Plants extracts and secondary metabolites, their extraction methods and use in agriculture for controlling crop stresses and improving productivity: A review

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ABSTRACT

This review is based on the analysis of a comprehensive literature search for existing studies and scientific works about the biological properties of plants secondary metabolites, extracts and their benefits as agents to control crop health and diseases and as alternative to chemical pesticides and antimicrobial agents. After an introduction of the major secondary metabolites and their categories, the review presents the different extraction methods of secondary metabolites from plants with a focus on the non-conventional approaches. In addition, this review compiles very recent published studies on plants extracts and secondary metabolites that have been tested as biostimulant for crop growth and against fungi, pests and insects causing damages to various corps.

Key words: Secondary metabolites, medicinal and aromatic plants, crop diseases, abiotic stress, antifungal, insecticide, pesticide, bio-stimulant.

INTRODUCTION

The first medical texts from Chinese, Egyptian and Mesopotamian on the use of medicinal plants indicate that there was already a therapeutic knowledge some 3,000 to 4,000 years ago. Through Greek, Latin and Arabic medicine (inheritors of ancient civilizations), the knowledge of medicinal plants and plant remedies keeps getting richer (Cragg and Newman, 2013). As proof, the manuscripts of Dioscoride in the first century AD compiled more than 500 species of plants in his famous "De Materia Medica" (Sneader, 2005). This book has been an authority in Europe and has been the basis of medical and botanical studies for more than 1500 years. Probably, the synthesis of acetylsalicylic acid (aspirin) is considered to be the most famous and well known example which was derived from the bark of the willow tree Salix alba L. (DerMarderosian et al., 2002).

During all that time, medicinal plants were only applied on an empirical basis, without mechanistic knowledge on their pharmacological activities or active constituents. It was only in the 18th century that Anton von Störrck, who investigated poisonous herbs such asaconite and colchicum, and William Withering, who studied foxglove for the treatment of edema, laid the basis for the rational clinical investigation of medicinal herbs (Sneader, 2005). This period has formed the basis of clinical, pharmacological and chemical studies of medicinal plants (Butler, 2004).

The plants have the so-called "secondary" metabolites as compared with primary metabolites such as proteins, carbohydrates and lipids. Although the roles of these compound species are still poorly understood, they are clearly involved in the relationship between the plant and the surrounding living organisms. They are probably essential elements of coevolution of plants with living organisms, such as parasites, pathogens and predators, but also are pollinators and disseminators. These different relationships gave extreme diversification of secondary compounds (Bennett and Wallsgroove, 1994; Bernhoft, 2010; Hartmann, 2007; Kimura et al., 2001). The typical medicinal or aromatic plants contain higher concentrations of more potent bioactive compounds or secondary metabolites that are used for humans as pharmaceuticals,
Table 1: Summary of different known groups of terpenes.

<table>
<thead>
<tr>
<th>Types of terpenes</th>
<th>Full formula</th>
<th>Reported biological activity</th>
<th>Representative structures</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemiterpenoid</td>
<td>C_{5}H_{8}</td>
<td>The chlorinated hemiterpenes utililactone and eputililactone were found in the leaves of Prinsepia utilis and the anti-platelet aggregation active compound pubesconoside A was reported from Ilex pubescens.</td>
<td><a href="#">Representative structures</a></td>
<td>(Zhang et al., 2011)</td>
</tr>
</tbody>
</table>

agrochemicals, food additives and as ingredients in cosmetics.

Agriculture uses large quantities of chemicals products as fertilizers, insecticides or herbicides and plants growth regulators. Insecticides and herbicides are spread in the environment to control insects, weeds, plant diseases and othersharmful factors that affect crops or livestock, as well as to fight against insects that transmit human diseases. They play a role of first plan in agriculture and public hygiene. Because of its benefits in terms of economic return and to improve human health and well-being, this chemical control technique was quickly adopted around the world (Carvalho, 2017). However, the awareness of secondary effects of chemical pesticides use on the environment and human health is currently causing a green revolution. Modern agriculture is changing; it tends to spread less pesticide and generally use more-selective and less-polluting products.

Most of the published review articles have focused on analyzing research works on the use of secondary metabolites from plants as an ingredient of pharmaceuticals or cosmetics and few of them in agricultural applications as treatment. Also, very limited research on controlling crop health and diseases carried out in the last decade has been published. In the current review, our focus is on how secondary metabolites from plants were valorized for use in agriculture sector as green and healthy alternatives to chemical pesticides and insecticides. In a first part, we will present the different classes of secondary metabolites extracted from medicinal plants. The second section is dedicated to the different extraction technics with an emphasis on non-conventional ones, their benefits and drawbacks. The last part of the review focuses on analyzing recent advances in the development and use of antifungal agents, pesticides, insecticides and biostimulants for controlling crop diseases.

MAJOR TYPES OF SECONDARY METABOLITES FROM PLANTS

Plants secondary metabolites have a prominent function in the protection against predators and microbial pathogens based on their toxic nature and repellence to herbivores and microbes. Other secondary metabolites are involved in defense against abiotic stress (e.g. UV-B exposure) and also important for the communication of the plants with other organisms (Schäfer and Wink, 2009), and are insignificant for growth and developmental processes (Rosenthal, 1991). Secondary metabolites will be discussed in the context of their chemical classes rather than their roles as phytoanticipin or phytoalexins, as several compounds fall into both categories in different species. Plant secondary metabolites can be divided into three chemically distinct groups: Terpenes, Phenolics and N and S containing compounds (Mazid et al., 2011).

The terpenes or terpenoids

The Terpenes are a group of very diverse natural products, generally insoluble in water. They are widely represented and are of considerable chemical interest. They constitute the odoriferous principle of plants. This smell is due to the release of highly volatile molecules containing 10, 15, 20 carbon atoms (Table 1). They are synthesized from acetyl-CoA or its glycolytic intermediates. The name terpene is a transposition in French coined from the German word Terpen (itself from Terpentint which designates turpentine) (Ahmed et al., 2017). Terpenes are divided into
### Table 1 Contd: Summary of different known groups of terpenes.

<table>
<thead>
<tr>
<th>Types of terpenes</th>
<th>Full formula</th>
<th>Reported biological activity</th>
<th>Representative structures</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Monoterpenoids    | C\(_{10}\)H\(_{16}\) | • Activity of Limonene and Perilic Alcohol against Pancreatic and Breast Cancers.  
• Antiviral  
• Many derivatives are anti-inflammatory.  
• Often analgesic.  
• Their oxygenated derivatives are antibacterial.  
• Vulnerary  
• Improve penetration of dermal drugs. | ![Isoprene](image1.png) ![α-Ionone](image2.png) ![Prenol](image3.png) ![Isovaleric acid](image4.png) | (Astani et al., 2010; Barreto et al., 2014; Crowell et al., 1996; de Cásia da Silveira e Sá et al., 2013; Guimarães et al., 2013; Kotan et al., 2007; Sapra et al., 2008) |
| Sesquiterpenoids  | C\(_{15}\)H\(_{24}\) | • Many of them have biological activity, including antimicrobial, antitumor, and cytotoxic properties. In plants, they play important ecological roles in interactions with insects and microbes and act as attractants, deterrents, antifeedants and phytoalexins.  
• Sesquiterpenoids have potential as anticancer agents. | ![Famesol](image5.png) | (Modzelewskab et al., 2005) |
| Diterpenoids      | C\(_{20}\)H\(_{32}\) | • Various effects depending on the molecule (anti-inflammatory, antioxidant, expectorant).  
• Found in plants of euphorbiaceae and work as skin irritants and internal toxins to mammals.  
• Disease resistance agents. | ![Retinol](image6.png) ![Cembren](image7.png) | (Khyade et al., 2015) |
| Triterpenoids     | C\(_{30}\)H\(_{48}\) | Several steroid alcohols (sterols) are important component of plant cell membranes, especially in the plasma membrane as regulatory channels and maintain permeability to small molecules by decreasing the motion of the fatty acid chains. | ![Hopane](image8.png) | (Ogbemudia and Thompson, 2014) |
| Polyterpenoids    | (C\(_{4}\)H\(_{0}\))\(_n\) | In long vessels called laticifers the rubber found provides protection against herbivores and a mechanism for wound healing. | Polyisoprene; lycopene; rubber | (Rohmer et al., 1993) |
monoterpenes, sesquiterpenes, diterpene, triterpenes and polyterpenes. Terpenoid find their origin mostly from both terrestrial and marine plants (50,000 terpenoids have been isolated so far) and also fungi (Yamada et al., 2015). However, only few of them have been identified in prokaryotes. Berthelot André (1981) was the first to isolate bacterial terpenes when he conducted an investigation on the characteristic odor of freshly plowed soil.

### Phenolics

Phenolic compounds present essential functions such as contribution to the color of plants and in the reproduction and growth of plants. They act as defense mechanisms against pathogens, parasites, and predators (Báidez et al., 2007). Plants synthesize phenolic compounds as a response to ecological and physiological conditions and mainly when they are attacked by pathogens, and insects or exposed to UV radiation and wounding (Chung et al., 2003; Crozier et al., 2006; Diaz Napal et al., 2010; Kennedy and Wightman, 2011).

The basic and common scaffold of all phenolic compounds is the aromatic ring bearing one or more hydroxyl groups. The classification of the plant phenolic compounds are based on the number of phenol units in the molecule (simple phenols or polyphenols). Based on this classification, they comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids (Soto-Vaca et al., 2012). Arising from a common intermediate, phenylalanine, or a close precursor, shikimic acid, more than 8,000 phenolic compounds have been discovered so far (Pandey and Rizvi, 2009) (Based upon their structure, phenolics have been divided into two main categories: flavonoids and non-flavonoids (Crozier et al., 2006).

#### Flavonoids

Flavonoids belong to a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in all vascular plants, where they can be in various organs: roots, stems, woods, leaves, flowers and fruits. They are represented by a group of more than 4,000 compounds, they are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Kumar and Pandey, 2013; Dixon et al., 1983). The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of chemical polymerization (Heim et al., 2002). Their activities are structure dependent and they help in combating oxidative stress and act as growth regulators.

Flavonoids are derivatives of the FLAVONE or 2-PHENYL CHROMONE ring bearing free phenol functions, ethers or glycosides. They are complex polyphenols whose structure consists of two aromatic rings (rings A and B) and an oxygenated heterocycle (ring C) (Table 2).

#### Non flavonoids

This class of polyphenols is composed of phenolic acids, tannins, polyphenolics, hydroxycinammates, stilbenes and their conjugated derivatives (Table 3). Phenolic acids subclass is the most populated non-flavonoid groupin plants. In addition, some phenolic acids are of microbial origin (Moorman et al., 1992). They occur in the form of

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### Table 2: Summary of known chemotypes from flavonoids.

<table>
<thead>
<tr>
<th>Class structure</th>
<th>Class name</th>
<th>Subclass name</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavones</td>
<td>Luteolin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>-</td>
<td></td>
<td>(Kim et al., 2016)</td>
</tr>
<tr>
<td>Apigni</td>
<td></td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonols</td>
<td>Kaempferol</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>-</td>
<td></td>
<td>(Kawser Hossain et al., 2016; Kim et al., 2016; Weng and Yen, 2012)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myricetin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>isorhamnetin</td>
<td>OH</td>
<td>OCH3</td>
<td>H</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavanones</td>
<td>Naringenin</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>(Kim et al., 2016)</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>H</td>
<td>OCH3</td>
<td>H</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavanonols</td>
<td>Taxifolin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>-</td>
<td></td>
<td>(Yuan et al., 2004)</td>
</tr>
<tr>
<td>Astilbin</td>
<td>O-Rhamnosyl</td>
<td>OH</td>
<td>OH</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engeletin</td>
<td>O-Rhamnosyl</td>
<td>H</td>
<td>OH</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Summary of known chemotypes from flavonoids.

<table>
<thead>
<tr>
<th>Class structure</th>
<th>Class name</th>
<th>Subclass name</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavanols</td>
<td>Catechin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>(Kim et al., 2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epicatechin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

|                 | Anthocyanidins | Pelargonidin | OH | H    | OH   | H  | (Kim et al., 2016; Panche et al., 2016) |
|                 | Cyanidin       |              | OH | OH   | OH   | H  |                                   |
|                 | Delphinidin    |              | OH | OH   | OH   | OH |                                   |

|                 | Isoflavones    | Genistein    | OH | OH   | OH   | -  | (Panche et al., 2016)            |
|                 | Daidzein       |              | OH | H    | OH   | -  |                                   |

Table 3: Different chemotypes of non-flavonoids.

<table>
<thead>
<tr>
<th>Chemotype</th>
<th>Representative member</th>
<th>R'</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-coumaric acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>(Heleno et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Protocatechualdehyde</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vanillin</td>
<td>H</td>
<td>H</td>
<td>OCH3</td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Veratricaldehyde</td>
<td>H</td>
<td>H</td>
<td>OCH3</td>
<td>OCH3</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferulic acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>OCH3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sinapic acid</td>
<td>OH</td>
<td>H</td>
<td>OCH3</td>
<td>OH</td>
<td>OCH3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Piccol</td>
<td>CH3</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

Benzoic acids

|                 | p-Hydroxybenzoic acid | OH | H  | H  | OH | H  |                                   |
|                 | Salicylic acid        | OH | OH | H  | H  | H  |                                   |
|                 | α-Resorcylic acid     | OH | H  | OH | H  | H  |                                   |
|                 | Protocatechuic acid   | OH | H  | OH | OH | H  |                                   |
|                 | Gentisic acid         | OH | OH | H  | H  | OH |                                   |
|                 | Isovanillic acid      | OH | H  | OH | OCH3 | H  |                                   |
|                 | Syringic acid         | OH | H  | OCH3 | OH | OCH3 |                                   |

Cinnamic acids

|                 | Caffeoyl quinic acid  | -  | OH | OH | -  | -  | (Trugo and Macrae, 1984)          |
|                 | p-Coumaroyl quinic acid | - | H  | OH | H  | -  |                                   |
|                 | Feruloyl quinic acid  | -  | OCH3 | OH | H  | -  |                                   |
|                 | Sinapoylquinic acid   | -  | OCH3 | OH | OCH3 | -  |                                   |

Chlorogenic acids
Table 4: Different classes of Nitrogen containing secondary metabolites (Evans and Evans, 2009).

<table>
<thead>
<tr>
<th>Class</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aporphine</td>
<td><img src="image" alt="Aporphine" /></td>
</tr>
<tr>
<td>β-carbolines</td>
<td><img src="image" alt="β-carbolines" /></td>
</tr>
<tr>
<td>Tryptamines</td>
<td><img src="image" alt="Tryptamines" /></td>
</tr>
<tr>
<td>Imidazole</td>
<td><img src="image" alt="Imidazole" /></td>
</tr>
<tr>
<td>Bataines</td>
<td><img src="image" alt="Bataines" /></td>
</tr>
<tr>
<td>Pyridine</td>
<td><img src="image" alt="Pyridine" /></td>
</tr>
<tr>
<td>Isoquinoline</td>
<td><img src="image" alt="Isoquinoline" /></td>
</tr>
</tbody>
</table>

esters, glycosides or amides, but rarely in free form. Variation in phenolic acids is happening in the number and location of hydroxyl groups on the aromatic ring (Pereira et al., 2009). Phenolic acids have two parent structures: hydroxycinnamic (derivatives include ferulic, caffeic, p-coumaric and sinapic acids) and hydroxybenzoic acid (derivatives consist of gallic, vanillic, syringic and protocatechuic acids) (Khoddami et al., 2013). They are produced in plants via shikimic acid through the phenylpropanoid pathway, as by-products of the monolignol pathway and as breakdown products of lignin and cell wall polymers in vascular plant (Buchanan et al., 2015; Butler and Bailey, 1973). The phenolic acids found in plant cell walls and lignin have a unique chemical structure of C6-C3 (phenylpropanoid type), whereas those of microbial origin are of the form C6-C1 (Phenylmethyl type) (Sarkanen and Ludwig, 1971).

Sulphur containing secondary metabolites

Sulfur-containing class of secondary metabolites are made from GSH, GSL, phytoalexins, thionins, defensins and allinin. These elements are directly or indirectly involved with the defense mechanisms of plants against microbial pathogens (Grubb and Abel, 2006; Halkier and Gershenzon, 2006; Saito, 2004). They are also involved in several types of chemical defenses, including constitutive, induced and activated defenses in a broad range of higher plant species, as well as mosses and algae (Burow et al., 2008).

The biosynthesis of these secondary metabolites occurs in two important pathways. One pathway, found in members of family Crucifereae e.g. Nasturtium, Cabbage and Broccoli, gives rise to the group of glucosinolate substrate hydrolyzed by myrosinase enzyme. The second pathway, found in members of Allium genus e.g. Allium cepa (Onion), Allium sativum (Garlic) and Allium porrum (Leeks), produces the alliin which is hydrolyzed by allinase enzyme (Ober et al., 2003).

Nitrogen containing secondary metabolites (Alkaloids)

Some plant secondary metabolites, such as alkaloids, cyanogenic glucosides, and non-protein amino acids, contain nitrogen atom within their chemical structure and are mostly biosynthesized from natural amino acids.

Alkaloids are found in approximately 20% of the species of vascular plants (herbaceous dicot, monocots and gymnosperms) and about 12,000 alkaloids were isolated from plants. They are generally toxic to some extents and are used by plants primarily in defense against microbial infection and herbivoral attack (Pagare et al., 2015). From the structural point of view, Alkaloids, with low molecular weight structures are very diverse class of compounds derived from amino acids having and contain a nitrogen atom within a heterocyclic ring (Table 4). They have been used for centuries owing to their large spectra of therapeutical effects on animals and human such as antibiotic and anticancer agents. They have also been used as narcotics, poisons and stimulants (Ziegler and Facchini, 2008).

PLANTS SECONDARY METABOLITES EXTRACTION METHODS

The recent advances in extraction of bioactive compound
Table 5: Benefits and drawback of non-conventional extration methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
</table>
| ASE    | • Short extraction time.  
• High pressure permits the extraction of thermally labile analytes, even at high temperature.  
• Low solvent use.  
• Either single or solvent mixture can be used.  
• All type of solvents can be used. Solvents do not generally need to be degassed. | • Very high cost of the equipment.  
• Generally, the extraction is not selective.  
• Extract clean up is usually necessary before the final analysis. | (Giergielewicz-Możajska et al., 2001) |
| SFE    | • Enhanced extraction efficiency.  
• Tunability of the solvent strength.  
• Low organic solvent consumption.  
• Preservation of bioactive properties and organoleptic properties of the extracts.  
• In-line integration with sample preparations and detection methods. | • High capital investment.  
• Large number of variables to optimize.  
• Strong dependence on matrix analyte interactions.  
• Difficulties in scale up and technology transfer.  
• Difficulty in implementing continuous extraction processes  
• Difficulty in extracting more polar compounds. | (Shams et al., 2015) |
| MAE    | • Reduced extraction time and solvent volume.  
• Improved recoveries of analytes and reproducibility were observed in MAE method but with caution of using proper conditions to avoid thermal degradation. | • Limited to small-molecule phenolic compounds such as phenolic acids (gallic acid and ellagic acid), Quecertin, isolavon and trans-resveratrol because these molecules were stable under microwave heating conditions up to 100°C for 20 min. | (Handa et al., 2008; Trusheva et al., 2007) |
| MAE    | • Reduction in extraction time and solvent consumption. | • Use of ultrasound energy more than 20 kHz may have an effect on the active phytochemicals through the formation of free radicals. | (Abdullah et al., 2012; Kaufmann and Christen, 2002; Mediani et al., 2013) |
| SWE    | • More polar target materials with high solubility in water at ambient conditions are extracted most efficiently at lower temperatures. | • Applying higher water flow rates is increasing the extract volume and consequently, lower concentration of the final extracts. | (Haghighi Asl and Khajenoori, 2013) |

Conventional extraction techniques

Bioactive compounds from plant materials can be extracted using various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing. To obtain bioactive compounds from plants, the existing classical techniques are: (1) Soxhlet extraction, (2) Maceration and (3) Hydrodistillation (Azmir et al., 2013). These extraction methods use organic solvents (such as hexane, acetone, methanol, ethanol, etc.) or water and are generally carried out under atmospheric pressure. The use of organic solvents and duration of the extraction process are the major drawback of the conventional methods.

Non-conventional extraction techniques

Alternative approaches to conventional extraction methods have emerged in an attempt to mitigate limitations of the conventional ones. These new methods include Accelerated Solvent Extraction (ASE), Supercritical Fluid Extraction (SFE), Microwave Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), Subcritical Water Extraction (SWE), Pulsed-Electric Field extraction (PEF), Enzyme-Assisted Extraction (EAE), Pressurized Liquid Extraction (PLE) and Rapid Solid-Liquid Dynamic Extraction (RSLDE). In this regard, a focus is given to ASE, SFE, MAE, UAE and SWE because they are the most used methods for extracting bioactive molecules from plants. Table 5 shows the advantages and disadvantages of these extraction methods.
**Accelerated solvent extraction**

Similar to Soxhlet extraction, ASE, also called pressurized liquid extraction or pressurized fluid extraction, is a new extraction technique that use organic solvents, but the use of elevated temperature and pressure with ASE allows the extraction process to be completed within a short time and with a small quantity of solvent (Gan et al., 1999). Particular attention should be paid to ASE performed at high temperature, which may lead to degradation of thermolabile compounds (Majekodunmi, 2015).

Rahmalia et al. (2015) used Cyclohexane-acetone solution at the ratio of 6:4 v/v with 5 min heating (50°C) and obtained the highest yield of bixin from *Bixa orellana* with 68.16% purity (Rahmalia et al., 2015). Tan et al. (2014) succeeded in obtaining higher recoveries (~94%) of flavonoids from Rheum palmatun using 80% aqueous methanol using ASE, suggesting the suitability of this method for quality control evaluation (Tan et al., 2014).

**Supercritical fluid extraction**

The pure matter has a critical point corresponding to a given pressure and temperature. When it is subjected to a pressure and a temperature superior to those of its critical point, it is in a phase called "supercritical" (SC) or supercritical fluid (SF). The SF has an intermediate behavior between the liquid state and the gaseous state. Absolutely, it has high density such as that of liquids, a coefficient of diffusivity between that of liquids and gases, and a low viscosity (as the one of gases). An example of SF is CO₂ that becomes SF at above 31.1°C and 7380 kPa.

In addition to its favorable physical properties, CO₂ is inexpensive, safe and abundant. However, CO₂ is a polar and has weak dissolution power and therefore cannot be used as a solvent especially for polar solutes. To overcome this limitation, it is sometimes accompanied by co-solvents such as ethanol or methanol. However, a major drawback of this method is the initial cost of the equipment which is very high.

Naudé et al. (1998) optimized the use of SC-CO₂ on *Wadelia calendulacea* and achieved its optimum yield at 25 MPa, 25°C temperature, 10% modifier concentration and 90 min extraction time.

In a more recent study by Patil et al. (2014), argon was used because it is inexpensive and more inert instead of carbon dioxide. They showed that the component recovery rates generally increase with increasing pressure or temperature. The highest recovery rates in the case of argon were obtained at 500 atm and 150°C.

**Microwave assisted extraction (MAE)**

In order to facilitate partition of analytes from a sample matrix into the solvent, MAE utilizes microwave energy, with frequencies range of approximately 300 MHz to 1000 GHz (Rostagno et al., 2007; Trusheva et al., 2007). Microwaves have the properties of penetrating biomaterials and interfering with polar molecules such as water present in biomaterials and then generate heat. Therefore, microwaves can cause temperature rise of an entire material by deep penetration into its matrix (Kaufmann and Christen, 2002). MAE can be considered as a selective method that favor polar molecules and solvents with high dielectric constant. In non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only (Handa et al., 2008).

**Ultrasound-assisted extraction (UAE)**

The power of ultrasound can significantly improve the solvent extraction of organic compounds contained within the bodies of plants and seeds. In fact, the mechanical effects of ultrasound provide a greater solvent penetration into cellular matrices and improve the transfer of mass because of micro-streaming process. An additional benefit of using ultrasound in the plant extraction is its ability to disrupt the biological cell walls which release the cell contents (Awad et al., 2012). Overall UAE with ultrasound ranging from 20 kHz to 2000 kHz, is an efficient extraction technique that drastically reduces process times, increases yields and often the quality of the extract (Awad et al., 2012).

In the past few years, diverse set of compounds have been extracted from several matrices by UAE, with special emphasis on the commercial production of bioactive compounds in the food industry. Indeed, Mason et al. (1996), Vilkhu et al. (2008) and Soria et al. (2010) have reviewed different industrial applications of ultrasound in the intensification of extraction of bioactive materials from herbs, oils from seeds and proteins from soy (Mason et al., 1996; Soria and Villamiel, 2010; Vilkhu et al., 2008).

**Subcritical water extraction (SWE)**

Extraction of bioactive molecules from plants using subcritical water is an extraction method in which water is used with temperatures of between 100°C (boiling point of water) and 374.1°C (critical point of water) and with a certain pressure, which varies according to the temperature, and which maintains the water in its liquid form (Chauhan et al., 2007; Negi et al., 2005). At room temperature, water is a polar solvent which has a dielectric constant (ε′) of 75.5. However, in an atmosphere pressurized at high temperatures of the order of 250-300°C, the polarity of the water decreases and the dielectric constant as well. It becomes equivalent to that of an organic solvent such as ethanol (ε′=24) (Luque de Castro et al., 1999). SWE allows the extraction of medium-polar to non-
Table 6: Illustrations of the antifungal activity of plant extracts.

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Extract and/or SM</th>
<th>Crops</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
</table>
| *Medicago sativa* L. (Alfalfa) | Saponins (medicagenic acid, 3-Glc medicagenic acid and 3-Glc-Ara hederagenin) | diverse crop | • Anti-fungal properties.  
• Effective against a wide range of crop pests such as nematodes, herbivorous insects and fungi.  
• Inhibition of mycelial growth. | (Abbruscato et al., 2014; Demirci and Dolar, 2006; Rafińska et al., 2017) |
| *Medicago sativa* L. | Isoflavones (medicarpin and coumestrol) | Alfalfa | • Inhibition of the synthesis of Nodulation factors.  
• Inhibits motility of *Pratylenchus penetrans*.  
• Growth-promoting properties.  
• Inhibition germination of *Allium cepa*. | (Baldridge et al., 1998; Macias et al., 1999; Morel et al., 2015; Zuanazzi et al., 1998) |
| *Salvia officinalis* | Essential oil | Apple | • Antifungal activity against *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer*. | (Ouadi et al., 2015) |

polar molecules without the use of organic solvents with solubilization improvement of the active principles (Livermore, 2002).

Extracts from grape seeds and cereals, such as wheat bran and rice bran, have been obtained using SWE. In more recent studies, SWE has been used to extract antioxidants, proteins, and anti-inflammatory from plants and foods. In many cases, it was demonstrated that SWE can provide higher extraction yields in shorter extraction times (up to 50% lower) than Soxhlet and SFE methods. However, the efficiency of extraction is influenced by factors such as temperature, pH, and pressure, among others (Carr et al., 2011).

**USE OF PLANTS EXTRACTS AND SECONDARY METABOLITES IN CONTROLLING CROP HEALTH AND DISEASES**

Phytochemical products, used in plant-based treatments, are increasingly subject to limitations of use because of their high cost, the unavailability of certain products on the local market and the potential impact on the environment including human and animal health. These various reasons motivated the search for alternative solutions to the use of conventional chemical pesticides. Bio-pesticides present an interesting alternative to the conventional chemical treatments, and in recent years, pesticide firms have invested significantly in companies producing bio-pesticides (Siegwart et al., 2015).

In this section, we have focused our literature analysis on the use of plants secondary metabolites and extracts as antifungal, biopesticide and bioinsecticides attacking plants, as general agents against crop diseases and as bio-stimulants of plants growth.

**Plants extracts as antifungal agents**

Since the last decade, there has been a growing scientific interest in the use of crude plants extracts or isolated secondary metabolites to treat fungal invasion of plants (Table 6). Most of the published studies demonstrated the fungicide activity of the tested compounds on a variety of crops.

**Saponins**

Saponins are a group of glycosidic secondary metabolites produced by a variety of plant species. They belong to three major chemical classes: steroid glycosides; steroid alkaloid glycosides and triterpene glycosides. Owing to their chemical, physical and physiological characteristics, saponins display a broad spectrum of biological effects including fungicidal, molluscicidal, nematocidal, antibacterial and antiviral activities (Tava and Avato, 2006).

Jarecka et al. (2008) evaluated the effect of total and individual saponins from *Medicago arabica*, *M. hybrida*, and *M. sativa* on growth and development of *Fusarium oxysporum f. sp. tulipae in vitro* and *in vivo* (Jarecka et al., 2008). The authors showed that the total amount of saponins originated from the roots of *M. hybrida*, and of
Table 6 Contd: Illustrations of the antifungal activity of plant extracts.

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Extract and/or SM</th>
<th>Crops</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cistus villosus</em>, <em>Ceratonia siliqua</em>, <em>Halimium umbellatum</em></td>
<td>Methanol extracts</td>
<td>Mandarin</td>
<td>• Antifungal activity.</td>
<td>(Talibi et al., 2012)</td>
</tr>
<tr>
<td><em>Thymus leptobotrys</em> Murb.</td>
<td>Essential oils chloroformic extract</td>
<td>in vitro antifungal test</td>
<td>• Total inhibition of <em>Penicillium digitatum</em>; <em>Penicillium italicum</em> and <em>Geotrichum candidum</em>.</td>
<td>(Ameziane et al., 2007)</td>
</tr>
<tr>
<td><em>Cinnamomum camphora</em> L.</td>
<td>Ethanol extract</td>
<td>Cucumber</td>
<td>• Antifungal activity against <em>C. lagenarium</em>.</td>
<td>(Chen and Dai, 2012)</td>
</tr>
<tr>
<td>Garlic, clove, garden quinine, Brazilian pepper, anthimandhaari, black cumin, white cedar and neem</td>
<td>Ethanolic extract of garlic, wheat leaf</td>
<td>• Strong reduction of the wheat leaf rust infection.</td>
<td>(Shabana et al., 2017)</td>
<td></td>
</tr>
<tr>
<td>Coriander</td>
<td>Aqueous extracts</td>
<td><em>Lactuca sativa</em> (Lettuce)</td>
<td>• Positive effects on the germination and development of lettuce (<em>Lactuca sativa</em>).</td>
<td>(Carmello and Cardoso, 2018)</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Aqueous extracts combined to Sodium hypochlorite</td>
<td><em>Lactuca sativa</em> (Lettuce)</td>
<td>• Complete inhibition of the mycelial growth of <em>Cercospora longissimi</em>.</td>
<td>(Carmello and Cardoso, 2018)</td>
</tr>
</tbody>
</table>

*Alfalfa* (*M. sativa*) at concentrations of 0.01, 0.05 and 0.1% presented strong antifungal effect against *F. oxysporum* f. sp. *tulipae* in artificially infested substrate. This work suggested that saponins originated from *Medicago* can act as fungicide.

In a study performed by Abbruscato et al. (2014), the antifungal properties of saponin extracts from alfalfa (*M. sativa L.*) against the causal agent of rice blast *P. oryzae* was investigated. Saponins from *M. sativa* tops and roots, the corresponding mixture of prosapogenins, and pure saponins and sapogenins were tested against *P. oryzae* isolates in in vitro experiments. All of the tested compounds showed a broad spectrum of antifungal activity (Abbruscato et al., 2014). The data presented in this work demonstrated the potential use of saponins, related prosapogenins, or aglycones from *M. sativa* for new fungicidal formulations.

**Isoflavones**

The isoflavones are a distinctive but large subgroup of flavonoids. These compounds possess a 3-phenylchroman skeleton that is biologically derived by rearrangement of the flavonoid 2-phenylchroman system. This class of flavonoids has been investigated for their property of protecting crops from fungal invasion.

Several studies have reported the antifungal activity of isoflavones (Hiroshi et al., 1973; Fukui et al., 1973). These researchers isolated a new isoflavone, named luteone, using amethanol extraction of immature fruits of *Lupinus luteus* (Leguminosae). They found that luteone has a significant inhibitory activity against the conidia of *Coelhobulus mimbeanils* (*Helminthosporium oryzae*).

Blount et al. (1992) tested the antifungal activity of isoflavones medicarpin, vestitone, 2′-hydroxyformononetin (2′-OHF), formononetin and daidzein against several fungal strains which are pathogens and non-pathogens of alfalfa. They reported that medicarpin caused little or no inhibition of the growth of the four known alfalfa pathogens. *Phytophthora megasperma* f.sp. *medicaginis* (Pmm) showed a very significant sensitivity to 0.1 mM vestitone and 2′-OHF, whereas it was strongly inhibited by medicarpin, vestitone, and 2′-OHF at 0.5 mM. *Phoma medicaginis* was strongly inhibited by 0.5 mM medicarpin and vestitone, whereas 2′-OHF was not a significant inhibitor. They suggested that the antifungal activity of the tested isoflavones is often based on the inhibition of spore development and mycelial hyphae elongation.

Massaoka et al. (1993) found a novel compound in the exudates from Fe-deficient alfalfa roots that dissolves ferric phosphate intensively. This isoflavonoid 2-(3,5,7-trihydroxyphenyl)-5,6-dihydroxybenzofuran, released by Alfalfa roots, works as a phytoalexin against root pathogens such as *F. oxysporum* f. sp. *Phaseoli* (Masaoka et al., 1993).

Weidenbörner and Jha (1994) investigated the effect of flavonoids on growth of fungi in vitro. They found that from the 10 tested flavonoids, the strongest antifungal activity is...
demonstrated by unsubstituted flavones and unsubstituted
flavanones. Hydroxyl and methyl groups in these
compounds reduce their antifungal properties.

Other extracts

Other plants crude extract obtained by various extraction
methods were studied for their antimicrobial effects and
proved potential antifungal agents.

Ameziane et al. (2007) studied the antifungal activity of
twenty-one medicinal and aromatic plants (whole plant, leaves, stem, bark, seeds, flower and fruit) used in southern
Moroccan traditional medicine against the principal post
harvest fungal pathogens (Penicillium digitatum, Penicillium
titalicum and Geotrichum candidum) of citrus fruits. They
evaluated in vitro the antifungal activity of grounded plants,
esential oils, methanolic and chloroformic extracts. Data
from this study showed that essential oils and the
chloroformic extract of the T. leptobotrys plant totally
inhibited the three pathogens (P. digitatum, P. italicicum, G.
candidum). They concluded that among the 21 plants
tested, T. leptobotrys, C. villosus, E. globulus and P. harmala
showed the highest antifungal activities against the tested
pathogens and that the plant powders showed a stronger
antimicrobial activity than essential oils and solvent
extracts.

In an attempt to find an alternative to the chemical
fungicides used in the control of G. candidum which is the
causal agent of citrus sour rot, Talibi et al. (2012) evaluated
the effectiveness of eight medicinal plants extracted in
different organic solvents, against the fungus under both in
vitro and in vivo conditions. The in vitro results of this
investigation showed that the methanol extracts of Cistus
villosus, Ceratonia siliqua and Halimium umbellatum
exhibited strong antifungal activity (minimum inhibitory
concentration values ranged between 0.156 and 1.25
mg/ml). As for the in vivo results, all the tested methanol
plant extracts significantly reduced the incidence of sour
rot caused by the fungus under the laboratory conditions.

Chen and Dai (2012) investigated several plants extracts
for their antifungal activity against C. lagenerarium, the causal
agent of anthracne in cucumber. They showed that the
ethanol extract of C. camphora consistently showed
significant inhibitory activity against C. lagenerarium in vitro
and in seedlings, and could be used as a potent
phytochemical fungicide.

Ouadi et al. (2015) studied the antifungal effect of
essential oil of Salvia officinalis on mycelial growth of three
fungi (Botrytis cinerea, Penicillium expansum and Rhizopus
stolonifer) responsible for apple rot in storage in fridges.
They used the essential oil of the aerial part of the plant and
evaluated its antifungal activity against mycelial growth of
isolated fungi using poisoned food technique and volatile
activity assay. The data showed that the essential oil has a
significant activity and inhibited the mycelial growth of all
strains in a dose-dependent manner. The obtained results
demonstrated that the essential oil of S. officinalis is an
effective antifungal agent against B. cinerea and R. stolonifer,
but especially for P. expansum. They concluded that the
obtained essential oil may be considered as a potential
alternative to synthetic fungicides for the protection of
apples from phytopathogenic fungi and may also prevent
the spoilage of other food commodities during storage.

Sales et al. (2016) reported the screening of the
antifungal potential of 131 extract forms from 63 plant
species performed in vitro using plate-hole method. To
control pineapple fusariosis in situ, preventive and post-
infection treatments were performed on detached
pineapple leaves of cv. Pérola (susceptible). This study
showed that among the analyzed 49 mother tincture
samples, 46% were effective against F. guttiforme and 29%
against Chalara paradoxa. The natural plant extracts,
mother tincture of Glycyrrhiza glabra (MTGG1), mother
tincture of Myroxylon balsamum (MTBT2), mother tincture
of Aloe vera (MTAV3), mother tincture of A. sativum
(MTAS4), resin of Protium heptaphyllum (RESAMS) and
crude extracts of Rhizophora mangle (CEMv6), exhibited
an antifungal activity against F. guttiforme. In the preventive
treatment against pineapple fusariosis, MTAV3, MTAS4
and MTGG1 were statistically similar to the treatment with
tebuconazole fungicide. The curative treatments with
MTAV3, MTAS4, MTGG1 and MTBT2 presented similar
activity to fungicide (P < 0.05). Based on the findings,
they concluded that mother tinctures can effectively control
phytopathogens.

Shabana et al. (2017) investigated the role of some plant
extracts (garlic, clove, garden quinine, Brazilian pepper,
anthimandhaari, black cumin, white cedar and neem) from
Egypt in controlling wheat leaf rust disease under in vitro
and in vivo conditions. Their ultimate aim was to develop
safe alternative control strategies to reduce dependency on
synthetic fungicides. In their study, in vitro-tested plant
extracts inhibited spore germination of wheat leaf rust P.
triticina by 93% or more. Neem extract caused 98.99%
inhibition to spore germination, which was not significantly
different from the fungicide Sumi-8 treatment (100%
inhibition). Foliar spray application of wheat plants at
mature stage with all plant extracts significantly reduced
the leaf rust infection (average coefficient of infection, ACI)
as compared with the untreated control and neem was the
most effective treatment. The study concluded that plant
extracts may be useful to control leaf rust disease in Egypt
as a safe alternative option to synthetic fungicides.

A very recent study by Carmello et al. (2018) evaluated
the potential use of the aqueous extracts of clove,
cinnamon, and coriander and the sodium hypochlorite in
the germination and initial development of lettuce (Lactuca
sativa) seeds and effects of these extracts on the mycelial
growth of the fungus Cercospora longissima. They showed
that the aqueous extract of coriander exerts positive effects
on the germination and development of lettuce seedlings,
Table 7: Illustrations of the pesticide activities of plant extracts.

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Extract or/and SM</th>
<th>Crops</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medicago sativa</em> L.</td>
<td>Saponins</td>
<td>Potato</td>
<td>• Deterrent activity against Potato beetle.</td>
<td>(Argentieri et al., 2008; Szczepaniak et al., 2001; Tava and Odoardi, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Insecticidal activity against European grape moth (<em>Lobesia botrana</em>) and summer fruit tortrix moth (<em>Lobesia botrana</em>) and summer fruit tortrix moth (<em>Adoxophyes orana</em>).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Toxic effect on the parasitic nematode <em>Xiphinema index</em>.</td>
<td></td>
</tr>
<tr>
<td><em>Medicago truncatula</em> L.</td>
<td>3-GlcA, 28-AraRhAxyL medicagenic acid</td>
<td>Rice</td>
<td>• Toxic activity against rice weevil (<em>Sitophilus oryzae</em>).</td>
<td>(Da Silva et al., 2012)</td>
</tr>
<tr>
<td><em>Ailanthus altissima</em> L., <em>Convolvulus krauseanus</em> Regel and Schmalh</td>
<td>Ethanol extracts</td>
<td>Bean leaves</td>
<td>• Insecticidal activities against the spider mite (<em>Tetranychus urticae</em> Koch).</td>
<td>(Chermenskaya et al., 2010)</td>
</tr>
<tr>
<td><em>Anabasis aphylla</em> L., <em>Ungernia severtzovii</em> B., <em>Ferula foetida</em></td>
<td>Ethanol extracts</td>
<td>Bean leaves</td>
<td>• Insecticidal activities grain aphid <em>Shizaphis graminum Rond.</em></td>
<td>(Chermenskaya et al., 2010)</td>
</tr>
<tr>
<td><em>Silenesus samyrca</em> Lazkov</td>
<td>Ethanol extract</td>
<td>Bean leaves</td>
<td>• Insecticidal activity Flower thrips <em>Frankluniella occidentalis Perg.</em></td>
<td>(Chermenskaya et al., 2010)</td>
</tr>
<tr>
<td><em>Robinia pseudoacacia</em></td>
<td>Ethanol extract</td>
<td>Cotton and Rape</td>
<td>• Insecticidal activity against aphids.</td>
<td>(Jiang et al., 2018)</td>
</tr>
<tr>
<td><em>Pongamia pinnata</em></td>
<td>Methanol and hydroalcohol extracts</td>
<td>Larvae Mosquito</td>
<td>• Mosquito larvicidal activity against <em>Culex quinquefasciatus, Aedes aegypti</em> and <em>Anopheles stephensi</em>.</td>
<td>(Kolli et al., 2013)</td>
</tr>
<tr>
<td><em>Litsea cubeba</em></td>
<td>Chlorobutanol</td>
<td>Corn</td>
<td>• Insecticidal activity against <em>Sitophilus zeamais</em> (maize weevil)</td>
<td>(Zhang et al., 2017)</td>
</tr>
<tr>
<td><em>Piper nigrum</em></td>
<td>Piperolein B, Piperchabamide D (extracted with chloroform)</td>
<td>Cabbage</td>
<td>• Insecticidal properties against <em>Plutella xylostella</em>.</td>
<td>(Hwang et al., 2017)</td>
</tr>
<tr>
<td><em>N. cataria</em></td>
<td>Essential oil</td>
<td>Various weeds and crop species</td>
<td>• Natural herbicide (strong inhibitory and phytotoxic effects on crops and weeds).</td>
<td>(Saharkhiz et al., 2016)</td>
</tr>
</tbody>
</table>

thereby promoting aerial part development. The aqueous extract of cinnamon and sodium hypochlorite completely inhibited the in vitro mycelial growth of *C. longissima* and can therefore act as an efficient alternative treatment for controlling this phytopathogen.

**Pesticides and insecticides from plants extracts**

Agricultural crops are under constant assault by insect pests, making insecticides essential to reduce losses. Synthetic insecticides are important, effective tools in modern crop management. However, in certain cases, they pose serious threats to the environment, animals and humans. They penetrate to groundwater, pollute streams and harm wildlife, including natural predators of the targeted pests. Older pesticides such as DDT killed bald eagles, birds, fish and even people (Carson).

Many plant species produce substances that protect them by killing or repelling the insects that feed on them. In this section, we will discuss selected literature works demonstrating the potential of plants extracts and secondary metabolites to fight against pesticides and insecticides causing harm to various crops (Table 7).

Szczepanik et al. (2001) compared the activity of total saponins extracted from the roots and from the aerial parts
of alfalfa (*M. sativa* L.) with different doses of substances included in the diet of the Colorado beetle larvae. In this study, the authors exposed the insect larvae to potato leaves treated with different concentrations of a saponin solution. They showed that the saponins considerably reduced the larval food consumption and the duration of insects’ feeding period. They suggested that the saponins used in their study acted as strong feeding deterrents against larvae.

Another study on the insecticidal activity of saponins can be found in the work of Argentieri et al. (2008). Absolutely, they investigated the nematicidal activity of saponins from *Medicago arborea* (tops), *M. arabica* (tops and roots) and *M. sativa* (tops and roots) against the plant parasitic nematode *Xiphinema index*. In the same study, the nematicidal activity of related prosapogenins and sapogenins on *X. index* was also described. The nematodes were exposed to test solutions and observed at different time intervals ranging from 1 to 48 h. The nematicidal activity of saponins was judged by the lack of mobility of the insects and the Nematode mortality was confirmed after lack of movement. The authors found that saponins, from *M. arborea*, *M. arabica* and *M. sativa* at different concentrations, possess nematicidal activity against the plant-parasitic nematode *X. index*, those from *M. arborea* tops being the less effective. This study suggested that the use of saponins from *Medicago* spp. for the development of new nematicidal formulations appears therefore as a reasonable possibility.

Chermenskaya et al. (2010) investigated insecticidal properties, inhibiting growth, behavior-modifying and also toxic activities of plant extracts of 123 plant species from 96 genera of 38 families, against three species of phytophagous pests: the western flower thrips *Frankliniella occidentalis* Perg. (Thysanoptera: Thripidae), the two narcissus mite were *Ailanthus altissima* L., roots of *Convolvulus krauseanus* Regel. (Homoptera: Aphididae) (Chermenskaya et al., 2010). The most active ethanol extracts against the spider mite were *Ailanthus altissima* L. (leaves) and roots of *Convolvulus krauseanus* Regel. and Schmalh., against the grain aphid – of *Anabasis aphylla* L., roots of *Ungeria severtzovii* (Regel) B. Fedtsch. and *Furula foetida* (Bunge) Regel, and against the thrips – of the *Silenes samyrica* Lazkov (aerial part). Extracts from these plants caused significant pest mortality, reduced reproductive potential and deterred pests from feeding on suitable host plants. The authors suggested that extracts of these plants could serve as the foundation for the development of new botanical insecticides.

Da Silva et al. (2012) studied the toxicity and specificity of saponin 3-GlcA-28-AraRhaxy-medigenenate from *M. truncatula* seeds for the insect *Sitophilus oryzae* which attacks several stored crops. They found that saponin 3-GlcA-28-AraRhaxy-medigenenate displayed a strong toxic activity towards the adults of the rice weevil *S. oryzae* at concentrations down to 100 μg/g of food. The data reported in their study showed specificity of saponin 3-GlcA-28-AraRhaxy-medigenenate for the rice weevil and suggested that this saponin has a specific mode of action, rather than acting via its non-specific detergent properties.

Koli et al. (2013) evaluated the mosquito larvicidal activity of *Pongamia pinnata* extracts against three mosquito vectors. The methanol and hydroalcohol extracts of bark part of *P. pinnata* L. were tested against four instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The mortality was observed 24 and 48 h after treatment, data were subjected to probity analysis to determine lethal concentration (LC50 and LC90) to kill 50 and 90% of treated larvae of tested species. The results from this work showed that larval mortality of *P. pinnata* against *C. quinquefasciatus*, *A. aegypti* and *A. stephensi* was found in both extracts with LC50 values of 84.8, 118.2 and 151.7 ppm for methanolic extracts and 97.7, 128.3 and 513 ppm for hydroalcohol extracts, respectively. The highest larval mortality was found in methanol extract of *P. pinnata* when compared with the hydroalcohol extract. The results of this investigation suggested that both methanol and hydroalcohol extracts have the potential to be used as an ideal eco-friendly approach for the control of disease vectors. This could lead to isolation of novel natural larvicidal compounds.

Zhang et al. (2017) investigated the insecticidal activity of different fractions of 95% ethanol extracts of *Litsea cubeba* fruit separated by liquid–liquid extraction. They examined the bioactivity of ethanol extracts in *n*-hexane, ethyl acetate, chloroform, and water against *Sitophilus zeamais* (maize weevil), and also assessed the repellent, fumigant, and contact toxicities of the four main components derived from chloroform extracts against this insect pest (Zhang et al., 2017). The main components in 95% ethanol extracts of *L. cubeba* fruits were water insoluble, water extractable, and chloroform extractable, and to a lesser extent ethyl acetate, and *n*-hexane extractable. Among these extracts, chloroform extract showed the highest bioactivity against *S. zeamais*. Twenty components were identified in chloroform extract, the main ones were laurine, 2,6-diisopropylaniline, chlorobutanol, and 6-methyl-5-hepten-2-one. All these compounds exhibited contact toxicity, fumigant toxicity, and repellent activity against *S. zeamais*, with chlorobutanol showing the overall strongest bioactivity. They concluded that these four compounds have the potential as natural insecticides.

Hwang et al. (2017) identified an environment-friendly larvicidal compounds isolated from *P. nigrum* against *Plutella xylostella*. They used a crude methanol extract from *P. nigrum* fruit. They demonstrated that piperolein B and piperchabamide D isolated from chloroform extract of *P. nigrum* are the major constituents of the extract, demonstrating insecticidal properties for the control of *P. xylostella* larvae and the most effective. They suggested that these plant-derived compounds should become useful
alternatives to synthetic chemicals after studying their insecticidal mechanisms.

Jiang et al. (2018) studied the chemical composition of the seed ethanol extract of Robinia pseudoacacia using GC×GC-TOF-MS and evaluated the aphidicidal activity of the seed extract against aphids in a laboratory bioassay and in a field test. The petroleum ether fraction from R. pseudoacacia seeds showed significant insecticidal activity against aphids. The GC×GC-TOF-MS analysis showed that 9, 12-octadecadienoic acid, 9, 12, 15-octadecatrienoic acid methyl ester and 9, 12-octadecadienoic acid methyl ester were the major components in the petroleum ether fraction. They concluded that R. pseudoacacia seeds extract could be considered as a promising source of plant insecticide for controlling aphids.

All these published studies and many others, strongly demonstrate the potential of isolated secondary metabolites or other plant extracts to be insecticide and pesticide agents and could serve as the foundation for the development of new plant based insecticides for agriculture use.

### Plant extracts as bio-stimulants for plants growth

Biostimulants are materials, other than fertilizers, that promote plant growth when applied in small quantities (Table 8). These environment friendly and natural substances promote vegetative growth, mineral nutrient uptake and tolerance of plants to biotic and abiotic stresses. The interest in this category of bioproducts in modern agriculture results from the trend to search for new preparations based on natural substances that could replace the application of synthetic chemicals in agriculture (Hwang et al., 2017).

A first series of studies by Zida et al. (2008b) reported that seed treatment with an aqueous extract of the plant Eliptica alba increased the yield of sorghum in Burkina Faso in several field trials (Zida et al., 2016, 2012, 2008). In a recent study, the same authors compared the antifungal effect of E. alba to hydropriming, measuring for the first time the level of fungal infection directly in planta using both a classical method (enumeration of fungal outgrowth from plant tissue on potato-dextrose agar) and a molecular method (amplicon sequencing of fungal 18S ribosomal DNA from seedlings) (Zida et al., 2018). The study showed that, with 6-h of soaking, hydropriming was an inherent component of seed treatment with the E. alba extract and contributed significantly to the overall observed increase of yield and emergence. An additional yield increase was caused by factor(s) derived from the plant E. alba, and may involve suppression of pathogenic fungi.

Pardo-García et al. (2014) investigated the impact of oak extracts on wine polyphenol production when they are applied over grapevines (Pardo-García et al., 2014). They used commercial aqueous French oak extract chosen for this study. This extract was obtained by macerating in water at high temperature French oak chips (Quercus sessiliflora Salisb) from natural seasoning toasted at a medium intensity level. This study showed that oak extract could be considered as an important biostimulant of grape polyphenols, since it affected grape composition, producing less alcoholic and acid wines with higher color intensity and lower shade, and also produces more stable color and higher content of polyphenols such as gallic acid, hydroxycynnamoyltartaric acids, acylated anthocyanins, flavanols and stilbenes.

Mahdavikia and Saharkhiz (2016) determined the

### Table 8: Illustrations of the biostimulation effects of plant extracts on crops growth.

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Extract or/and SM</th>
<th>Crops</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peppermint</td>
<td>Water extract</td>
<td>Tomato</td>
<td>• Decrease in seed germination and the growth of tomato seedlings</td>
<td>(Mahdavikia and Saharkhiz, 2016)</td>
</tr>
<tr>
<td>Ascophyllum nodosum</td>
<td>-</td>
<td>Tomato</td>
<td>• Biostimulant (induce drought tolerance in tomato)</td>
<td>(Goñi et al., 2018)</td>
</tr>
<tr>
<td>Moscatel vine-shoots</td>
<td>Aqueous extract</td>
<td>Grapevines</td>
<td>• Viticultural biostimulant (improve non-aromatic wines quality in terms of their aroma and phenolic composition)</td>
<td>(Sánchez-Gómez et al., 2017)</td>
</tr>
<tr>
<td>Quercus sessiliflora Salisb. (French oak chips)</td>
<td>Aqueous extract</td>
<td>Grapevines</td>
<td>• Biostimulant of grape polyphenols</td>
<td>(Pardo-García et al., 2014)</td>
</tr>
<tr>
<td>Eliptica alba</td>
<td>Aqueous extract</td>
<td>Sorghum</td>
<td>• Antifungal effect</td>
<td>(Zida et al., 2016, 2018, 2012, 2008)</td>
</tr>
<tr>
<td>Maize grain with Mg foliar treatment</td>
<td>Aqueous and alcoholic extracts</td>
<td>Sandy Soil</td>
<td>• Biostimulant (promotes the growth and yield of sunflower seeds with a very good quality of oil)</td>
<td>(Rehman et al., 2018)</td>
</tr>
</tbody>
</table>
morphophysiological and biochemical responses of tomato to stress caused by allelopathic compounds of peppermint. Water extract of Peppermint (*Mentha x piperita* L. CV. *Mitcham* from Iran) causes oxidative stress through generation of reactive oxygen species (ROS), and activates antioxidant enzymatic and non-enzymatic machinery in the target tissue as a defense mechanism to counter the peppermint extract-induced stress. They demonstrated that different concentrations of peppermint water extract caused decrease in seed germination and the growth of tomato seedlings; especially the 10% (v/v) concentration showed potent allelopathic and phytotoxic effects.

Sánchez-Gómez et al. (2017) conducted a study to determine if the aromatic character of Moscatel can be transmitted to Airén white wines, when Moscatel vine-shoots aqueous extracts were foliarily applied to Airén grapevines. For this purpose, two Moscatel extracts were applied in the form of non-toasted (MVS) and toasted (MVSToasted), and the quality of the resulting wines was studied in terms of their aroma and phenolic composition. They strongly showed that Moscatel aqueous extracts application from non-toasted and toasted vine-shoots applied over leaves of Airén grapevines had a positive effect on wine quality, from the oenological parameters, highlighting its reduction in the alcohol degree content, to volatile and phenolic composition. These results have demonstrated that there is a "feedback" effect to the wine of the compounds present in vine-shoots, when they were applied to grapevines leaves, independently of the variety used (Airén or Moscatel). Therefore vine-shoot extracts could be considered as viticultural biostimulant, since they could enhance the non-aromatic wines, such as Airén, which was pointed in previous studies.

A very interesting work conducted by Goñi et al. (2018) addressed the role of *Ascophyllum nodosum* Extracts (ANE) biostimulants in maintaining crop productivity during the periods of drought. They investigated whether or not ANE biostimulants are the same in terms of their ability to induce drought tolerance in tomato and also looked at the effects of biostimulants on some of the molecular players involved in mediating drought tolerance in tomato. The authors used three commercially available liquid seaweed extracts of *A. nodosum* (ANE A, ANE B and ANE C) manufactured using different methods that were applied to plants as biostimulant treatments. Clear phenotypic differences were observed between ANE formulations at the end of the drought period with ANE A maintaining better plant growth without symptoms of drought stress.

Rehman et al. (2018) evaluated the beneficial effects of seed soaking maize grain extract (aqueous and alcoholic extraction) in combination with or without foliar Magnesium on growth, yield performance, seed oil and fatty acid. This study was not only essential for vegetative growth, seed yield and oil quality in sunflower plants under sandy soils conditions, but also for obtaining strong progeny for the following seasons. They concluded that using maize grain extract as organic biostimulant and foliar Mg can be a novel strategy for achieving optimum growth, seed and oil yields with desired quality.

**CONCLUSIONS**

Medicinal or aromatic plants contain metabolites that are very specific and sometimes extremely at a high concentration. For economic reasons, the biochemical study of these compounds has been the subject of intense efforts, contributing to the development of several areas of phytochemistry with close links to the pharmacy and dye industry. These secondary metabolites might play a role in the defense against herbivores and pesticides, and some of them have been shown to play essential roles in the relationships between plants and their environment. They are probably essential elements of evolution of plants with living organisms, such as parasites, pathogens and predators, but also pollinators and disseminators.

Secondary metabolites can be classified into several major groups: among them are phenolics, terpenes and steroids, and nitrogen compounds including alkaloids. Each of these classes contains a very large and diverse set of compounds that have a very wide range of biological activities.

The extraction processes of secondary metabolites from plants are based on the difference in solubility of the compounds present in a mixture and in a solvent. There are several techniques for extracting products with high added value present in plants. These techniques can be called conventional (used for a long time) and non-conventional (developed more recently). Conventional techniques (maceration, Soxhlet, and reflux extraction) use organic solvents (such as hexane, acetone, methanol, ethanol, etc.) or water and are generally carried out under atmospheric pressure. The new techniques (ASE, SFE, MAE, UAE, SWE, PEFE, EAE, PLE and RSLDE) use high pressures and/or temperatures.

The use of chemical plant protection products has considerably reduced the work difficulties for the farmer in the field, while allowing sufficient production and at a lower cost. Agriculture production losses, before crop harvest, due to pests (insects, micro-organisms) and weeds are major concerns for farmers. Consequently, it is obvious that synthetic chemical plant protection products have many advantages. However, their use may be the cause of environmental problems and public health, especially since the risks inherent in some of them are poorly evaluated. One of the tools for reducing conventional pesticides for agricultural use is the use of phytosanitary products of biological origin. Secondary metabolites from plants and other plant extracts, such as essential oils, present interesting biological activities against numerous harmful pests and microorganism. In addition, some of the secondary metabolites have a beneficial effect as plant
growth regulators. These plant-derived biopesticides and biostimulants offers many application opportunities in organic and conventional farming.

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