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Natural Products from Plants as Potential Source Agents for Controlling *Fusarium*

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1. Introduction

At present, due to the fast expansion of the world's population, agricultural practices have been focused on increasing food production. Crop protection from competing weeds, insects and diseases has been necessary to obtain higher yields in agriculture. However, as a consequence of pest infection, a decrease in production performance has happened manifested in low food quality [1]. In order to solve this situation, synthetic chemical products have been widely used for controlling infectious diseases. Nonetheless environmental pollution due to their slow biodegradation, phytotoxicity, carcinogenicity and toxic waste in agricultural products is an important drawback [1, 2]. Agriculture production is currently trending to use eco-friendly methods for controlling diseases and pest infection [3, 4].

The growing demand of pesticide-free agricultural products has led to the search of novel, affordable, and less toxic strategies for pest control. Amongst those strategies biological agents, mineral salts and vegetable products have gained interest in the industry. Natural products are an important source of novel active chemical agents that could delay or inhibit pathogen growth and / or toxin production [5, 6]. Generally speaking, the plant derivatives (essential oils, extracts, fractions and compounds) are generally considered as non – phytotoxic and potentially effective for controlling pathogenic fungi in plants, [7]. Some of these natural substances have showed antifungal, fungistatic or fungicidal activities which allow protected crops to have an extended shelf life by preventing enzymatic or metabolic processes of microorganisms. These fermentative or degradative microbiological processes can result not only in changes in odor taste, color and texture but also can cause potential harm to the consumer [8].

Natural compounds can be useful for crop and food protection. The use of botanicals for the management of the phytopathogens is gaining ground. Plant extracts and essential oils may

have an important role to play in the preservation of foodstuffs against fungi. Recent literature has shown that biological activity of many plant-extracts, essential oils and their individual components is related with the inhibition of the growth of various fungi. From the 1970s there has been an increased interest on the study of the defensive mechanisms of plants for protecting against pathogenic agents or adverse environmental conditions. Phytochemical research has led to the isolation of active constituents synthesized by plants as a response to biotic or abiotic stresses, evidencing that these substances have insecticidal, fungicidal, bactericidal or herbicidal action. Throughout their evolution, plants have developed several defense mechanisms to prevent infections due to pathogens; also, plants synthesize a large number of secondary metabolites to protect themselves against biotic and abiotic stresses and for the maintenance of structure and vital functions. These are reasons to consider plants as an important source of new biopesticides [9, 10].

Fungi are a major cause of plant diseases and are responsible for significant economic losses to the food industry. These pathogens can cause local or systemic symptoms on their hosts. The most common symptoms are die-back (extensive necrosis of twigs), root-rot (disintegration or decay of the root system), leaf-spots (localized lesions on leaves consisting of death and collapsed cells), damping-off (rapid death of young seedlings), blight (general and extremely rapid browning death of leaves, branches, twigs, and floral organs), anthracnose (necrotic and sunken ulcer-like lesions of the stem, leaf, fruit, or flower), canker (localized necrotic lesion), basal stem rot (disintegration of the lower part of the stem), soft rots and dry rots (maceration and disintegration of fruits, roots, bulbs, tubers, and fleshy leaves) and decline (plants growing poorly, small and yellowish or red leaves) [11].

Species of the genus *Fusarium* are examples of phytopathogenic and toxine-producing fungi that have been reported to be widespread throughout the world, which can cause health problems associated with cell toxicity, cancer and adverse effects on growth and development of animals and humans [12, 13]. The genus *Fusarium* is a soilborne, necrotrophic, plant pathogenic fungus with many species causing serious harm to the plants. *Fusarium* infections are responsible for destroying crops and dramatically reducing production yields [9, 11]. *Fusarium* species have the ability to synthesize toxic mycotoxins, such as zearalenone, fusarins, fumonisins or trichothecenes which are detrimental to the consumer's health [14, 15, 16]. Some of these toxins, such as enniatin and fusaric acid are phytotoxins, whereas others, such as the mycotoxins, trichothecins and fumonisins, are toxic to animals [11].

Fusarium species are typically found in plants prior to harvest attacking cereals and often forming mycotoxins in the kernels. *F. oxysporum* causes primarily vascular wilts on many crops, whereas numerous species, especially *F. solani*, cause root and stem rots and rots of seeds that are accompanied by the production of mycotoxins. Moreover, *Fusarium* species causing disease in immunocompromised human population have been reported [11, 17]. The species *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. semitectum*, *F. tricinctum* and *F. sporotrichioides* are found in cereals; *F. nygamai*, *F. verticilloides* and *F. subglutinans* in corn; *F. thapsinum* and *F. chlamydosporum* in sorghum, while *F. nygamaia* and *F. fujikuroi* are found in rice. In legumes *F. chlamydosporum* and *F. tumidum* are typically encountered. *F.*

solani usually attack potatoes. The species *F. acuminatum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani* and *F. sambucinum* can attack a variety of substrates including fruits, vegetables and ornamental plants [18].

This chapter consists of a literature survey of the antifungal potential against *Fusarium* of substances obtained from different plant species. The chapter is organized as follows: in section 2 the main control methods against *Fusarium* spp are explained; in section 3 the main antifungal assays employed to evaluate the fungicide potential of different substances are described; in section 4 the results of antifungal activity of some plant natural products with potential for controlling *Fusarium* are presented and the potential role of these substances for sustainable plant disease management is discussed.

2. Control methods

The methods used to combat, control and prevent diseases in many crops by different strains of fungi, usually are divided into three groups according to the origin of the substance that makes the control. Currently, it is common the integral use of all kinds of control methods, practice that is known as integrated pest management. All methods have advantages and disadvantages, but the choice of a specific one for a particular crop depends on the state of the disease. In the following, a description of the chemical, biological and physical methods for controlling *Fusarium* is presented.

2.1. Chemical methods

Since the appearance in Europe of the fungus that caused the aggressive downy mildew disease of grape in the late 1870's, many researchers have focused their efforts on the search for chemical entities that could control the diseases caused by fungal pathogens [11]. In particular, chemical fungicides have been used widely to control diseases caused by *Fusarium*, but all have a problem: high toxicity and accumulation of the active substance. In the following, the kinds of chemical compounds more commonly used for the treatment of diseases caused by *Fusarium* are presented.

2.1.1. Halogenated hydrocarbons

Halogenated hydrocarbons are used as soil fumigants because of its fast spread. These compounds are used before the sowing of seeds as a sterilization system. The compounds most commonly used are methyl bromide **1** and trichloronitromethane **2**, known as Chloropicrin, that were successfully employed in the treatment of root rot caused by different types of *Fusarium* species in crops of strawberry (*Fragaria vesca*), raspberry (*Rubus idaeus*), chile (*Capsicum annuum*), onions (*Allium cepa*), snuff (*Nicotiana tabacum*) [19]. Recent studies at University of Chile showed the combined use of the methyl bromide and chloropicrin in the successful control of vascular wilt in tomato plants by *Fusarium oxysporum* f. sp. *lycopersici* and of stem and root rot caused by *Fusarium solani* [20] at doses between 70 g/m² and 100 g/m².

However, the Environmental Protection Agency classifies chloropicrin as a highly toxic and non-selective fungicide. The trichloronitro methane decomposes in the presence of light and heat and produces toxic gases like hydrogen chloride and nitrogen oxides. These compounds cause eye and skin irritation and adverse effects on the nervous system. Humans exposed for a long time to decomposition vapors of chloropicrin suffer severe headaches and pulmonary edema. Chloropicrin has a low accumulation in water due to its high volatility [21].

In the other hand, the Vienna Agreement of 1985 and the 1987 Montreal Protocol amended in London and Nairobi, classified the methyl bromide as a substance that ends up the ozone layer. Ozone (O_3) is a molecule consisting of three oxygen atoms, formed naturally in the upper layers of the atmosphere by the sun's energy; ozone is a very unstable molecule, the solar radiation decomposes the ozone into molecular oxygen and atomic oxygen, which react to form O_3 again. The ozone's concentration in the atmosphere depends on a dynamic equilibrium between the rate at which forms and the speed with which destroys. When methyl bromide reaches the ozone layer, sunlight decomposes the halogenated hydrocarbon generating bromine radical. The bromine radical reacts with an oxygen atom of the ozone molecule, inducing a radical reaction that destroys ozone molecules quickly. For the serious environmental consequences generated by the destruction of the ozone layer many countries have outright the use of methyl bromide as a pesticide; however the countries that permit the use of this substance as a pesticide should be implement environmental care measures. Exposure to methyl bromide causes headache, vomiting, skin irritation and damage to the central nervous system [22].

2.1.2. EBDC's

EBDC's (Ethylenebisdithiocarbamates) are a group of non-systemic (surface acting) fungicides. EBDC active ingredients approved for their use are mancozeb, maneb, zineb and zineb ethylene thiuram disulphide adduct (metiram) [23]. The exact mechanism of action of EBDCs on fungi is not known. It is supposed that they act as fungicides when they are metabolised to an isothiocyanate radical (containing nitrogen-carbon-sulphur atoms) which inactivates the sulphhydryl (sulphur-hydrogen) groups in amino acids (building blocks for proteins) contained within individual fungal pathogen cells [23]. Ethylenebisdithiocarbamates has been used for many years to control different diseases caused by *Fusarium* species in various crops like potato, guava and tomato.

EBDCs have relatively low acute toxicity. They are categorised by the World Health Organization (WHO) as Class III unlikely to present an acute hazard in normal use. However, some studies of toxicity in mice of EBDC's and some of their degradation products (like ethylenethiourea (ETU)) show that the principal target organ upon repeated exposure to all of the EBDCs is the thyroid [24]. For example, EBDCs and ETU altered thyroid hormone levels and/or weights in rats at the lowest dose after three months of dietary feeding. Other organs affected by ETU are liver at higher doses and pituitary gland: prolonged dietary feeding of ETU produces thyroid and pituitary tumors in rats and mice,

and liver tumors in mice [23]. ETU is considered an industrial contaminant of the EBDCs' industries.

2.1.3. Neonicotinoids

Chemical structures of neonicotinoids are obtained by synthetic methods from nicotine, an alkaloid derived from ornithine and obtained naturally from *Nicotiana tabacum* used since the mid-sixteenth century as a pesticide in multiple crops. Different synthetic series of neonicotinoids has been obtained. Imidacloprid **3** is an example of a type of neonicotinoid that has been used as a contact fungicide systemic in the treatment of vascular wilt caused by *F. oxysporum* and *F. moniliforme* [25, 26]. According to WHO imidacloprid is classified as moderate hazard or class II, the LD₅₀ corresponds to 450 mg/kg. According to the EPA, neonicotinoid insecticides are classified as low toxicity to mammals [27]. However, countries like Germany and France banned its use because there are evidences that it causes collapse of bee colonies [28].

2.1.4. Benzimidazoles

The benzimidazoles are organic compounds resulting from the fusion of an aromatic ring and an imidazole ring, widely known for its effective use as dewormers of mammals and some of its derivatives are recognized as important antifungal substances. Benzimidazoles interfere with cell division and intracellular transport mechanisms of pathogenic fungi. The active substance with antifungal activity of more widespread use with chemical structure derived from the benzimidazole is known as benomyl **4**. It is a systemic foliar fungicide selectively toxic to microorganisms and invertebrates. Benomyl is used to treat vascular wilt of various crops (as tomato and carnation) caused by different special forms of *F. oxysporum*, and to treat potato dry rot caused by *F. graminearum* and *F. sambicinum*. Benomyl inhibits *F. oxysporum* growth in a percentage close to 60% [29]; and *F. graminearum* and *F. sambicinum* growth in around 90% [25].

However, benzimidazoles show low mobility in soil and do not volatilize, therefore, they produce high accumulation. Their agrochemical registration was canceled in the United States and the European Union. Since 1982 the use of benomyl has been restricted in Sweden and New Zealand for the birth of children with malformation whose mothers were exposed to this pesticide. In Latin America it has been registered the use of benzimidazoles; however, since 2006 in Brazil is no longer authorized to use fungicide whose active ingredient is benomyl. According to the World Health Organization, benomyl is a fungicide that it is safe for mammals the LD₅₀ is greater than 10000 mg/kg. Other international institutions such as the EPA and the Academy of Sciences of the United States of America classified the benomyl as teratogenic substance, and one of the twelve chemicals responsible for cancer in the USA [30].

2.1.5. Phenyl pyrroles

Phenyl pyrroles are contact systemic fungicide used to control fungal phytopathogens, formulated mainly for *Botrytis* control in blueberries, tomatoes and grapes crops, and also to

control the sour rot complex formed by species of *Aspergillus*, *Alternaria*, *Rhizopus* and *Penicillium* in grape growing. They act by interfering the life cycle of the fungus, mainly in the processes of conidia germination, germ tube development, penetration and development of mycelium in the tissues of the host plant [31]. There are reports of the use of fludioxonil **5** in the control of several species of *Fusarium* which cause rot and common scab of potato [32]. Studies in Canada about potato crops in field conditions show the inhibition of about 70% and 90% of *F. solani* var. *coeruleum* and *F. sambusinum* growth, respectively [32]. Fungicides containing the active substance fludioxonil are classified by WHO as pesticides that do not present acute hazard in normal use, with LD₅₀ greater than 5000 mg/kg [33]. Syngenta home manufacturer of a large number of fungicides with fludioxonil specified in the product data sheets that are moderately toxic to fish and should avoid contact with aquatic environments [31].

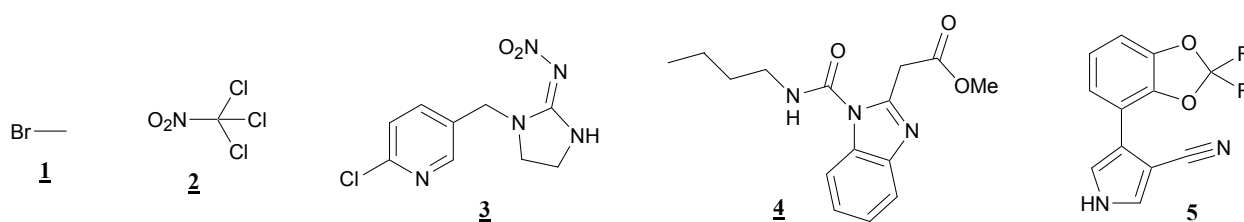


Figure 1.

2.2. Biological methods

Biological control is defined as the use of living organism to eliminate or control other. These control methods become an option that reduces the risks to health and to the ecosystem, and in many cases produce effects comparable to synthetic chemical pesticides. Biological controllers alternatively can be used in combination with synthetic pesticides to reduce substantially the amount of chemical product applied. In the case of diseases caused by *Fusarium* species, the biological control methods most commonly used involve the use of antagonistic organisms, particularly fungi and bacteria. Below, it is presented the antagonistic organisms more commonly used for *Fusarium* control.

2.2.1. *Trichoderma* spp.

Trichoderma species are a beneficial fungus that occurs naturally in all soils. In particular, *T. harzianum* is the most used specie for biological control of *Fusarium*, which acts as antagonist. In many markets worldwide, several products that contain *T. harzianum* are available. The fungus is applied to seeds or to plants in the crops, then it colonizes the roots and forms a kind of protective glove. *Trichoderma* spp. and the roots form a symbiosis: the fungus feeds and lives with the exudates produced by the roots and the fungus gives to the roots protection. The protection process consists of any of the following three ways [34]:

- The first one is given when the antagonist fungus consumes the root exudate of the host. As the exudate is the chemical signal that alerts the fungal pathogen to attack the plant, the infection does not happen.

- When any fungal pathogen gets to cross the protective glove, it is destroyed and used as food by *Trichoderma* spp.
- The third type of protection is by exclusion, considering that *Trichoderma* occupies all the space near to the roots, constituting a physical barrier and excluding from this area any fungal pathogen.

The previous considerations are very important because the biological control is a preventive but not curative methodology, therefore, when damage appears in plants must first be applied the chemical fungicide and seven days after *Trichoderma* should be applied. For use this antagonist fungi is necessary a pH between 4 and 8 and a temperature between 48 and 95 °F [34].

The Panamerican Agricultural School in Honduras has recorded, produced and developed a commercial fungicide based on *T. harzianum* spores known as Trichozam®. It is used in the treatment of root and stem rot caused by *Fusarium* species particularly in the follow crops: lettuce (*Lactuca sativa*), sweet chile (*Capsicum frutescens*), tomato (*Lycopersicon esculentum*), snuff (*N. tabacum*), potato (*Solanum tuberosum*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*); in all cases the recommended dose is 240 g/ha [34]. In addition to *T. harzianum*, *T. lingnorum* strain has been used and processed in commercial products for control of phytopathogens of the genus *Fusarium* [35]. Together with the facts presented above, there is experimental evidence of control of *Fusarium* with *Trichoderma* spp. in cultures of Papaya in Mexico (*Carica papaya*) [36], bean in Colombia (*Phaseolus vulgaris*) [37] employing the antagonist fungus alone or in combination with other antagonists. It is important to make clear that the antagonistic fungi are not toxic for mammals.

2.2.2. Antagonistic bacteria

Free-living bacteria or associations that inhabit the rhizosphere can control natural inhabitants of soil as phytopathogenic fungi, like *Fusarium* species. The mechanisms of action of these organisms are not clear; however, taking into account experimental evidence, some authors suggest the mechanism is related to mycelial growth inhibition and stimulation of plant defense-related enzymes [38]. Microorganisms that have been most studied belong to the genera *Azospirillum*, *Azobacter*, *Klebsiella*, *Pseudomonas* and *Bacillus*. Fungicides based in a combination of some of these bacteria have been used successfully in treatment of alfalfa wilt caused by *Fusarium* spp. [39]. Moreover, these organisms have the ability of produce growth promoting substances, since they belong to a group of organisms called plant growth stimulators. These substances stimulate the germination of seeds and accelerate plants growth, especially in the early stages, induce root initiation and increase the formation of roots and root hairs. The main substances that are produced are stimulating hormones like auxins, gibberellins and cytokines [39].

2.3. Physical methods

In addition to chemical and biological control, there are physical methods, which normally are used as prevention methods and always should be used in combination with other

methods of control. In the case of *Fusarium* control has been used three physical control methods:

1. Rotate crops: this method consist in planting of successive different crops in one field, following a defined order. In contrast, monoculture planting is repeated the same species in the same field year after year. The crop rotation is a practice that has positive effects on the crops, raise the production due to: Reducing the incidence of pest and diseases, to stop their cycle's life. Provides a better nutrients soil profile. Allows balancing the production of waste and when the crop is contaminated with a pathogen, the crop rotation provides a partial reduction of pathogen inoculums [37]. In the specific case of affected crops by *Fusarium* (like tomato and potato), the crop rotation is very common, the idea is to rotate the crops commonly attacked by *Fusarium* by other crops that are not attacked by the same pathogen.
2. Planting bed: this method is commonly used in many countries of Latin America to control *F. oxysporum* f. sp. *dianthi*. This technique involves the use of different planting substrates, which form a high bed that isolates the seed from the ground, natural habitat of the pathogens of *Fusarium* genus [40].
3. Solarization: according to the FAO, soil solarization is a term that refers to soil disinfestation by heat generated from solar energy captured. Soil solarization is a hydrothermal process that takes place in moist soil which is covered by a plastic film and exposed to sunlight during the warmer months [40]. The efficiency of soil solarization to control soil pests depends on the relationship between exposure time and temperature. This method is based on the fact that many pathogens are mesophiles in which a threshold temperature of 37 °C is critical and the accumulation of the effects of heat at that temperature or higher is lethal. It is important to note that there are thermophilic and thermotolerant organisms that can survive and even thrive at this temperature. This method has the advantage that it is not dangerous for farmers and does not transmit toxic waste to the consumers, being easy to educate farmers about their use. However, some disadvantages of this method are the lack of sufficient irrigation water and the survival of the pathogen in the deeper soil layers. For *Fusarium* control, experimental evidence shows that the use of the solarization combined with chemicals fungicides gives good results. For example, when solarization was used combined with fumigation with methyl bromide (at lower doses than those normally used) was observed the mycelial growth inhibition in carnation crops affected by *F. oxysporum* f. sp. *dianthi* [41].

It is clear that chemical control methods are most effective for the treatment of diseases caused by *Fusarium* species, however, exist many problems by toxicity of these substances in the short, medium and long term. It is also clear that the integrated management of different methods for controlling *Fusarium* pathogens has shown good results, however, there is still much to do in the search for methods of biological and physical control for gradually decrease the use of synthetic pesticides. In this way, plants are an important option, even more if one considers that from ancient times have been used in an empirical way in the maintenance of different crops by many civilizations.

3. Bioassays for antifungal activity evaluation

Several methods for testing antifungal susceptibility are currently used. So far, key areas for the application of antifungal bioassays include control of crop pathogens in phytopathology and human pathogenic fungi in antimycotic chemotherapy. Fungicidal testing includes either *in vitro* methods, such as minimum fungicidal testing methods or animal models [42, 43].

The available methods for detecting activity are not equally sensitive or not based upon the same principle; therefore results will be profoundly influenced by the method. The choice of assay constitutes the first arising difficulty when working with fungi. One of the most inherent problems is that the single methodologies do not really produce comparable results. The standardization of antifungal susceptibility testing methods is crucial for the evaluation and development of antifungal drugs and agrochemicals, because the successful use of a fungicide usually also requires the dissemination of its correct application procedure [42, 44].

The ability of a compound to kill a pathogen as opposed to simply inhibiting its growth is an apparently desirable quality, particularly in the setting of decreased immunity. Although several studies have characterized the fungicidal activity of antifungal agents, there is no standardized method for doing so [43].

Below is a description of the main features of methods to evaluate antifungal activity. The most used assays to detect antifungal substances are bioautography, disk diffusion, agar dilution and dilution tests. These antifungal test methods have been classified into three main groups: dilution, diffusion and bioautographic methods [44, 45].

3.1. Dilution methods

Dilutions assays, especially those that are carried out in microwell plates, are one of the most useful and efficient methodologies to evaluate antifungal activity of different substances [45, 46, 47].

In the dilution methods, the compounds are mixed with an appropriate medium that has been previously inoculated with the fungal strain. The assay can be carried out in liquid as well as in solid media. The results of these assays can be measured in many ways; being the minimal inhibitory concentration (MIC) and half effective concentration (EC₅₀) the most common forms of reporting results. Minimal inhibitory concentration (MIC) is defined as the lowest concentration capable to inhibit any fungal growth. Half effective concentration is defined as the the median concentration that causes 50 % of maximal response in a given system.

In liquid or broth-dilution methods, turbidity and redox-indicators are most commonly used. Turbidity can be estimated visually or achieved more accurately by measuring the optical density at 405 nm. However, test samples that are not entirely soluble may interfere with turbidity readings, emphasizing the need for a negative control or sterility control. The

liquid-dilution method also determines whether a compound or extract has a fungicidal or static action at a particular concentration. The serial dilution test yielded the best reproducible results on the MIC and was recommended as general standard methodology for testing natural products. The microdilution method is more sensitive and allows detecting the MIC more exactly [45].

3.2. Agar diffusion or disk diffusion methods

This technique is one of the most widely employed for antifungal activity screening, due to its simplicity and low cost. It is primarily used to determine if a compound or a compound mixture (like crude extracts, fractions and essential oils) possesses any activity. This assay is based on the use of disks containing solutions of the substances to be evaluated. The tested substance, at a known concentration, is in contact with an inoculated medium, and the diameter of the clear inhibition zone around the reservoir (inhibition diameter) is measured at the end of the incubation period. The results of this assay also can be reported as Minimal Inhibitory Quantity (MIQ) which is defined as the minimal quantity of substance that causes some detectable inhibition of fungal growth. One of the major shortcomings of these methodologies is that, as for all diffusion assays, the concentration of the compound or compound mixture tested is unknown [42, 44].

The possibility to test up to six extracts per plate against a single microorganism and the use of small sample volumes are specific advantages of diffusion assays [45]. The diffusion method is not appropriate for testing non-polar samples or samples that do not easily diffuse into agar. The antimicrobial potency of different samples may not always be compared, mainly because their differences in physical properties, such as solubility, volatility and diffusion characteristics in agar. Additionally, size of inhibition zones might be influenced by volatilization of antimicrobial active test material. Due to the absolute values of inhibition zones have only relative importance, the agar diffusion method is appropriate as pre-test only and should not be used for compounds of high lipophilicity, such as volatile sesquiterpenes [48]. Furthermore, agar-diffusion methods are difficult to run on high-capacity screening platforms.

The composition of the medium could influence the activity of the tested substances. The agar diffusion assay is limited to substances with considerable water solubility. Growth media and compound doses employed in this test system vary much and hamper the interpretation of results. On the other hand, the disk diffusion method was used as a laboratory routine to perform a susceptible test for licensed drugs. There has been much research interest in agar-based antifungal susceptibility via disk diffusion method due to their relative ease and the lack of need for specialized equipment [49].

The inhibition zones are usually distorted as this application procedure does not guarantee the test compound to be evenly distributed across the disk. However, if the solvent has not been removed properly and causes inhibition effects by itself, the zones are highly concentric to the disk. The peculiarity of this phenomenon facilitates the experienced researcher to become aware of the deficiency in his work [42].

Conventionally, diameters of inhibition zones are presented to document the observed antifungal activity. In interpreting these diameters, should be considered that variable diffusion properties of the test compound may affect the outcome, especially if results from this assay are used to compare MIC values of different compounds. There exist modifications of this method, such as the agar well diffusion, including the hole-plate (diffusion of the aqueous test compound solution into the agar medium from a vertical hole in the agar layer) and the cylinder method (stainless-steel or ceramic cylinders placed on top of the agar medium) [50]. These two modifications have their merits when the test compound shows good solubility in aqueous solvents. However, as the majority of active compounds are better soluble in organic solvents, the addition of a specific portion of organic solvent to obtain an aqueous suspension of the test compound is required. This modification has the evident advantage that pure organic solvent can be used for the stock solution, which gets completely lost during the preparation of the disks after efficient drying [42].

3.3. Bioautographic method

This technique was introduced by Homans and Fuchs (1970) and is preferably carried out on thin-layer plates (TLC), but is also applicable on polyacrylamide gels [51, 52]. The bioautography can be done in three ways: (a) direct bioautography, where the microorganism grows directly on the thin-layer-chromatographic plate (TLC); (b) contact bioautography, where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact and (c) agar-overlay bioautography, where a seeded agar medium is applied directly onto the TLC plate. TLC has an enormous potential for separating mixtures of low-molecular weight compounds, reason that bioautography allows localizing substances with antimicrobial activity of an extract on the chromatogram; it supports a quick search for new antimicrobial agents through bioassay-guided isolation [44].

Autobiography on TLC plates facilitates the evaluation of a wide range of filamentous fungi to antifungal testing. Preference is given to those fungi that are characterized by pigmented hyphae, spores or conidia; if contrast is poor, it can be enhanced by treatments with iodine vapor [42]. The fungus is applied to the plates in a suspension, that usually consists of malt extract broth or glucose medium with mineral salts added [51]. However, the nutrient medium composition may have to be adjusted to the specific requirements of each test fungus.

Wedge and Nagle published the application of 2D-TLC as efficient approach to obtain improved separation of compounds with a concomitant gain in sensitivity of the assay [53]. Diffusion assays are generally less suitable to assess the quality of the antifungal activity in comparison to positive controls, despite their quick and versatile application.

Apart from the advantages of rapidly detecting active compounds in mixtures and high sensitivity, the depicted bioautography also points to a potential disadvantage of this diffusion assay. Its applicability is limited to microorganisms that easily grow on TLC plates. The diffusion effects may significantly hamper a comparison of activities between

different compounds with differing chemical properties. Another factor that may also affect results is the stability of the compound on the TLC plate as the duration of the assay may last for several days and exact quantization of the amounts of the compound that survived on the TLC plate are rarely performed due to the amount of effort required. This qualitative technique is not directly applicable in current high capacity screening designs and does not give data of values for Minimal Inhibitory Concentration (MIC). When pure compounds are evaluated at different quantities in this assay, the results of its activity can be reported as Minimal Inhibitory Quantity (MIQ) which is defined as the minimal quantity of substance that causes some detectable inhibition of fungal growth [54, 55].

3.4. Other methods

Flow cytometry (FC) has been described as an excellent tool for studying the susceptibility of different microorganisms, including fungi [48, 49]. The main advantages of FC are: 1) it yields higher susceptibility and precise results and 2) FC assays combine the speed of cell-by-cell analysis of very large populations with the independence from long incubation times, resulting in faster tests. However, there are still determinant disadvantages such as: the extremely high cost of the FC equipment, besides that, and in spite of the evolutions made in recent times regarding the user-equipment interface, the techniques still require an experience and skilled operator in order to obtain optimal results [56].

4. Plant natural products as potential agents for controlling *Fusarium*

Natural plant products have been used since the fifteenth century by different communities to control different pests. Today, interest in botanical pesticides has come back, reason why many phytochemical investigations have been focused on finding new products with pesticidal properties. It is important to note that many antifungal activity assays are used to determine the fungicidal potential of a substance, therefore, the results of antifungal activity forextracts, fractions, essential oils and pure compounds are reported in many ways, making difficult to compare results, in order to establish which substances are most promising to control fungi. The following sections present a review of studies of antifungal activity of different products from plants evaluated against different *Fusarium* species, carried out from 2000 to 2012, taking into account the studies that used dilution, diffusion and bioautography assays as methods for determine the antifungal activity.

4.1. Crude extracts and fractions

This section presents a review of the main results of antifungal activity of plant extracts and fractions that have been carried out in recent years. Some crude extracts from species of families Asteraceae, Rubiaceae, Rosaceae, Rutaceae, among other have excellent activities *in vitro* and *in vivo* against plant fungal pathogens of genus *Fusarium*. The reports of antifungal activity of extracts and fractions of plants against *Fusarium* species have been carried out mainly against *F. oxysporum*, *F. verticillioides*, *F. dimerum*, *F. proliferatum* and *F. solani*, using different assays.

The diffusion method is the most employed technique in the screening to the antifungal activity in extracts or fractions, because it is a fast - low cost assay and allows an approach to the presence of active compounds. Table 1 summarizes the main results of antifungal activity against species of *Fusarium* genus of different extracts and fractions obtained from some plant species of different families. It is shown the results of the antifungal activity as inhibition zones or minimum inhibitory quantity MIQ.

The antifungal activity of extracts of four plants from Lake Manzalah in Egypt was tested *in vitro* against *F. oxysporum*. Extracts were obtained from dried leaves employing different solvents (methanol, ethanol, water and chloroform). The antifungal activity was measured using disk diffusion method. All aqueous extracts showed the highest inhibitory activity against *F. oxysporum* with inhibition zones between 38 and 48 mm; therefore, polar compounds are responsible for the antifungal activity [57].

In a study of the antifungal activity against *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *dianthi*, 100 extracts and fractions obtained from Colombian species belonging to the families Lauraceae, Rutaceae, Piperaceae and Myristicaceae were tested by disk diffusion method using quantities of 500 µg of extract or fraction [58, 59]. The results of this study, expressed as MIQ, proved that the ethanolic extract and chloroform fraction from *Compsonera capitellata* wood (Myristicaceae), ethanolic extract of the bark of *Zanthoxylum monophyllum* (Rutaceae), chloroform fraction from alkaloid extract of *Z. quinduense* bark

Specie	Part of plant	Sample	Measured variable	<i>Fusarium</i> species	Results (Concentration)	Reference
Ceratophyllaceae						
<i>Ceratophyllum demersum</i>	Leaves	Aqueous	Inhibition diameter (ID)	<i>F. oxysporum</i>	45.0 mm (300 mg/mL)	57
		Chloroform			23.0 mm (300 mg/mL)	
		Ethanolic			24.0 mm (300 mg/mL)	
		Methanolic			11.0 mm (300 mg/mL)	
Lauraceae						
<i>Ocotea callophylla</i>	Leaves	Ethanol	Minimum inhibitory quantity (MIQ)	<i>F. oxysporum</i> f. sp. <i>dianthi</i>	250 µg	58
<i>Ocotea macrophylla</i>	Steam	Alcaloids fracction	MIQ	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	500 µg	59
		Chloroform			500 µg	
Moraceae						
<i>Maclura tictoria</i>	Leaves	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	500 µg	59
Myristicacea						
<i>Compsonera capitellata</i> .	Leaves	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>dianthi</i>	100 µg	58 y 59
		Petroleum ether			100 µg	
		Chloroform		<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	500 µg	
		iso-propil acetate			500 µg	
	Wood	Ethanolic		<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	250 µg	
		Hexane			250 µg	

		Chloroform		<i>F. oxysporum</i> f. sp. <i>dianthi</i>	100 µg	
		iso-propil acetate		<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	100 µg	
Piperaceae						
<i>Piper eripodon</i>	Fruits	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>dianthi</i>	250 µg	58
<i>P. holtoni</i>	Leaves				250 µg	
<i>P. hispidum</i>	Roots				250 µg	
<i>P. aduncum</i>	Leaves				250 µg	
<i>P. bogotense</i>	Leaves				100 µg	
<i>P. bogotense</i>	Fruits				250 µg	
<i>P. artanthe</i>	Aerial part				100 µg	
<i>P. artanthe</i>	Wood				250 µg	
<i>P. arboreum</i>	Aerial part				100 µg	
Pontederiaceae						
<i>Eichhornia crassipes</i>	Leaves	Aqueous	ID	<i>F. oxysporum</i>	44.0 mm (300 mg/mL)	57
		Ethanolic			12.0 mm (300 mg/mL)	
		Methanolic			14.0 mm (300 mg/mL)	
Potamogetonaceae						
<i>Potamogeton crispus</i>	Leaves	Aqueous	ID	<i>F. oxysporum</i>	48.0 mm (300 mg/mL)	57
		Chloroform			11.0 mm (300 mg/mL)	
		Ethanol			16.0 mm (300 mg/mL)	
		Methanol			21.0 mm (300 mg/mL)	
<i>Potamogeton pectinatus</i>	Leaves	Aqueous	ID	<i>F. oxysporum</i>	38.0 mm (300 mg/mL)	57
		Chloroform			28.0 mm (300 mg/mL)	
		Methanol			26.0 mm (300 mg/mL)	
Rosaceae						
<i>Rubus ulmifolius</i>	Shoots	Metanolic	ID	<i>F. dimerum</i>	10.7 mm (35 mg/mL)	61
				<i>F. solani</i>	24.3 mm (35 mg/mL)	
		Fenols fraction		<i>F. sp</i>	11.7 mm (35 mg/mL)	
				<i>F. solani</i>	18.0 mm 35 mg/mL)	
Rutaceae						
<i>Zanthoxylum quinduense</i>	Leaves	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	500 µg	59
		Alkaloids – CHCl ₃			250 µg	
	Wood	Ethanolic			500 µg	
		Ethyl acetate			100 µg	
		Acetone			500 µg	
<i>Z. monophyllum</i>	Bark	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	250 µg	58 y 59
				<i>F. oxysporum</i> f. sp. <i>dianthi</i>	250 µg	
<i>Z. rhoifolium</i>	Bark	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	500 µg	59
<i>Esenbeckia runyonii</i>	Wood	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>dianthi</i>	250 µg	58
Rubiaceae						
<i>Uncaria guianensis</i>	Leaves	Ethanolic	MIQ	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	500 µg	59

Table 1. Results of diffusion method (inhibition grown or minimum inhibitory quantity) of plant extracts and fractions against *Fusarium* species

(Rutaceae) and ethyl acetate fraction from *Z. quinduense* wood were substances that showed the highest antifungal activity against *F. oxysporum* f. sp. *lycopersici* [59]. The extracts that showed the higher antifungal activity against *F. oxysporum* f. sp. *dianthi* are Leaves of *C. capitellata* (Myristicaceae), petroleum ether fraction from *C. capitellata* leaves, chloroform fraction from *C. capitellata* wood, *Piper bogotense* leaves and aerial parts of *P. artanthe* and *P. arboreum* [58].

Plants species of *Rubus* genus are known to have antimicrobial properties mainly due to their high content in phenolic compounds [60]. At 2008 it was investigated the micropropagation of *R. ulmifolius* and it was evaluated the *in vitro* antifungal activity of shoots against different species of genus *Fusarium*. The methanolic extract was fractionated by column chromatography on sephadex LH-20 and it was obtained fractions enriched in phenols. The antimycotic activity of the crude methanolic extract and fractions were tested using the disk diffusion method. The methanol extract showed low activity against *F. dimerum* and *Fusarium* sp. with inhibition zones of 10.7 and 11.7 mm at 25 µl, equivalent to 100 mg of dried plant material. The crude extract showed good activity against *F. solani* (24.3 mm) at 25 µl, equivalent to 100 mg of dried plant material. The fractions with higher polarity, rich in tannins, showed moderate inhibition against *F. solani* with IZ of 12.5 and 19.0 mm at 25 µl (35 g/L) [61].

The dilution method is used to determine more specifically the activity of an extract or fraction. In this technic, minimum inhibitory concentrations MIC are determined by agar-dilution method or microdilution method. The results of the antifungal activity using dilution methods are summarize in Table 2.

Specie	Part of plant	Sample	Measured variable	<i>Fusarium</i> species	Results (Concentration)	Reference
Achariaceae						
<i>Xylothea kraussiana</i>	Leaves	Acetone	Minimum inhibitory concentration MIC	<i>F. oxysporum</i>	0.63 mg/mL	62
		Methanol			0.32 mg/mL	
		Hexane			0.32 mg/mL	
		Dichloromethane			0.32 mg/mL	
Asteraceae						
<i>Baccharis glutinosa</i>	Aerial part	Methanolic	% inhibition	<i>F. verticiloides</i>	67 % (8,4 mg/mL)	64
<i>Flourensia microphylla</i>	Leaves	Ethanolic	% Inhibition	<i>F. oxysporum</i>	85 % (100 µl/l)	65
<i>F. cernua</i>					80 % (100 µl/l)	
<i>F. retinophylla</i>					90 % (100 µl/l)	
Anacardeaceae						
<i>Harpephyllum caffrum</i>	Leaves	Acetone	MIC	<i>F. oxysporum</i>	0.08 mg/mL	62
		Methanol			0.63 mg/mL	
		Hexane			0.32 mg/mL	
		Dichloromethane			0.16 mg/mL	
Caparaceae						
<i>Capparis decidua</i>	Fruits	Ethanolic	% Inhibition	<i>F. oxysporum</i>	70 % (50 mg/mL)	66
Caesalpinaceae						
<i>Peltophorum pterocarpum</i>	Leaves	Aqueous	% Inhibition	<i>F. equiseti</i>	74 % (3,33 mg/mL)	67
				<i>F. graminearum</i>	69 % (3,33 mg/mL)	
				<i>F. proliferatum</i>	60 % (3,33 mg/mL)	
				<i>F. semitectum</i>	60 % (3,33 mg/mL)	

Combretaceae							
<i>Bucida buceras</i>	Leaves	Acetone	MIC	<i>F. oxysporum</i>	0.02 mg/mL	62	
		Methanol			0.63 mg/mL		
		Hexane			0.32 mg/mL		
		Dichloromethane			0.04 mg/mL		
Euforbiaceae							
<i>Emblica officinalis Gaertn</i>	Leaves	Aqueous	% Inhibition	<i>F. equiseti</i>	75 % (3,33 mg/mL)	67	
					<i>F. graminearum</i>		71 % (3,33 mg/mL)
					<i>F. lateritium</i>		64 % (3,33 mg/mL)
					<i>F. moniliforme</i>		69 % (3,33 mg/mL)
					<i>F. oxysporum</i>		79 % (3,33 mg/mL)
					<i>F. proliferatum</i>		89 % (3,33 mg/mL)
					<i>F. semitectum</i>		80 % (3,33 mg/mL)
<i>F. solani</i>	76 % (3,33 mg/mL)						
Myrtaceae							
<i>Eucalyptus globulis Labill.</i>	Leaves	Aqueous	% Inhibition	<i>F. equiseti</i>	62 % (3,33 mg/mL)	67	
					<i>F. graminearum</i>		69 % (3,33 mg/mL)
					<i>F. lateritium</i>		76 % (3,33 mg/mL)
					<i>F. moniliforme</i>		66 % (3,33 mg/mL)
<i>Prosopis juliflora Swartz</i>	Leaves	Aqueous	% Inhibition	<i>F. proliferatum</i>	81 % (3,33 mg/mL)	67	
					<i>F. equiseti</i>		76 % (3,33 mg/mL)
					<i>F. graminearum</i>		72 % (3,33 mg/mL)
					<i>F. lateritium</i>		70 % (3,33 mg/mL)
					<i>F. moniliforme</i>		90 % (3,33 mg/mL)
					<i>F. oxysporum</i>		80 % (3,33 mg/mL)
<i>F. semitectum</i>	81 % (3,33 mg/mL)						
<i>F. solani</i>	80 % (3,33 mg/mL)						
Olinaceae							
<i>Olinia ventosa</i>	Leaves	Acetone	% Inhibition	<i>F. oxysporum</i>	0.08 mg/mL	62	
		Methanol			0.63 mg/mL		
		Hexane			0.32 mg/mL		
		Dichloromethane			0.16 mg/mL		
Rubiaceae							
<i>Breonadia salicina</i>	Leaves	Acetone	MIC	<i>F. oxysporum</i>	0.32 mg/mL	62	
		Methanol			0.08 mg/mL		
		Hexane			0.16 mg/mL		
		Dichloromethane			0.16 mg/mL		
<i>Mimusops elengi L.</i>	Leaves	Aqueous	% Inhibition	<i>F. equiseti</i>	86 % (3,33 mg/mL)	67	
					<i>F. graminearum</i>		70 % (3,33 mg/mL)
					<i>F. lateritium</i>		78 % (3,33 mg/mL)
					<i>F. moniliforme</i>		79 % (3,33 mg/mL)
					<i>F. oxysporum</i>		86 % (3,33 mg/mL)
					<i>F. proliferatum</i>		93 % (3,33 mg/mL)
					<i>F. semitectum</i>		84 % (3,33 mg/mL)
<i>F. solani</i>	80 % (3,33 mg/mL)						
Verbenaceae							
<i>Lantana camara</i>	Stems	Ethanolic	% Inhibition	<i>F. oxysporum</i>	60 % (5,0 mg/mL)	66	
	Leaves				70 % (5,0 mg/mL)		
	flowers				80 % (5,0 mg/mL)		

Table 2. Results of dilution method (percentage of inhibition or minimum inhibitory concentration) of plant extracts and fractions against *Fusarium* species

The antifungal activity of acetone, methanol, hexane and dichloromethane leaf extracts of six African plants, *Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum*, *Olinia ventosa*, *Vangueria infausta* and *Xylothea kraussiana* were evaluated against *F. oxysporum*. The microdilution assay was used to determine the minimum inhibitory concentration (MIC) values for each plant extracts. All plant extracts were active against the phytopathogenic fungi. *B. buceras* had the best antifungal activity against *F. oxysporum*, with minimum inhibitory concentration (MIC) of 0.02 g/L. The number of active compounds in the plant extracts was determined using bioautography method, this compounds was separated with CEF had similar Rf values of 0.70, 0.85 and 0.95 in acetone, hexane, DCM and methanol extracts, respectively [62].

The genus *Baccharis* belonging to the Asteraceae family is mainly distributed in central and South America and it is characterized by the presence of phenolic compounds with significant activity against different pathogens [63]. The antifungal activity of *B. glutinosa* was evaluated against *F. verticillioides*. Sample of aerial part was extracted with 70% methanol and sequentially partitioned with hexane, ethyl acetate and n-butanol. The crude and partitioned extracts were evaluated in their capacity to inhibit the radial growth of fungi. The results showed that the partitioned ethyl acetate extract exhibited the highest antifungal activity. The ethyl acetate extract inhibited completely the growth of *F. verticillioides* at 0.8 g/L (Table 2) [64]. Some other Mexican endemic species of the Asteraceae family presented fungicidal activity against *F. oxysporum*. The antifungal activity of ethanol extracts from *Flourensia microphylla*, *F. cernua*, and *F. retinophylla* were tested against micelial growth Inhibition of *Fusarium oxysporum*. The three *Flourensia* species showed growth inhibition of *F. oxysporum* at 10 μ l l⁻¹ and *F. microphylla* had the highest inhibition at this concentration. *F. cernua* and *F. microphylla* had the highest efficiency [65].

Aqueous extracts of 46 plants belonging to 32 different families were tested for antifungal activity against eight species of *Fusarium* genus (*F. equiseti*, *F. moniliforme*, *F. semitectum*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani* and *F. lateritium*). Table 2 shows that only 12 plants exhibit significant antifungal activity (inhibition percentages greater than 60%). The antifungal activity of aqueous extracts varied among the test pathogens and it was compared with that of the synthetic fungicides. *F. proliferatum* was the strain that showed the highest susceptibility for the aqueous extracts [67].

4.2. Essential oils

Amongst alternatives for natural biological control, are found essential oils and their components, which have showed therapeutic activities and toxicity facing fungi, bacteria and insects. These substances could be an alternative to inhibit pathogen fungi growth such as *Fusarium* species. They have as advantages specificity, evaporation (avoiding residues) and biodegradability; and furthermore they are considered non aggressive from the standpoint of health. Although their action mechanisms are not totally clear, it has been reported that chemical components present in essential oils produces the following effects:

protein denaturalization in the cell membrane, precipitation of cell proteins and enzymatic inhibition, provoking the loss of amino acids [68, 69, 70]. Thus, each component in an essential oil has its own contribution upon the whole biological activity. Amongst those reported compounds that showed antimicrobial properties are found thymol, carvacrol, geranial, citronellal, geraniol, linalool, citronellol and lavandulol [6].

Despite the advantages that have essential oils for control of fungal pathogens, their use as commercial products is still incipient, due to its high cost-benefit ratio due to the low extraction yields of essential oils. Another reason is the low development of efficient formulations to maintain its effective concentration for long periods of time, due to that essential oils: they are very complex mixtures, they have high evaporation rate and they degrade quickly even at room temperature. At present they has proposed the use of waxes that are widely used in the food industry for incorporate the essential oils. It has also been proposed to prepare emulsions with controlled release of essential oils, as part of the solution to counteract some of the above mentioned disadvantages [71].

The following will be a review of essential oils that could be considered as an alternative for plant disease control produced by *Fusarium* genus fungi. Among the plants that are important for its antifungal activity are found the plants of the families Annonaceae, Apiaceae, Asclepidaceae, Asteraceae, Caryophyllaceae, Cupresaceae, Chenopodiaceae, Geraniaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rosaceae, Rutaceae, Solanaceae Umbeliferaceae, Verbenaceae and Zingiberaceae. Lamiaceae family is the one with the largest number of antifungal activity reports, with studies of species belonging to genera *Mentha*, *Ocimum*, *Origanum* and *Thymus*.

Also was observed that *Fusarium* species that have been object of the highest number of studies are: *F. oxysporum*, *F. solani* and *F. moniliforme*. The results of antifungal activity evaluation for essential oils are reported as follows: inhibition diameter, inhibition percentage, minimal inhibitory concentration (MIC); minimal fungicidal concentration (MFC); concentration producing 50% of inhibition (IC50); half effective concentration (EC50). However, during the review were found other reported variables such as: antifungal index, biomass production inhibition percentage and conidium inhibition percentage; but they will not be considered for not being the variables most common to report the results of antifungal activity.

The information from the review are shown on Tables 3 and 4, according to the type of assay used, such as previously was described in section 3. In the tables is possible to see that the assay most commonly used for evaluate the antifungal activity of essential oils was the dilution method (Table 3).

Melaleuca alternifolia (Myrtaceae) and *Salvia lanigera* (Lamiaceae) were the species with the best results of antifungal activity in dilution assay with MIC values of 0.23 µg/mg and 0.63 L/mL, respectively [2, 91]. While for the diffusion test, the best results were for essential oils of *Piper betle* (Piperaceae) and *Lippia berlandieri* (Verbenaceae) with MIC values of 0.20 µL/mL and 0.50 µL/mL respectively [112, 114].

Specie	Plant part	Isolation Method	Fungi	Variable	Result	Reference
Annonaceae						
<i>Artabotrys odoratissimus</i>	Leaves	Hydrodistillation	<i>F. oxysporum</i>	Percentage of growth inhibition	100% (500 µL/L)	72
<i>Monodora myristica</i>	Seeds		<i>F. moniliforme</i>		66% (500 µg/mL)	73
Apiaceae						
<i>Carum carvi</i> L.	Whole plant	Steam distillation	<i>F. moniliforme</i>	Percentage of growth inhibition	92% (1000 µg/mL)	74
<i>Citrus carvi</i> L.	No report		<i>F. verticillioides</i>		88% (1 µg/mL)	75
			<i>F. oxysporum</i>	94% (1 µg/mL)		
<i>Cuminum cyminum</i>	Comercial		<i>F. solani</i>	MFC	72 µg/mL	6
			<i>F. oxysporum</i>		202 µg/mL	
			<i>F. verticillioides</i>		96 µg/mL	
			<i>F. paoe</i>		75 µg/mL	
			<i>F. equiseti</i>		99 µg/mL	
<i>Foeniculum vulgare</i> L.	Comercial		<i>F. solani</i>	MFC	77 µg/mL	6
			<i>F. oxysporum</i>		151 µg/mL	
			<i>F. verticillioides</i>		73 µg/mL	
			<i>F. paoe</i>		69 µg/mL	
			<i>F. equiseti</i>		63 µg/mL	
	Whole plant	Steam distillation	<i>F. moniliforme</i>	Percentage of growth inhibition	83% (1000µg/mL)	74
<i>Heraclum persicum</i>	Comercial		<i>F. solani</i>	MFC	70 µg/mL	6
			<i>F. verticillioides</i>		952 µg/mL	
			<i>F. equiseti</i>		795 µg/mL	
<i>Pimpinella anisum</i> L.	Whole plant	Steam distillation	<i>F. moniliforme</i>	Percentage of growth inhibition	100% (500µg/mL)	74
<i>Pituranthos tortuosus</i>	Aerial part	Hydrodistillation	<i>F. graminearum</i>	MIC	3,6 µg/mL	76
Asteraceae						
<i>Achillea fragrantissima</i>	Whole plant		<i>F. moniliforme</i>		89% (1000µg/mL)	74
<i>Achillea millefolium</i>			<i>F. moniliforme</i>		67% (1000µg/mL)	
<i>Bidens pilosa</i> Linn. var. Radiata	Leaves	Steam distillation	<i>F. solani</i>	Percentage of growth inhibition	78% (400 µg/disco)	77
	Flowers		<i>F. oxysporum</i>		89% (400 µg/disco)	
			<i>F. solani</i>		98% (400 µg/disco)	
<i>Calendula ofricinalis</i> L.	Whole plant		<i>F. oxysporum</i>		95% (400 µg/disco)	74
<i>Matricaria chamomilla</i> L.			<i>F. moniliforme</i>		76% (1000µg/mL)	
			<i>F. moniliforme</i>		76% (1000µg/mL)	
Caryophyllaceae						
<i>Silene armeria</i> L.	Flowers	Hydrodistillation	<i>F. oxysporum</i>	MIC	500 µg/mL	7
			<i>F. solani</i>		125 µg/mL	
Cupresaceae						
<i>Juniperus excelsa</i>	Berries	Hydrodistillation	<i>F. tricinctum</i>	MIC	10 µg/mL	78
<i>Metasequoia glyptostroboides</i> Miki ex Hu	Floral cones		<i>F. solani</i>		500 µL/mL	79
			<i>F. oxysporum</i>		500 µl/mL	
Chenopodiaceae						
<i>Chenopodium ambrosioides</i>	Leaves	Hydrodistillation	<i>F. oxysporum</i>	Percentage of growth inhibition	100% (100 µg/mL)	80

Specie	Plant part	Isolation Method	Fungi	Variable	Result	Reference	
Geraniaceae							
<i>Pelargonum roseum</i> L.	No report		<i>F. oxysporum</i>	Percentage of growth inhibition	86% (1 µg/mL)	75	
			<i>F. verticillioides</i>		74% (1 µg/mL)		
Lamiaceae							
<i>Hyptis suaveolens</i>	Leaves	Hydrodistillation	<i>F. oxysporum</i> sp. <i>gladioli</i>	Percentage of growth inhibition	100% (0,6 µL/mL)	81	
<i>Lavandula angustifolia</i> Mill	Leaves	Hydrodistillation	<i>F. solani</i> var. <i>Coeruleum</i>	EC50	520 µg/mL	82	
	No report		<i>F. verticillioides</i>	Percentage of growth inhibition	69% (1 µg/mL)	75	
<i>Mentha arvensis</i>	Aerial part	Hydrodistillation	<i>F. oxysporum</i>	Percentage of growth inhibition	100% (3.12 µg/mL)	83	
<i>Mentha longifolia</i> ssp. <i>longifolia</i>	Leaves		<i>F. acuminatum</i>	MIC	63 µg/mL	84	
			<i>F. oxysporum</i>		63 µg/mL		
			<i>F. tabacinum</i>		31 µg/mL		
<i>Mentha pulegium</i>		<i>F. solani</i> var. <i>Coeruleum</i>	EC50	400 µg/mL	82		
<i>Mentha piperita</i>	Commercial		<i>F. oxysporum</i> sp. <i>gladioli</i>	Percentage of growth inhibition	83% (300 µg/mL)	70	
<i>Mentha viridis</i>	No report	Steam distillation	<i>F. moniliforme</i>		77% (1000 µg/mL)	74	
<i>Nepeta cataria</i> L	No report		<i>F. oxysporum</i>		98% (1 µg/mL)	75	
			<i>F. verticillioides</i>		92% (1 µg/mL)		
<i>Ocimum basilicum</i> L	No report	Steam distillation	<i>F. moniliforme</i>		67% (1000 µg/mL)	74	
	No report		<i>F. oxysporum</i>		75% (1 µg/mL)	75	
			<i>F. verticillioides</i>		78% (1 µg/mL)		
<i>Ocimum basilicum</i> Yatta	Leaves	Hydrodistillation	<i>F. verticillioides</i>		100% (5 µL/mL)	85	
	Flowers		<i>F. verticillioides</i>		100% (5 µL/mL)		
<i>Ocimum basilicum</i> Sagana	Leaves		<i>F. verticillioides</i>		100% (5 µL/mL)		
	Flowers		<i>F. verticillioides</i>	100% (5 µL/mL)			
<i>Origanum glandulosum</i>	Aerial part	SFME	<i>F. oxysporum</i>	MIC	56 µg/mL	86	
			<i>F. oxysporum</i>		57 µg/mL		
<i>Ocimum gratissimum</i> Sagana	Leaves	Hydrodistillation	<i>F. verticillioides</i>	Percentage of growth inhibition	75% (5 µL/mL)	80	
<i>Ocimum gratissimum</i> Yatta.	Flowers		<i>F. verticillioides</i>		75% (5 µL/mL)		
	Leaves		<i>F. verticillioides</i>		75% (5 µL/mL)		
<i>Ocimum gratissimum</i>	Leaves		<i>F. moniliforme</i>	100% (500 µL/mL)	87		
<i>Origanum dictamnus</i>	Leaves			<i>F. solani</i> var. <i>Coeruleum</i>	EC50	76 µg/mL	82
<i>Origanum majorana</i>	Leaves			<i>F. solani</i> var. <i>Coeruleum</i>	120 µg/mL		
<i>Origanum vulgare</i>	Leaves			<i>F. solani</i> var. <i>Coeruleum</i>	50 µg/mL		
<i>Origanum vulgare</i> ssp. <i>vulgare</i>	Aerial parts		<i>F. acuminatum</i>	MIC	63 µg/mL	88	
			<i>F. oxysporum</i>		63 µg/mL		
			<i>F. tabacinum</i>		31 µg/mL		
<i>Orthosiphon stamineus</i> Benth	Leaves		<i>F. solani</i>	500 µg/mL	89		
	Stems			500 µg/mL			
<i>Plectranthus cylindraceus</i>	Aerial part	Steam distillation	<i>F. oxysporum</i>	Percentage of growth	100% (125 µg/mL)	90	

Specie	Plant part	Isolation Method	Fungi	Variable	Result	Reference
				inhibition		
<i>Rosmarinus officinalis</i>	Leaves	Hydrodistillation	<i>F. solani</i> var. <i>Coeruleum</i>	EC50	668 µg/mL	82
<i>Salvia lanigera</i>	Aerial part		<i>F. oxysporum</i>	MIC	100 µL/mL	91
<i>Salvia officinalis</i>			<i>F. moniliforme</i>		0.63 µL/mL	92
<i>Salvia sclarea</i> L	No report		<i>F. verticillioides</i>	Percentaje of growth inhibition	65% (1 µg/mL)	75
<i>Teucrium marum</i> subsp. <i>marum</i>	Aerial part	Steam distillation	<i>F. oxysporum</i> Sch	MIC	450 µg/mL	93
<i>Thymus capitatus</i>	Leaves	Hydrodistillation	<i>F. solani</i> var. <i>Coeruleum</i>	EC50	71 µg/mL	82
<i>Thymus spathulifolius</i>	Whole plant		<i>F. acuminatum</i>	MIC	125 µg/mL	94
			<i>F. tabacinum</i>		125 µg/mL	
			<i>F. oxysporum</i>		63 µg/mL	
<i>Thymus vulgaris</i>	Whole plant	Commercial	<i>F. moniliforme</i>	Percentaje of growth inhibition	100% (600 µg/mL)	73
			<i>F. sp</i>		100% (200 µg/mL)	70, 95
			<i>F. oxysporum</i> sp. <i>gladioli</i>		100% (100 µg/mL)	
<i>Thymus vulgaris</i> L	No report		<i>F. oxysporum</i>	Percentaje of growth inhibition	98% (1 µg/mL)	75
			<i>F. verticillioides</i>		98% (1 µg/mL)	
	Whole plant	Steam distillation	<i>F. moniliforme</i>		100% (250 µg/mL)	84
	No report	Hydrodistillation	<i>F. oxysporum</i>		100 % (0.7 µL/mL)	96
<i>Zataria multiflora</i>	Herb	Commercial	<i>F. solani</i>	MFC	153 µg/mL	6
			<i>F. oxysporum</i>		184 µg/mL	
			<i>F. verticillioides</i>		99 µg/mL	
			<i>F. paoe</i>		145 µg/mL	
			<i>F. equiseti</i>		99 µg/mL	
Lauraceae						
<i>Cinnamomum zeylanicum</i>	Commercial		<i>F. sp</i>	Percentaje of growth inhibition	68% (200 µg/mL)	95
			<i>F. oxysporum</i> sp. <i>gladioli</i>		100% (100 µg/mL)	
	Bark	Hydrodistillation	<i>F. solani</i>	MIC	150 µg/mL	97
	Leaves	Commercial	<i>F. proliferatum</i>		50 µg/mL	98
Bark	<i>F. proliferatum</i>		50 µL/mL			
<i>Cinnamomum zeylanicum</i> L.	Whole plant	Steam distillation	<i>F. moniliforme</i>	Percentaje of growth inhibition	100% (1000 µg/mL)	74
<i>Laurus novocanariensis</i>	Leaves	Hydrodistillation	<i>F. oxysporum</i>	EC50	280 µg/ml	99
			<i>F. moniliforme</i>		440 µg/ml	
			<i>F. solani</i>		410 µg/ml	
Myrtaceae						
<i>Leptospermum scoparium</i>	Leaves	No report	<i>F. circinatum</i>	Percentaje of growth inhibition	62% (26 µg/ml de air)	100
<i>Melaleuca alternifolia</i>	Leaves and flowers	Comercial	<i>F. culmorum</i>	MIC	0.23 µg/mg	2
			<i>F. graminearum</i>		0.12 µg/mg	
<i>Pimenta dioica</i> L. Merr	No report		<i>F. oxysporum</i>	Percentaje of growth inhibition	100% (1µg/mL)	75
			<i>F. verticillioides</i>		100% (1 µg/mL)	

Specie	Plant part	Isolation Method	Fungi	Variable	Result	Reference
<i>Syzygium aromaticum</i>	Clove	Comercial	<i>F. proliferatum</i>	MIC	50 µL/mL	98
Poaceae						
<i>Cymbopogon citratus</i>	Leaves	Hydrodistillation	<i>F. moniliforme</i>	Percentaje of growth inhibition	100% (800 µg/mL)	73
<i>Cymbopogon flexuosos</i>	Aerial parts		<i>F. oxysporum</i>		MIC	100% (3.12 µg/mL)
	Leaves			MIC	1,1 µL/mL	101
<i>Cymbopogon nardus</i> L.	No report		<i>F. verticillioides</i>	Percentaje of growth inhibition	86% (1 µg/mL)	75
					76% (1 µg/mL)	
Rosaceae						
<i>Agrimonia eupatoria</i>	Whole plant	Steam distillation	<i>F. moniliforme</i>	Percentaje of growth inhibition	75% (1000 µg/mL)	74
Rutaceae						
<i>Citrus reticulata</i> Blanco	Peel of ripe fruits	Hydrodistillation	<i>F. oxysporum</i>	MIC	200 µL/mL	102
Solanaceae						
<i>Cestrum nocturnum</i> L.	Flowers	Hydrodistillation	<i>F. solani</i>	MIC	250 µg/mL	103
Umbeliferaceae						
<i>Trachyspermum ammi</i> Lin	Seeds	Hydrodistillation	<i>F. oxysporum</i> <i>f.sp. lycopersici</i> ,	MIC	240 µg/mL	104
			<i>F. oxysporum</i> <i>f.sp. cubense</i>		240 µg/mL	
			<i>F. oxysporum</i> <i>f.sp. capsici</i>		240 µg/mL	
Verbeneaceae						
<i>Vitex agnus-castus</i>	Unripe fruits	Hydrodistillation	<i>F. tricinctum</i>	MIC	178µg/mL	8
	Ripe fruits				89 µg/mL	
	Leaves				178µg/mL	
Zingiberaceae						
<i>Zingiber cassumunar</i> Roxb	No report		<i>F. verticillioides</i>	Percentaje of growth inhibition	67% (1 µg/mL)	75
<i>Zingiber officinale</i>	Rhizomes	Hydrodistillation	<i>F. moniliforme</i>		100% (1000 µg/mL)	73

Table 3. Essential oils with antifungal activity against *Fusarium* species evaluated by the diluttion assays.

Specie	Plant part	Isolation Method	Fungi	Variable	Result	Reference
Asclepidaceae						
<i>Periploca laevigata</i>	Root	Hydrodistillation	<i>F. oxysporum</i>	Diameter zone inhibition	48 mm (50 µg/mL)	105
			<i>F. solani</i>		55 mm (50 µg/mL)	
Asteraceae						
<i>Achillea gypsicola</i>	Whole plant	Hydrodistillation	<i>F. aveneum</i>	Percentaje of growth inhibition	60% (1000 µg/mL)	106
			<i>F. culmorum</i>		82% (1000 µg/mL)	
			<i>F. oxysporum</i>		69% (1000 µg/mL)	
			<i>F. sambucinum</i>		79% (1000 µg/mL)	
			<i>F. solani</i>		66% (1000 µg/mL)	
<i>Haplopappus baylahuen</i>	Leaves		<i>F. oxysporum</i>		76% (100 µg/mL)	107
<i>Tanacetum aucheranum</i>	Aerial parts		<i>F. acuminatum</i>		63% (1.5 µL/mL)	108

			<i>F. chamydosporum</i>		67% (1.5 µL/mL)	
			<i>F. culmorum</i>		67% (1.5 µL/mL)	
			<i>F. equiseti</i>		66% (1.5 µL/mL)	
			<i>F. graminearum</i>		73% (1.5 µL/mL)	
			<i>F. nivale</i>		83% (1.5 µL/mL)	
			<i>F. proliferatum</i>		64% (1.5 µL/mL)	
			<i>F. sambucinum</i>		78% (1.5 µL/mL)	
			<i>F. semitectum</i>		84% (1.5 µL/mL)	
Tanacetum chiliophyllum var. Chiliophyllum			<i>F. acuminatum</i>		78% (1.5 µL/mL)	
			<i>F. chamydosporum</i>		75% (1.5 µL/mL)	
			<i>F. culmorum</i>		78% (1.5 µL/mL)	
			<i>F. equiseti</i>		67% (1.5 µL/mL)	
			<i>F. graminearum</i>		81% (1.5 µL/mL)	
			<i>F. nivale</i>		83% (1.5 µL/mL)	
			<i>F. oxysporum</i>		71% (1.5 µL/mL)	
			<i>F. proliferatum</i>		75% (1.5 µL/mL)	
			<i>F. sambucinum</i>		84% (1.5 µL/mL)	
			<i>F. scirpi</i>		63% (1.5 µL/mL)	
			<i>F. semitectum</i>		86% (1.5 µL/mL)	
			<i>F. tabacinum</i>		68% (1.5 µL/mL)	
			<i>F. verticillioides</i>		68% (1.5 µL/mL)	
	Lamiaceae					
Origanum acutidens Hans	Aerial parts	Hydrodistillation	<i>F. culmorum</i>		81% (25 mg/disc)	1
			<i>F. equiseti</i>		70% (25 mg/disc)	
			<i>F. nivale</i>		86% (25 mg/disc)	
			<i>F. oxysporum</i>		82% (25 mg/disc)	
			<i>F. sambucinum</i>		89% (25 mg/disc)	
			<i>F. semitectum</i>		87% (25 mg/disc)	
Origanum vulgare	Inflorescences	Hydrodistillation	<i>F. avenaceum</i>	Diameter zone inhibition	20 mm (500 µg/mL)	109
	Leaves				22 mm (500 µg/mL)	
Origanum onites	Leaves and flowers	Hydrodistillation	<i>F. semitectum</i>	MFC	1.8 µg/mL	110
					<i>F. oxysporum</i>	
Thymus algeriensis Boiss. Et Reut.	Aerial parts	Hydrodistillation	<i>F. solani</i>	MIC	1 µL/mL	111
Piperaceae						
Piper betle L. var magahi	Leaves	Hydrodistillation	<i>F. oxysporum</i>	MIC	0.50 µL/mL	112
Rutaceae						
Zanthoxylum monophyllum	Fruits	Steam distillation	<i>F. oxysporum</i>	EC50	0.140 µL/mL air	113
Zanthoxylum fagara					0.183 µL/mL air	
Zanthoxylum rhoifolium					0.245 µL/mL air	
Verbenaceae						
Lippia berlandieri Shauer	Leaves and flowers	Steam distillation	<i>F. oxysporum</i> <i>lycopersici</i>	Percentage of growth inhibition	100% (0.20 µL/mL)	114

Table 4. Essential oils with antifungal activity against *Fusarium* species evaluated by the diffusion assays.

For the Myrtaceae family, the best results were obtained for *M. alternifolia* oil, with values lower than 1% w/w, which were attributed to the presence of terpinen-4ol [2]. Another species from the same family having a high activity is *Syzygium aromaticum*; which at 100 mg/L of oil inhibits 100% of growth of *F. oxysporum* sp. *gladioli* and at 50 µL/mL of oil inhibits totally the growth of *F. proliferatum* [70, 98]. Other oil that showed the best results in the dilution test was the obtained from *Salvia officinalis*, which presented a MIC and MLC of 0.63 µg/mL, which indicates a fungicide effect [92]. In this same genus, the oil of *S. lanigera* presented a moderated effectiveness at 100 mg/L. In this two studies, activity is attributed to the presence of phenolic compounds (Thymol and carvacrol) [91].

Piper betle (Piperaceae) is another species with very good results, with a value MIC of 0.50 mg/L, where once more, the activity is attributed to phenolic compounds. Due to OH presence, it is able to form hydrogen bonds with the active spot enzymes and increases the activity via enzyme denaturalization [112].

The species *Citrus carvi*, *Foeniculum vulgare*, *Pimpinella anisum* and *Piruranthos tortuosus* of Apiaceae family, presented a strong inhibitory power against the growth of *Fusarium* species. The essential oil *P. tortuosus* is one of the oils showing the lowest MIC with a value of 3,6 mg/L. In regard to these results, the authors of this paper mentioned that the activity is related with the high monoterpenoids contents in this oil. These substances have the capacity of altering the morphology of hyphae and aggregates, reducing the diameter each time they interact with cell membranes of pathogen agents [76].

Rutaceae family also presented good results in the diffusion test, being *Zanthoxylum* genus the most representative. The species *Z. monophyllum*, *Z. fagara* and *Z. rhoifolium* were evaluated against *F. oxysporum*, and it was found that essential oils from the fruits of these species show similar or higher results compared to the positive controls used. *Z. monophyllum* was the most active, followed by *Z. fagara* and *Z. rhoifolium*. These results are attributable to the presence of some compounds that are in these fruits essential oils in low concentrations such as (E)-caryophyllene, T-muurolol, and α -cadinol, compounds that have been previously reported as antifungal substances by other researchers [113].

As is observed in Tables 3 and 4, Asteraceae and Lamiaceae families have the highest number of reports of antifungal activity against *Fusarium* species. For the Asteraceae family, two genera (*Achillea* and *Tanacetum*) showed moderate results, against to a large number of *Fusarium* species. Both studies showed a correlation of results with a high monoterpenoids content such as: camphor, 1,8-cineole, piperitone, borneol and α -terpineol [106, 108].

In the Lamiaceae family there were the highest inhibition percentages with small amounts of oils from *Hyptis suaveolens* L, *Lavandula angustifolia* Mill, *Mentha arvensis*, *Nepeta cataria* L, *Ocimum basilicum*, *Ocimum gratissimum*, *Origanum majorana* L, *Salvia scalrea* and *Thymus vulgaris*. An example of the use of essential oils from species of this family as an alternative to control *F. oxysporum*, is the integration of the oil from *H. suaveolens* species in hot water and ultraviolet radiation, as a treatment which gives excellent results with a high reduction of pathogen population in artificially inoculated in gladiolus corms [81]. From the same

family, the potential of *M. arvensis* as antifungal, is observed taking into account that when using a concentration of 3.12 mg/L of this oil, there would be a 100% inhibition in *F. oxysporum* growth, this is an effect attributed to its high menthol contents (71.50%) [83]. In this family, the *Ocimum* genus is one of the most studied, and an example of this is the report by Dambolena, where all oils assessed came from leaves and flowers, had inhibitory effects upon the growth of *F. verticillioides*. Authors report that inhibition degree depends widely upon composition and concentration of components of each of those oils. Oils with a high eugenol contents showed the best results. These results are consistent to other prior works that have shown that *O. basilicum* oil has antifungal and antibacterial activities due to its oxygenated monoterpenoids high contents, following the rules that antifungal activity is related with content to phenols > alcohols> aldehydes> ketones> esters> hydrocarbons contents [85].

The review confirmed the importance of establish the criteria for obtain the essential oils and how are affect the results of th antifungal activity when the oils are obtained from different forms and from plants cultivated in different conditions. Also it is important establish the criteria for evaluate the antifungal activity. The most important factors are: trial type, time of vegetal matter collection, parts of the plant used and maturity status, and extraction methods to obtained essential oils [5], [8], [87]. An example of this is found in the study of *Ocimum onites*, where it was established that there are differences in antifungal activities of the oils, when are used fresh or dried fruits, being the most active of fresh fruits oil [110]. Additionally this species is the one showing the lowest MFC values with 1.8 y 6.0 µg/mL facing *F. semitectum* and *F. oxysporum* species respectively.

4.3. Pure compounds

Several reviews have discussed the activity of isolated natural products against a wide range of fungi including human pathogenic species such as *Candida albicans* or *Aspergillus* species, or plant pathogens such as *Colletotrichum* and *Cladosporium* species [115, 116, 117]. However it is difficult to find a comprehensive review of natural products specifically active against *Fusarium* plant pathogens. Here we describe some of these natural agents that according to their activity against *Fusarium* have the potential to be used either directly as pure constituents, or they can serve as starting point for generating more potent and selective antifungals.

4.3.1. Alkaloids

Plant alkaloids are biologically active entities that often confer chemical protection against pathogen infections or herbivory [118]. Different types of nitrogen-containing substances have been found to display antimicrobial [119, 120], antifungal, antiparasitary [121] and antiviral [122] effects. Chinese traditional phytomedicine is a vast source of bioactive scaffolds, and berberine **6** is one interesting representative, originally isolated from *Coptis chinensis* rhizome [123]. It is a yellow, bitter, and a very active agent against bacteria, fungi, protozoa, trypanosome and mammalian cells from higher organisms. The biological action is thought to be pleiotropic, inhibiting mainly protein synthesis but also intercalating into

DNA strands [124]. Berberine has an spore germination half inhibitory concentration (SPIC₅₀) of 599 mg/L against *F. oxysporum*, being also active against other fungi such as *Botrytis cinerea*, *Alternaria solani* and *Monilinia fructicola* [125]. Dehydrocorydalmine and oxyberberine, two berberine structurally-related alkaloids were found to be less active against *Fusarium* species with SPIC₅₀ close to 1000 mg/L. Nonetheless two other interesting alkaloids sharing some similarity to berberine, are corydalmine **7** and isocorypalmine **8** isolated from *Corydalis chaerophylla* which displayed an SPIC₅₀ close to 300 and 800 mg/L respectively against *F. nudum* [126]. Certainly isoquinolines are attractive chemotypes with antifungal activity and studies of their mechanism of action and structure-activity relationships can boost the development novel *Fusarium* inhibitors.

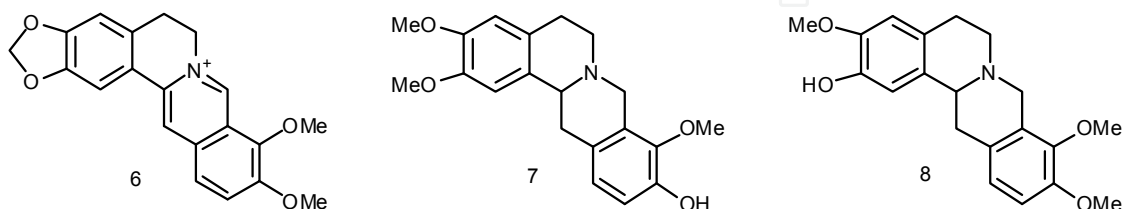


Figure 2.

Another type of interesting alkaloid which has attracted the attention of researchers because of their pronounced biological activity specifically against lower organisms is the class of pyrrolizidines [127]. Against a species of *Fusarium*, a notable antifungal activity was observed for floridinine **9** obtained from *Heliotropium floridum*, showing an inhibitory concentration of 500 mg/L against *F. oxysporum* [128]. Europine **10** isolated from *H. bovei* showed an SPIC₅₀ of 740 mg/L against *F. moniliforme*, while 7-acetyeuropine was inactive [129]. It is interesting to see that a related alkaloid, lycopsamine isolated from *H. megalanthum* was inactive against *Fusarium* while being active against other phytopathogenic fungi [130]. From the plant *Senecio jacobaea*, retrorsine and retrorsine-*N*-oxide were isolated and showed to reduce the growth of *F. oxysporum* but were not able to arrest their development [131]. The pyrrolizididine alkaloids are thought to be involved in crucial ecological relations, for example, these alkaloids can be sequestered by some butterflies offering them with chemical protection against depredators [132]. It can also be hypothesized that the insects also utilize them for preventing microbial infection.



Figure 3.

Tropane alkaloids are interesting phytochemicals with a wide array of biological activity typically present in species of the Solanaceae family. Hyoscyamine **11** isolated from traditionally medicinal plant *Hyoscyamus muticus* was show to inhibit several species of pathogenic fungi including *F. dimerum*, *F. nivale* and *F. oxysporum* in an autobiography assay

[133]. *Ent*-norsecurinine **12** isolated from *Phyllanthus amarus* is a bioactive scaffold for anti-fungal drug development, as it is very active against a wide range of phytopathogenic fungi [134]. Specifically, against *F. nudum* infecting *Cajanus cajan*, the alkaloid displayed an SPIC₅₀ of around 250 mg/L. The *allo*- form of securinine was found to be less active with an SPIC₅₀ close to 450 mg/L [135, 136].



Figure 4.

Steroidal alkaloids have also been found to display activity against *Fusarium* species. *Solanum* glycoalkaloids solasonine **13** and solamargine were able to slow down the growth of three different species of *Fusarium* [137]. Tomatidine, the aglycone of α -tomatine **14**, was found to be less active against *F. subglutinans*, than the parent glycoside [138]. Tomatidine inhibited almost 90% of the growth of the phytopathogenic fungi at a concentration of 0.30 mM. However the strain *F. oxysporum f. sp. lycopersicum* was found to produce an extracellular hydrolase named tomatinase which is able to hydrolyze α -tomatine rendering it inactive [139].

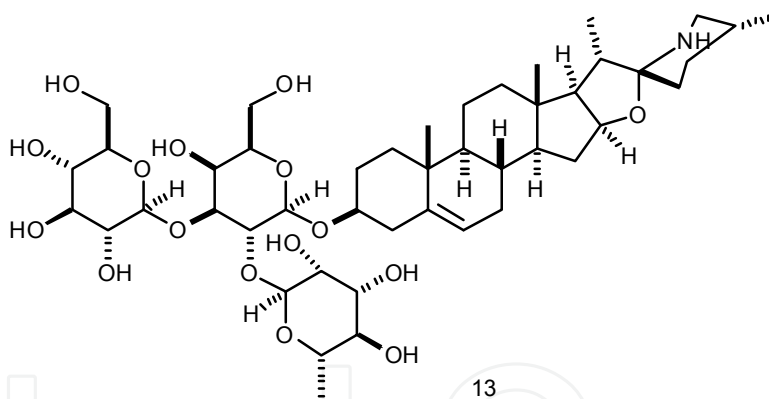


Figure 5.

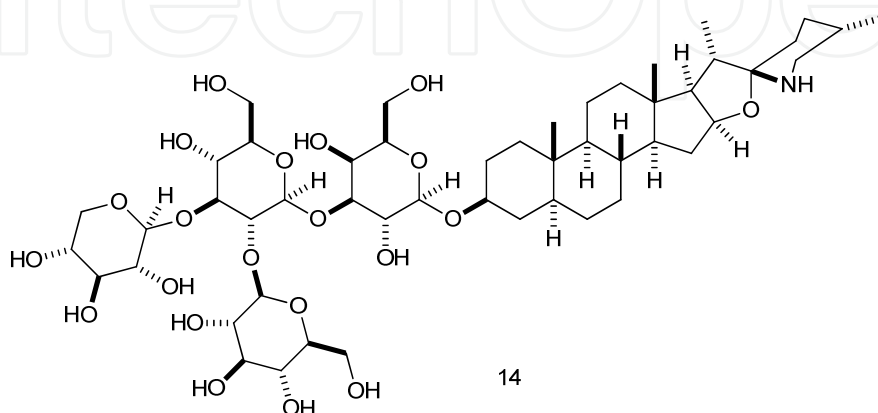


Figure 6.

Another type of steroidal alkaloids which have also been found to be active against *Fusarium* is the ceveratrum alkaloids. The seeds of *Schoenocaulon officinale*, commonly known as sabadilla powder have been used traditionally as insecticide in South America [140]. This plant possesses cevadine **15** and veratridine, which are active against *F. graminearum* [141] however being less effective against *F. oxysporum*. Venenatine **16** is an indole alkaloid isolated from the bark of *Alstonia venenata* which displayed antifungal activity against a wide array of phytopathogenic fungi [142]. Against the strain *F. udun* a SPIC₅₀ close to 400 mg/L was found. This alkaloid was particularly active against the fungi *Ustilago cynodontis*. In another study a closely related alkaloid, Δ^3 -alstovenine was isolated and shown to be inactive against *F. udun* even at 1000 mg/L while sustaining a marked inhibition against *Helminthosporium maydis* and *Erysiphe pisi* [143]. Fistulosin **17**, an oxyndole alkaloid, was isolated from roots of Welsh onion (*Allium fistulosum* L.). This compound exhibited antifungal activities against different phytopathogenic filamentous fungi, especially varieties of *Fusarium oxysporum* at 1.62 ± 6.5 g/L of MICs. Fistulosin inhibited protein synthesis and had a slight inhibitory effect on DNA synthesis, but no inhibitory effect on RNA synthesis [144].

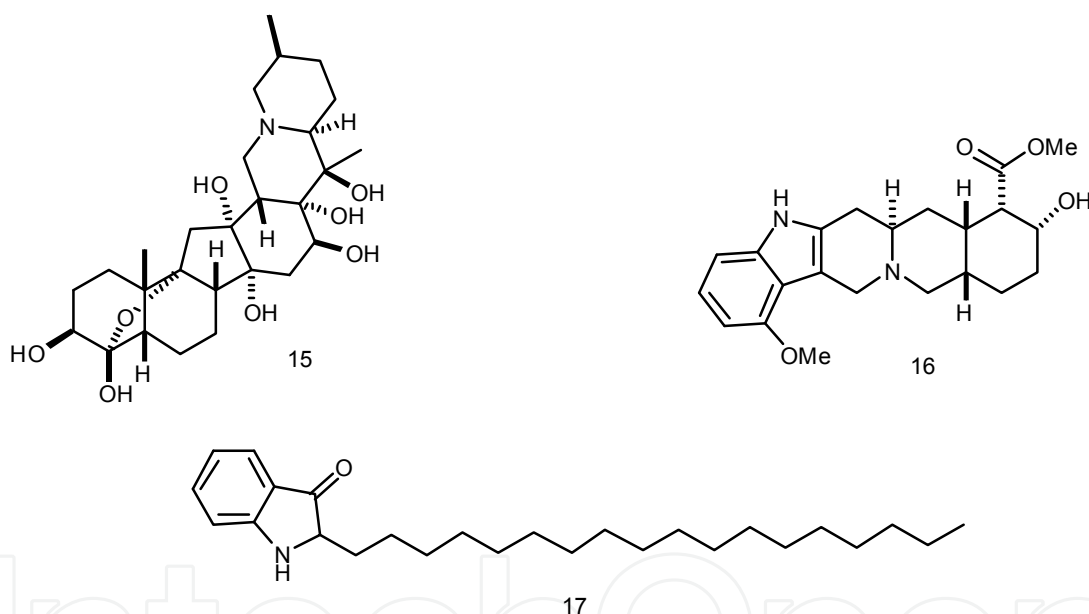


Figure 7.

The antifungal activity of four aporphine alkaloids isolated from *Ocotea macrophylla* was evaluated using the disk diffusion method against *F. oxysporum* f. sp. *lycopersici*. The inhibitory activity against the growth of the fungi was moderate at 5 g/L for (S)-3-methoxynordomesticine **18**, while the other alkaloids were inactive, suggesting that the presence of electron withdrawing substituents on the nitrogen atom decrease the antifungal activity [145]. The antifungal activity against the same phytopathogenic strain was evaluated by direct bioautography in a TLC bioassay [42,146] for the compounds isolated from *Z. monophyllum* and *Z. quinduense*. The minimum growth inhibitory amount was determined for each compound taking into consideration a growth inhibition with less than 100 μ g. Among the evaluated compounds three benzophenanthridine alkaloids (norchelerytrine **19**, 6-

acetonyl-dihydrochelerythrine **20** and chelerythrine **21**), a berberine alkaloid (jathrorrhizine **22**) and a quinolone alkaloid (thalifoline **23**) were found to display inhibitory properties [147-149].

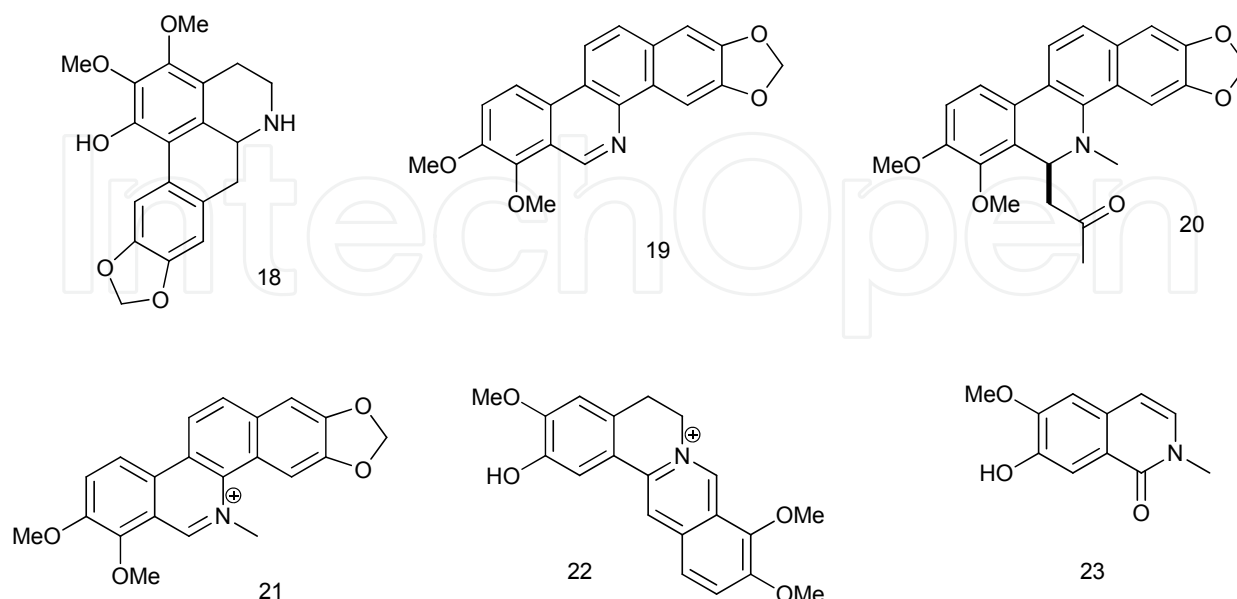


Figure 8.

4.3.2. Phenolic compounds

A comprehensive study from 1998, evaluated several flavones on the growth inhibition of five different species of *Fusarium*: *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium poae*, *Fusarium avenaceum* and *Fusarium nivale* [150]. Unsubstituted flavone showed the highest growth inhibition among all flavones [151], preventing 70% of the growth in comparison with the control against all the *Fusarium* strains tested except *F. culmorum*. Interestingly the combinations of oxygenated substituted flavones with unsubstituted flavone or flavanone were more active than the pure constituents [150]. This synergistic effect was not observed for others flavonoids, indicating the specific substitution on the flavonoid skeleton was responsible for this effect.

Oxygenated flavonoids isolated from the stems and leaves of *Ficus sarmentosa* also showed inhibition of the mycelium growth of *F. graminearum* [152]. Luteoline **24** was able to inhibit half the growth of the fungi at a concentration of 56 mg/L. The reduction of luteoline to a flavanone, the substitution on C-3 or the methoxylation of phenolic in C-3' produces as result a significant loss in activity. Other flavonoids and phenols have also shown to inhibit the growth of *F. culmorum*, notably taxifolin **25** [153].

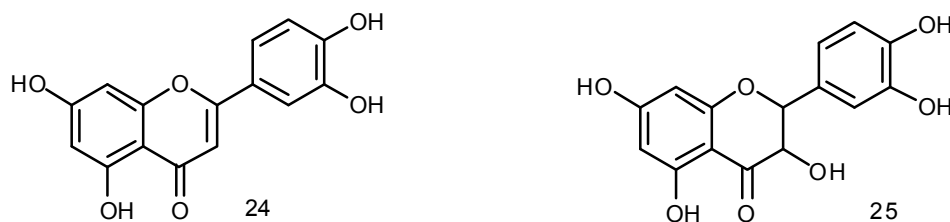


Figure 9.

Glycosylated flavonoids trifoline **26** and hyperoside **27** have been isolated from the medicinally important species *Camptotheca acuminata*, source of useful metabolites for cancer chemotherapy such as camptothecin [154]. These two galactosides inhibited half of the growth of *Fusarium avenaceum* at a concentration of 75 mg/L with a complete inhibition at 125 mg/L for trifolin and more than 150 mg/L for hyperoside. They showed also inhibition against other fungal pathogens such as *Pestalotia guepinii* and *Drechslera* sp. In general it is well accepted that flavonoids alter the growth of *Fusarium*, some flavonoids can induce sporulation in *F. solani* [155] and they are therefore thought to be involved in host-pathogen regulatory relations. There is also specificity in the activity of flavonoids, some fungi being more sensitive to certain flavonoids than others, and also synergistic effects have been observed [156].

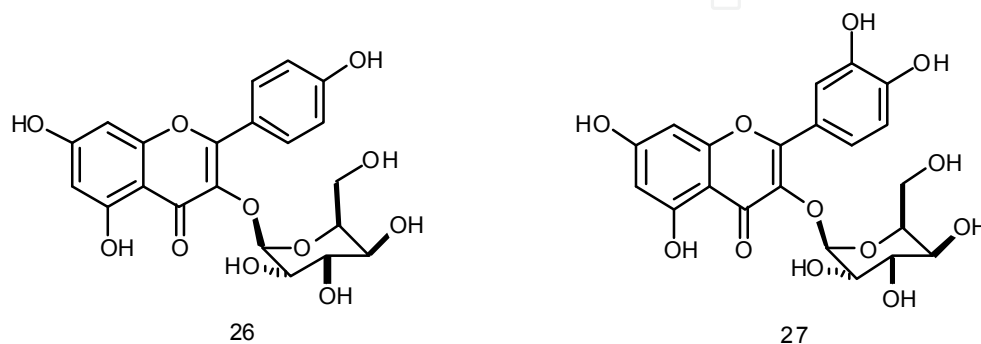


Figure 10.

Non-flavonoid phenols have also attracted the attention of researchers, as other biosynthetic pathways have evolved extremely potent phenols either from shikimate or acetate precursors or by mixed routes. In this class we can find simple phenols, mono and poly-prenylated phenols, lignans, coumarins, stilbenes and others. For example rotenone **28** and related metabolites known as rotenoids, are well established insecticides and piscicides [157]. Specifically rotenone had the highest anti-fungal activity from the rotenoids isolated from *Pachyrhizus erosus* against *F. oxysporum*, significantly inhibiting its growth at 250 mg/L [158]. Some lignans have also demonstrated activity against *Fusarium* pathogens. (-)-Taxiresinol **29** obtained from the medicinally important *Taxus baccata*, source of the anticancer agent paclitaxel, displayed 60% inhibition of the growth of *F. solani* at 200 mg/L [159]. Aryltetraline lignans isolated from the same plant were found to be less active as antifungals however they displayed higher cytotoxicity towards cancer cell lines.

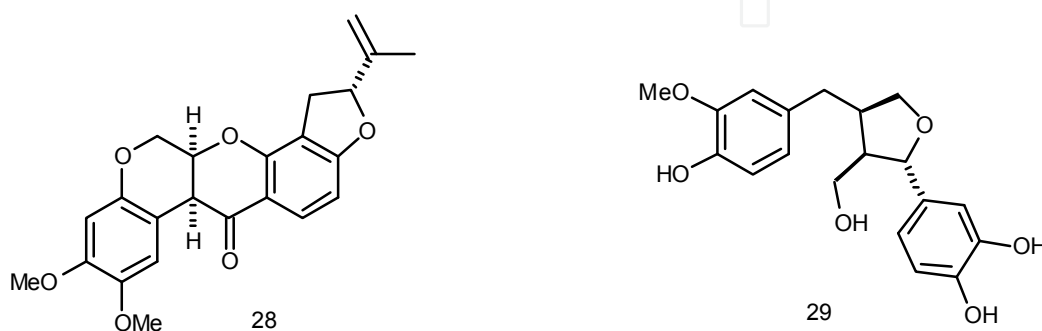


Figure 11.

Three naphthoquinones isolated from *Moneses uniflora* showed interesting antifungal activity against *F. tricinctum*. Chimaphylin **30**, 8-chlorochimaphylin **31** and 3-hydroxychimaphylin **32** showed complete inhibition of the fungi at a concentration between 12.5 and 25 mg/L, which is remarkable for such relatively simple chemical structures [160].

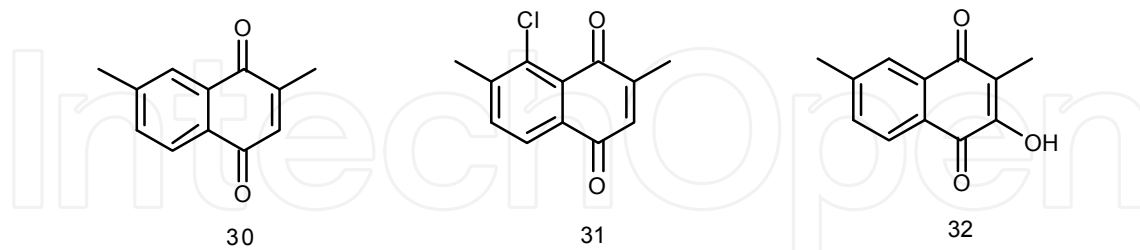


Figure 12.

Surangin B **33** is a coumarin isolated from *Mammea lonicifolia* that showed significant antifungal activity against many different pathogenic species with very low inhibitory concentrations [161]. Surangin B reduced to half the level of spore germination at 2.3 μ M. It was found that the mechanism of action of this interesting coumarin was through inhibition of electron transport chain in the mitochondrion. Another coumarin with antifungal activity is osthol **34**, isolated from species of the genera *Angelica* and *Cnidium*. *F. graminearum* is sensitive to this coumarin at a concentration from 25 to 100 mg/L, with an IC_{50} around 57 mg/L [162]. Osthol was shown to reduce considerably intracellular glucose levels which were detrimental to fungal development. In a research carried out in Colombia, the activity of phenolic derivatives obtained from different plant species has been reported against *F. oxysporum* f. sp. *dianthi*. Direct bioautography in a TLC bioassay showed that the minimum amount required for the inhibition of fungal growth was 1 μ g for cumansenic acid **35** obtained from *Piper cf. cumanense* [163], 5 μ g for evofolin-C **36** isolated from *Z. quinduense* [147], and 2 μ g for uvangoletin **37** and chrysin **38** obtained from *P. septuplinervium* [164].

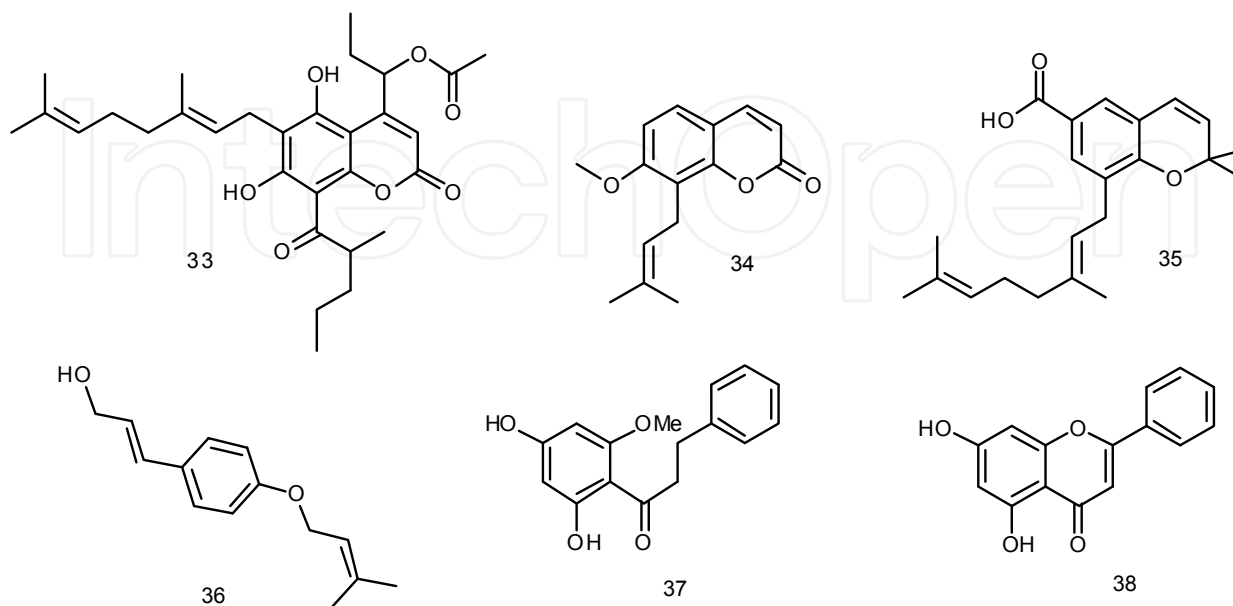


Figure 13.

4.3.3. Terpenes

The antifungal activity of essential oils is well documented [165], however less is known about the effects of isolated compounds from the oils. A study from 2005 reported the inhibitory activity of several isolated essential oil components using six different phytopathogenic fungi, including *F. oxysporum*. Specifically against this pathogen, chlorothymol **39**, thymol **40**, carvacrol **41** and carveol **42** inhibited half the growth of the fungi with an IC₅₀ less than 30 mg/L [166]. Other monoterpenoids such as geraniol, citronellol, eugenol, and vanillin were of moderate potency (around 100-200 mg/L for IC₅₀), while benzyl alcohol, camphor, carvone, menthol, cinammaldehyde, borneol, cineol were found to be less active.

On another study of antifungal potential of compounds usually encountered in essential oils, aromatic aldehydes such as benzaldehyde **43** and salicylaldehyde **44** showed a remarkable inhibition of *F. sambucinum* [167]. Their minimum inhibitory concentration (MIC) was found to be 40 mg/L and 4 mg/L respectively for several strains of this pathogen when tested in a vapour phase assay. Interestingly cinammaldehyde was confirmed to be less active against *Fusarium* species, with an MIC value superior to 400 mg/L. Moreover when the compounds were tested in dissolution in the media, cinammaldehyde **45** and thymol were able to inhibit completely the growth of the pathogenic fungi at 0.1 and 1% concentrations [167].

In a recent study of antifungal metabolites isolated from ethnomedicinal *Wardburgia ugandensis*, several sesquiterpenoids were found to inhibit the growth of different species of *Fusarium* [168]. Polygodial **46**, warburganal **47** and mukaadial **48** were potent inhibitors of the growth of pathogenic fungi showing MIC values below 25 mg/L against *F. solani* and 100 mg/L against *F. oxysporum*. Interestingly lactonic related natural products were much less active, suggesting that free aldehyde groups are essential for the antifungal effect, and this observation is supported by the fact that aromatic aldehydes are also highly active antifungals.

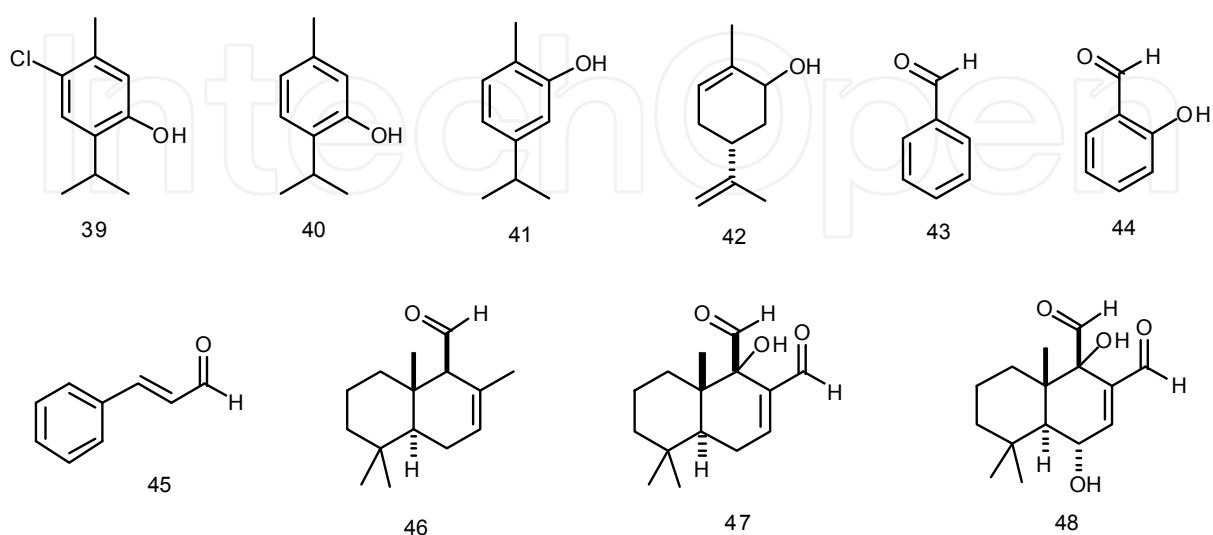


Figure 14.

Clerodane diterpenoids isolated from seeds of *Polyalthia longifolia* have shown moderate antifungal activity towards *Fusarium sp* [169]. 16 α -hydroxy-cleroda-3,13(14)-Z-diene-15,16-olide **49** and 16-oxo-cleroda-3,13(14)-E-diene-15-oic acid **50**. These diterpenoids were slightly more active against bacteria than against fungi, displaying MIC values of 100 mg/L and 50 mg/L respectively against *Fusarium sp*.

Costunolide and parthenolide are active sesquiterpene lactones typically found in *Magnolia* species. Parthenolide **51** was found to inhibit the growth of *F. culmorum* at 50 mg/L, while not inhibiting *F. oxysporum* [170]. In contrast costunolide was found inactive to both *Fusarium* species even at the highest concentration tested of 1000 mg/L. 1,10-epoxy parthenolide **52** was found to be slightly less active than parthenolide with MIC value of 230 mg/L against *F. culmorum*, being also inactive against *F. oxysporum*.

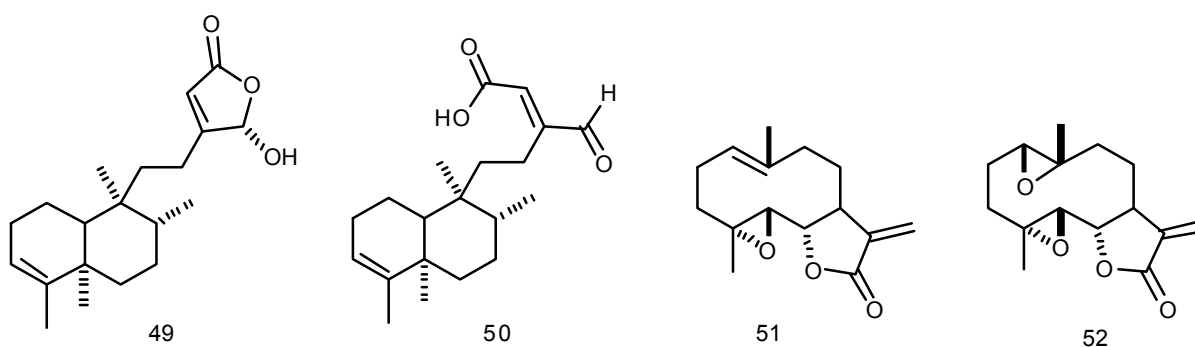


Figure 15.

5. Conclusions

The control of diseases caused by microorganisms such as bacteria and fungi in commercially important crops continues to be a large problem worldwide. Although different methods of control exist (chemical, physical, biological and combinations of these), these are still insufficient or have been found ineffective. Reports related to resistance mechanisms developed by the plagues are increasing; in many cases, the emergence of resistant strains is associated with a badly use of control products (under- or overdose). Especially, synthetic chemical control methods are associated with higher pollution and residues in crops, and in many cases poor selectivity of the pesticides causes alterations of biological balance of ecosystems. These observations have justified the growing research in the field of natural products in order to find effective and safe control methods for plagues that affect different products of economic importance.

To evaluate the antifungal power of a substance different methods have been developed. For these methods for antifungal activity evaluation, it should indicate that they are complementary methods, because some provide qualitative information and others shows quantitative data. The criterion for the selection of a test depends mainly on the characteristics of the sample to prove, principally purity and solubility. The bioautography method is inexpensive, very useful for screening large numbers of samples (especially crude

extracts). Although results are not entirely quantitative, it can give information about how many and which substances in a mixture showed antifungal activity. Dilution methods are employed for quantitative analysis and require little amount of sample, therefore, are suitable for evaluating compounds which do not present problems of solubility.

As a result of the intense research activity, a large number of species with potential for controlling phytopathogenic organisms have been identified. According to this review, approximately 150 plants species belonging to 30 plant families and about 50 compounds have promisory antifungal activity. These substances should be postulated as interesting agents for the control of *Fusarium* species.

The antifungal bioactivity was observed in crude extracts, fractions of varying polarity and essential oils. Studies have identified the individual compounds responsible for the activity. These results demonstrate that not a single type of extract, fraction or compound is responsible for antifungal activity, but sometimes they work in a synergistic fashion and therefore there is the possibility of using the whole extract instead of the pure compounds, which will be a cheaper and more feasible strategy in rural settings with low income.

The numerous reports of antifungal activity of natural products, contrast with the poor number of publications in relation to the mechanisms of antifungal activity. Therefore, the challenge is not only finding potential species to pest control, but determining the biochemical mechanisms that these products target. Understanding these mechanisms is important to developing products with higher selectivity. In the investigations in this field, it is necessary to involve computational methodologies and structure-activity relationships (SAR) that may lead to the identification of highly effective natural products, synthetic derivatives or structural analogues.

Although there have been found extracts, essential oils and pure compounds with antifungal activity comparable to that shown by current commercial products, these have not been converted into commercial products because there is still no large-scale production. To achieve production levels that are marketable missing including the following steps: a) studies of propagation and domestication of wild species that have shown activity, b) studies of agronomic crop species; c) study methods of harvesting and processing of plant material for obtaining the extracts; d) conducting formulation studies for extracts, oils and compounds.

The development of these activities will complement the work done so far as prospecting phase and lead to obtaining a standardized and reliable product that can be used by farmers for the partial or total replacement of the traditional synthetic products. In addition to not only provide a reliable product from the point of view of activity, it must be competitive in price, so search should focus on crude extracts or fractions, because with these substances is possible avoid separation process which could raise production costs.

We should take advantage of the fact that different types of substances and structures show antifungal activity, and if the mechanisms of action are varied, the commercial products based on these substances will be possible to avoid development of resistance by the

pathogen. Thus, the window of permanence of the products on the market will be higher. Although it is unrealistic to develop a unique product to control *Fusarium*, it is plausible to think that natural products used in combination with other methods could have a dramatic impact achieving higher level of protection and therefore greater productivity with fewer environmental problems.

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