phytotoxins extracted from the root and stem of *Caesalpinia mimosoides* and found that the phytotoxins had significant toxic effects on cress, alfalfa, lettuce, foxtail fescue, timothy, and barnyard grass.

4.1.2 Enzyme assay

In enzyme bioassay, usually, the inhibition effect of phytotoxins on specific enzyme activity is evaluated [65, 66]. Mizushina *et al*. [46] employed an enzyme assay method to identify a plant phytotoxin, Solanapyrone A, as an inhibitor of DNA polymerase β and λ. Gutiérrez-Martínez *et al*. [67] employed an enzyme assay method for assessment of phytotoxins from leaves and roots of *Phaseolus vulgaris* plants. Ruan and Peters [68] reported a rapid and very sensitive assessment technique for Rhizobitoxine based on cystathionase enzyme assay.

4.1.3 Cell bioassay

Cell-based assays or cell bioassays are among the most complex and accurate methods for measuring the biological activity of various compounds, especially phytotoxins. Moreover, membrane potential or cytotoxicity evaluation can be attributed to the cell bioassay method [47]. Galun and Breiman [69] reported the use of cell bioassay as a quantitative method for determination of phytotoxins in different plants.

4.2 Analytical methods

Analytical methods are based on the quantification and identification of phytotoxins according to their chemical characteristics. These sensitive and specific methods can accurately identify the compounds extracted from plants. However, many analytical techniques are costly and time-consuming and require organic solvents and advanced equipment. Another disadvantage of these

methods is the need to use certified standards to identify any phytotoxins or derivatives [70]. In other words, the accurate identification of phytotoxin compounds is not possible without the use of approved standards.

Unlike bioassays, analytical methods cannot determine phytotoxin toxicity and adverse effects on other organisms. Therefore, in phytotoxins studies, the simultaneous application of bioassays and analytical methods is of particular importance to phytotoxin isolation and characterization. Quantitative analytical methods are available for isolation, identification, and detection of phytotoxins. A specific extraction and isolation method was developed for enhancing the efficiency of phytotoxin analysis [71]. Table 1 presents various successful analytical techniques developed to analyze and detect phytotoxins from different plant species.

4.2.1 Chromatography

Chromatographic methods such as liquid chromatography (LC), liquid chromatography coupled with mass spectrometry (LC-MS), and gas chromatography (GC) are among the most efficient analytical methods for the identification and detection of different chemicals and phytotoxins. Usually, plant-based toxins and secondary metabolites have complicated structures with low molecular masses. Typically, in chromatographic methods, depending on the analytical instruments used, separation and identification of secondary metabolites and phytotoxins are performed based on polarity, molecular mass, and electrical charges. The separation of these compounds requires sensitive and accurate methods, and chromatography is an effective tool of use in these circumstances [72]. The most important and routinely used chromatographic methods for the analysis of plant phytotoxins are briefly described below.

4.2.1.1 Gel chromatography

In the previous decades, in the absence of modern analytical instruments, this method was used to separate and identify compounds. Today, with the development of advanced chromatography devices, it is not very popular among researchers. The separation of different compounds in this chromatographic method is according to molecule size (Figure 3). Meepagala *et al*. [73] successfully used silica gel chromatography for assessment of furanocoumarin phytotoxin extracted from the leaves of *Amyris elemifera* plants.

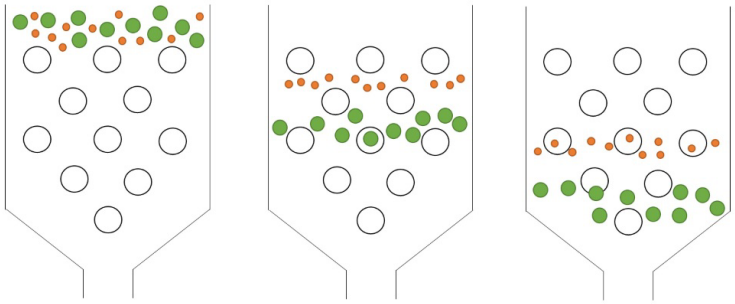

Figure 3. Schematic diagram of gel chromatography method

4.2.1.2 High performance liquid chromatography (HPLC)

Generally, the volatility of plant phytotoxins is low; therefore, HPLC is an appropriate method for analyzing these compounds. The use of HPLC with MS is one of the most suitable options for detecting and determining the secondary metabolites in different plants [74]. Depending on the type of samples evaluated by the HPLC, different solvents can be used to analyze and detect the compounds. In an HPLC system, the selected solvents (mobile phase) are passed through a column (stationary phase) at high pressure using the pumps (Figure 4). The HPLC stationary phase has very fine particles that play an essential role in detecting and separating metabolites in the sample [75]. Generally, setting the right temperature for sample analysis, an appropriate flowrate, selecting solvents, columns, and a proper detector by adjusting the appropriate wavelength, are the most critical factors affecting the efficiency and quality of detection and identification of samples examined by the HPLC [76]. An appropriate solvent can usually be used for detecting samples with simple compounds. However, for samples with complex structures and multiple compositions, a combination of various solvents is used. Many plant compounds and secondary metabolites can absorb wavelengths in the UV range. Therefore, the use of UV detectors is highly acceptable in the analysis of phytotoxins. Recently, the use of HPLC for the analysis of plant samples, especially phenolic compounds, has been considered by researchers [77-79]. Qasim *et al*. [44] reported a high-performance liquid chromatographic method for analyzing and quantifying phenolic compounds from various medicinal plants.

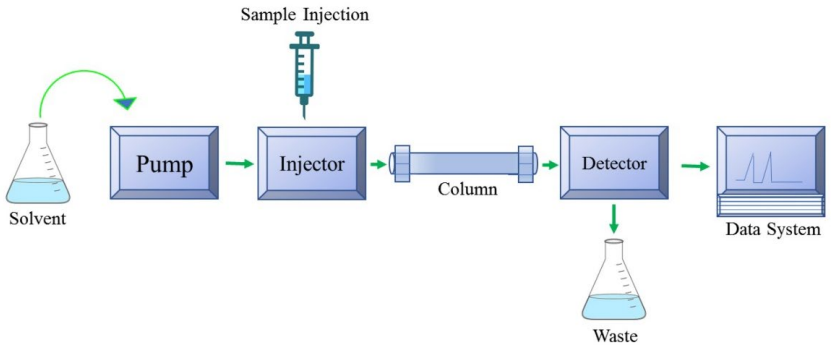

Figure 4. Schematic diagram of high-performance liquid chromatography (HPLC)

4.2.1.3 Gas chromatography (GC)

Gas chromatography is an applicable instrumental method for analyzing and identifying different volatile metabolites and compounds in the gas phase. A detector located in an oven is used to detect the sample compounds (Figure 5). Usually, helium or nitrogen gas is used to carry the volatilized samples through a capillary column. Chuah *et al*. [80] employed a GC-MS method for identification of three phytotoxins in Napiergrass (*Pennisetum purpureum*) and evaluated the toxicity of these compounds on the germination and seedling growth of Chinese Sprangletop (*Leptochloa chinensis*) using a bioassay method. Shanmugapackiam *et al*. [81] applied a GC-MS analytical method for detection of phytotoxins from *M. grisea*.

4.2.1.4 Thin layer chromatography (TLC)

TLC is a simple and relatively fast method for the initial detection and isolation of plant compounds including phytotoxins (Figure 6). Shanmugapackiam *et al*. [81] used thin layer chromatography for

the detection of toxic volatile compounds from leaf, neck and finger blast disease caused by *Magnaporthe grisea*.

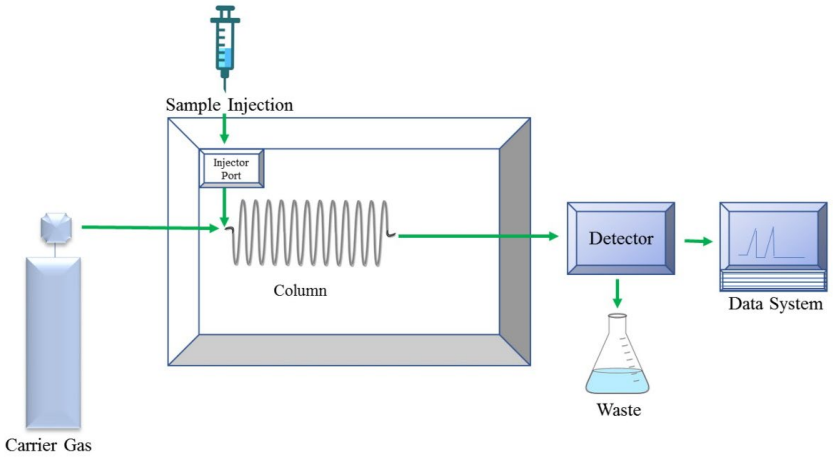

Figure 5. Schematic diagram of gas chromatography (GC)

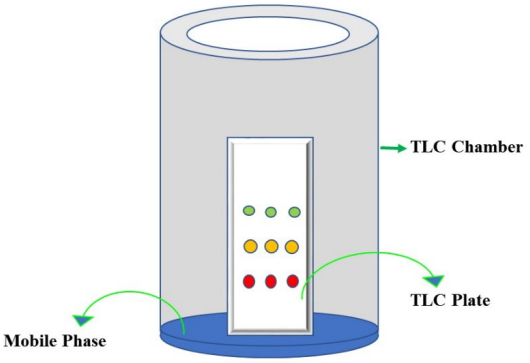

Figure 6. Schematic diagram of thin layer chromatography (TLC)

4.2.1.5 Liquid chromatography with mass spectrometry (LC/MS)

Mass spectrometer (MS) is one of the most complex and sensitive systems for analyzing chemical compounds and plant toxins. It offers very high accuracy in the identification and detection of different compounds based on their particle mass [82]. Coupling HPLC with MS is commonly done to evaluate and identify highly complex compounds. In this method, HPLC is used to separate the compounds in the sample, and MS is used to detect compounds [83]. Shimizu *et al*. [84] described an effective general LC-MS-based method for analyzing plant secondary metabolites. Nam *et al*. [85] investigated an LC-MS/MS method to analyze soybean metabolites and identified 476 different metabolites from leaf extract based on this method.

Recently, a relatively new method based on time-of-flight mass spectrometry (TOF-MS) has been considered for the evaluation and identification of plant phytotoxins. The advantage of this method is the identification of compounds for which no standard reference is available [86]. Oh *et*

al. [87] successfully developed a liquid chromatography quadrupole time-of-flight mass spectrometry (QTOF-MS) method to analyze certain metabolites of rice plants.

Table 1. Successful analytical and bioassay methods developed for analysis and detection of phytotoxins from different plant species over the period 1994-2020

Detection method	Detected phytotoxin	Target species	Reference
High performance liquid chromatography (HPLC)	(-)-Epicatechin	*Actinidia deliciosa*	[16]
	Methyl gallate	*Caesalpinia mimosoides*	[64]
	Salsolol and balanochalcone	*Ambrosia salsola*	[87]
	Indole-3-carboxaldehyde and (6R,9S)-3-oxo-a-ionol	*Asystasia gangetica*	[52]
	Phenols, flavnoids and tannins	*Capparis cartilaginea*	[44]
	Quercetin, chlorogenic acid, p-coumaric acid, m-coumaric acid, benzoic acid, caffeic acid, syringic acid, vanillic acid, ferulic acid, cinnamic acid, and gallic acid	*Brassica napus*	[29]
Reverse-phase HPLC	5,7-dihydroxyphthalide and altechromone	*Rumex maritimus*	[62]
Ultrahigh-pressure liquid chromatography mass spectrometry (UHPLC/MS)	Terpenoids	*Ligularia cymbulifera*	[63]
Gas chromatography mass spectrometry (GC/MS)	2,4 di-tert-butylphenol, cis-9-octadecenoic methyl ester, and phthalic acid, mono-(2-ethylhexyl) ester	*Pennisetum purpureum*	[79]
	Glycosinolates	*Brassica napus*	[88]
	Auxins, abscisic acid, cytokinins, jasmonic acid, salicylic acid	*Medicago truncatula*	[89]
GC/MSD	Sesamin and ashantin	*Artemisia arborescens*	[61]
liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS/MS)	Luminacins	*Streptomyces sp.*	[43]
High-speed counter-current chromatography (HSCCC)	Naphthopyranones paepalantine, paepalantine-9-O-beta-D-glucopyranoside, and paepalantine-9-O-beta-D-allopyranosyl-(1-->6)-O-beta-D-glucopyranoside	*Paepalanthus bromelioides*	[90]
Droplet counter-current chromatography (DCCC)	Salicylic acid, scopoletin, rutin, and quercetin	*Medicago sativa*	[91]
Gel chromatography	Borrelidin	*Streptomyces sp.*	[92]
Silica gel chromatography	(*S*)-4-(3-hydroxybutyl) phenol [(+)-rhododendrol] and (*E*)-4-((1*R*,4*R*)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one [3-hydroxy-α-ionone]	*Cassia alata*	[93]

Table 1. Successful analytical and bioassay methods developed for analysis and detection of phytotoxins from different plant species over the period 1994-2020 (continued)

Detection method	Detected phytotoxin	Target species	Reference
Thin-layer chromatography	Monoterpenes	*Mentha piperita*	[94]
	Flavonoids	*rategus oxyacantha* *Humulus lupulus* *Thea sinensis*	
Hydrophilic interaction liquid chromatography coupled to electrospray ionization mass spectrometry (MS) (HILIC-ESI -MS)	Sucrose, raffinose, stachyose, maltoheptose, UDP-glucose, 1,4-dideoxy-1,4-imino-d-arabinitol, N-methyl-1-deoxynojirimycin, 2-amino-2-deoxy-d-glucose, N-acetyl-d-glucosamine, glucosaminic acid, l-methionine, l-alanyl-l-alanine	*Cucurbita maxima*	[95]
Liquid chromatography coupled to MS-Q3 (AEC-MS-QqQ-MS/MS)	Trehalose-6-phosphate	*Arabidopsis thaliana*	[96]
Liquid chromatography-electrospray ionization tandem mass spectrometry (RP-ESI-QqQ-MS/MS)	Auxins, abscisic acid, cytokinins, gibberellins	*Lactuca sativa*	[97]
Capillary electrophoresis mass spectrometry (CE-MS)	Amino acids, amines, purine bases, organic acids, sugars and sugar phosphates, nucleotides, coenzymes	*Oryza sativa*	[98]
	Sugar phosphates, amino and organic acids, coenzymes and nucleotides	*Nicotiana tabacum*	[99]
Capillary electrophoresis (CE)	Nephroloxic and carcinogenic aristolochic acids	*Aristolochia debilis*	[100]
Capillary zone electrophoresis (CZE)	Aristolochic acids	Some medicinal plants	[101]
Enzyme assay	Toxoflavin	*Burkholderia glumae* BGR1	[102]
Enzyme-linked immunosorbent assay	Cleistanthin A	*cleistanthus collinus*	[103]

5. Future Perspectives

The wide range of phytotoxins in nature and the capabilities of these compounds will soon attract more attention from the researchers. Despite the development of technology for detecting and identifying phytotoxins and their impact on the environment, agricultural ecosystems, and human life in general, a deep understanding of phytotoxins and unknown dimensions of these compounds still requires field laboratory and clinical research. In the coming years, new advanced techniques based on biochemistry, robotics, biosensors, nanotechnology, and enzyme-based methods for better understanding phytotoxins and their function will be rapidly developed. There seems to be a promising future for the replacement of many chemical pesticides by phytotoxins.

6. Conclusions

Plant toxins as natural compounds have the potential to be used as different plant growth promoters as well as pest management. Furthermore, phytotoxin study may lead to the synthesis of new products such as biopesticides or growth regulators. Therefore, a sound knowledge of the detection and identification of plant toxins is critical. Based on the different factors, there are various technologies used to analyse these toxins. Generally, bioassay and analytical methods are two fundamental techniques of phytotoxin analysis.

7. Acknowledgements

We would like to show our gratitude to Mrs. Zoleikha Mehdizade (Department of Biology, Payame Noor University, 19395-3697 Tehran, Iran) for sharing her pearls of wisdom with us during the course of this review.

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