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# Natural Fungicides Obtained from Plants

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## 1. Introduction

Fungicides belong to a group of pesticides which inhibited fungal growth either causing damage to the cells or preventing the fungal development. As pesticides, they offer great economic and social benefits through the protection and preservation of materials, food and the prevention of diseases. Since pesticides are designed specifically to fight harmful or even dangerous life forms and therefore are toxic to them, they may present hazards to the environment by their potential effect upon non-target organisms, including humans, particularly when misused. The need to balance these benefits against the risks presents a challenge to the EPA (Environmental Protection Agency) unlike other chemicals.

The widespread use of synthetic fungicides (chemical fungicides) in agriculture is a relatively recent phenomenon, and most of the major developments have taken place during the last 60 years. It has been the major way of fungal disease control in the world during the past decades and nowadays it plays an important role in crop protection. However, the chemical residues are liable to remain on the plant or within its tissues following fungicidal treatment. Fungicide residues in plants and their fruits pose a great health risk to the consumer, led to the search for safe alternatives to synthetic fungicides. In days gone by, farmers often ignored or did not recognize the effect that fungal pathogens had on the yield and quality of their crops. Nowadays, however, the losses are unacceptable, and farmers rely on the use of chemicals to control fungal diseases. As a consequence, commercial fungicides have become an important component of the total agrochemical business, with world-wide sales in 1996 of about $5.9 billion, equivalent to 18.9% of the total agrochemical market (Clough & Godfrey, 1998; Wood Mackenzie, 1997).

Economic losses caused by plant diseases are one of the main problems in crop management and postharvest storage. Some chemical fungicides are able to present curative and preventive action at the same time. Generally, they have a high persistent action; therefore they can develop a preventive action for long time. In spite of their effective action depend on dose and mode of plant application; it is known that they present a high effectively. This fact together to the simple application, handle and low cost, these chemical products have been used from approximately 1950. However, the massive and continue use of these products, together to the lack of controlled and adequate conditions for using them it have generated numerous problems such as new fungal pathogen strains resistant to fungicides and the increase of waste residues and the toxic effects for humans and animals.

The use of synthetic fungicides has been the major commercial means of postharvest decay control for several decades. However, the chemical residues that are liable to remain on the

fruit or within its tissues following fungicidal treatment and the 1986 report from the US National Academic of Sciences (Research Council, Board of Agriculture, 1987) indicating that fungicide residues on food pose a great health risk to the consumer, led to the search for safe alternatives to synthetic fungicides. The fact that the effectiveness of synthetic fungicides has been reduced by the frequent development of resistance by the pathogens further highlighted the need for new substances and methods for the control of storage diseases. Naturally occurring plant products are important sources of antifungal compounds with low toxicity to mammals and safe to the environment which may serve as substitutes for synthetically produced fungicides. It was later suggested that these compounds might be developed either as products by themselves or used as starting point for synthesis (Knight et al., 1997).

## 2. Chemicals alternative to conventional fungicides

The continuing development of fungicide resistance in plant and human pathogens necessitates the discovery and development of new fungicides. Several preharvest and postharvest pathogens have developed resistance to commonly used fungicides. Hence, a wide range of chemicals has been evaluated for their potential for use as alternative to the current fungicides, e.g. plant extracts and some compounds obtained from plants. Discovery and evaluation of natural product based fungicides for agriculture is largely dependent upon the availability of miniaturized antifungal bioassay. Essentials for natural product bioassays include sensitivity to very small quantities, selectivity to determine optimum target pathogens, and adaptability assays should be relevant to potential pathogen target sites in the natural infection process of the host and applicable to the agrochemical industry. Bioassays should take advantage of current high throughput technology available to evaluate dose-response relationships, commercial fungicides standards, modes of action, and structure activity studies (Wedge & Smith, 2006).

Since the early 1970s, agriculture worldwide has struggled with the evolution of pathogen resistance to disease control agents. Increased necessities for repeated chemical applications, development of pesticide cross-resistance and disease resistance management strategies have characterized the use of agricultural chemicals to-date. As a consequence, producers are currently attempting to control agricultural pests with a decreasing arsenal of effective crop protection chemicals. In addition, the desire for safer pesticides with less environmental impact has become a major public concern. Particularly desirable is the discovery of novel pesticide agents from new chemical classes that are able to operate using different modes of action and, consequently, against plant pathogens with resistance to currently used chemistries. In this regard, evaluating natural products and extracts as a source of new pesticides is one strategy for the discovery of new chemical moieties that have not previously been created by synthetic chemists (Wedge & Smith, 2006).

In January 2009, European Parliament agreed the text of a Regulation to replace 91/414/EC which Plant Protection Products have been regulated up to now. The new Regulation, originally proposed in July 2006, maintained risk assessment but also introduced the concept of hazard assessment or 'cut-off criteria' for the approval of active substances. In addition it proposed the concept of comparative assessment and substitution for those active substances meeting certain 'less stringent' hazard criteria. According to this Regulation, the potential impact in terms of percentage reductions in available active fungicides from 31 to 43% not approved (Richardson, 2009).

Under this situation there have been investigated new methods to control plant diseases as an alternative way to chemical fungicide application either eliminating these synthetic compounds from agriculture or meanly controlling their use together with natural fungicide substances in an unique strategy plan called Integrated Plant Management (IPM).

## 3. Plants contain naturally fungicides

Plants possess a range of tools for combating fungal infections. Defence mechanisms include physical barriers and chemical defence includes many naturally occurring compounds and systems to prevent microbial attacks by using several types of pre-formed compounds and induced response plant defence compounds.

Plants have defensive response by means of some formed or pre-formed substances belong to the commonly called plant secondary compounds (secondary metabolism). Secondary metabolism is known as all those compounds that they are not involved in primary metabolic processes such as respiration and photosynthesis. However, many compounds do have roles intermediate between primary and secondary metabolism. For instance, some plant secondary compounds like plant growth substances are associated with plant growth and development (Seigler, 1998). In fact, secondary metabolites are compounds with a restricted occurrence in taxonomic groups that are not necessary for a cell (organism) to live with its environment, ensuring the survival of the organism in its ecosystem (Verpoorte, 2000).

There has been much speculation over many years about why organisms take the trouble to produce secondary metabolites or natural products which often have considerable structural complexity. All such structures serve the producing organisms by improving their survival fitness (Williams et al., 1989). In this case, it seems likely that the fungi and bacteria which produce strobilurins, oudemansins, myxothiazols and/or melithiazols, which are substances with antifungal properties synthesized by fungi or bacteria give themselves an advantage by doing so, presumably by suppressing other organisms which compete for nutrients in the same environment (Williams et al., 1989).

Secondary metabolites are now thought to mediate chemical defence mechanisms by providing chemical barriers against animal and microbial predators. Plants produce numerous chemicals for defence and communication, and can elicit their own form of offensive chemical warfare by targeting the proliferation of pathogens. These chemicals may have general or specific activity against key target sites in bacteria, fungi and viruses.

These substances are classified into different ways: based on chemical characteristics, plant origin or biosynthetic origin. From a chemical point of view, the compounds can be divided into a number of groups based on typical characteristics such as alkaloids and phenolic compounds, principally, Based on biosynthetic origin, there are terpenoids, phenylpropanoids, polyketides and other compounds belong to other minor groups must be considered. Therefore, the total number of these compounds is very elevated. A great number of those are used to prevent animal consumption. However, many of these defensive substances are toxic for the plants themselves and they are inactivated as glycosides or by polymeration or they are confined in intercellular spaces.

## 4. Classification of fungicides in plants

Natural compounds with antifungal activity belong to the secondary metabolism in plants as above mentioned. This type of metabolism can be classified into three important groups:

1. Preformed compounds (preformed resistance in plants)

Substances naturally forming part of the plant cell composition have antifungal properties. Phytonzide is normally called to all the substances naturally occurring in plants which have antimicrobial properties inhibiting the growth and development of bacteria and fungi. These compounds are present in the plants in their biologically active form. These include phenolics, terpenoids and steroids. Some preformed compounds are directly toxic, while others exist as conjugates such as glycosides that are not directly toxic but become toxic following disruption of the conjugate. On the other hand, some plant preformed compounds are toxic as glycosides, but lose toxicity when deglycosylated.

2. Inducible preformed compounds (inducible preformed resistance in plants)

Some substances which are normally present in healthy tissues but they can be further induced in the host in response to pathogen attack or presence, as well as to other stresses. On the other hand, there are compounds that occur as inactive precursors and are activated in response to pathogen attack. These preformed compounds or phytoanticipins differ from the inducible phytoalexins that are synthesized from remote precursors in response to pathogen attack (Prusky, 1997).

3. Phytoalexins – Induced inhibitory compounds

Inducible resistance mechanisms are active, energy-requiring systems typified by specific recognition of an invader that ultimately leads to the production of proteins or metabolites that are antagonist to the invader. Such active resistance mechanisms are usually referred as the hypersensitive response (HR).

In this group, we can include other induced inhibitory compound like pathogenesis-related proteins (PR), active oxygen species and lectins, although they are not phytoalexins. Therefore they are in a chapter apart (see below).

### 4.1 Preformed compounds (preformed resistance in plants)

Preformed resistance involves the presence in healthy tissues of biologically active, low-molecular-weight compounds whose activity affords protection against infection (Barkai-Golan, 2001). These compounds belong to the secondary metabolism which includes all the compounds belong to the three categories described above. It could also be included other defensive physical structures (physical resistance) like structural barriers (cuticle, epicuticular wax, cell wall and processes like lignification and suberization of cells at the wound areas).

#### 4.1.1 Plant extracts

Plant extracts is a group of substances extracted from different parts of plants which contain a great many of compounds with antimicrobial properties. These compounds can be obtained from roots, barks, seeds, buds, leaves and fruits. Aromatic plants are especially rich

in those substances and they have been used in foods as flavouring agents (Davidson, 1997). Most of these compounds are terpenes and have fungicide properties are described below.

Plant extracts can be directly used or substances responsible for the antimicrobial properties can be isolated. A new extract with potent antifungal properties is the extract obtained from *Aloe vera*, which has been found to have antifungal activity against four common postharvest pathogens: *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea* and *Alternaria alternata* (Barkai-Golan, 2001).

Among the compounds belonging to plant extracts, some important examples have been selected and explained below. They can be classified in other groups, mainly as phenolic compounds or essential oils.

Few studies have focused on the mechanism by which plant extracts and their essential oils inhibit microorganisms. The terpenes present of the essential oils are the primary antimicrobials. Many of the most active terpenes, for example, eugenol, thymol and carvacrol, are phenolic in nature. Therefore, it would seem reasonable that their modes of action might be related to those of other phenolic compounds. Essential oils may inhibit enzyme systems in yeasts, including those involved in energy production in cells and synthesis of their structural components.

Eugenol [2-methoxy-4-(2-propenyl)-phenol] is a substance obtained from clove (*Syzygium aromaticum* L.), which contain 95% of eugenol as the main volatile oils. Cinnamic aldehyde [3-phenyl-2-propenal] is another antimicrobial substance obtained from cinnamon (*Cinnamomum zeylanicum* J Presl.). This plant also contains eugenol (only 8% of volatile oil and 75% of the first one). Both eugenol and cinnamon inhibits spores of *Bacillus anthracis*, while eugenol and aqueous clove infusions inhibited outgrowth of germinated spores of *B. subtilis* in nutrient agar (Davidson, 1997). In addition, clove extracts has antifungal properties. Thus, it inhibited growth initiation for over 21 days at 25ºC of *Aspergillus* and *Penicillium* species. Cinnamon was the next most effective spice, inhibiting three *Penicillium* species for over 21 days (Davidson, 1997).

The genus *Allium* contains various compounds with resistance to fungal disease. Some are constitutive inhibitors, such as the phenolic compounds catechol that is present in the outer bulb layers of pigmented *Allium cepa* (onion) cultivars were it confers resistance to *Colletotrichum* (Link & Walker, 1933). Onion also produces a class of cyclopentane phytoalexin upon pathogen infection, designated tsibulins (Dmitriev et al., 1990), which accumulate in bulb scales at infection sites during incompatible interactions with *B. cinerea*. Tsibulins inhibited spore germination and germ tube elongation of *B. cinerea in vitro*. The $ED_{50}$ values were lower than the actual phytoalexin content in bulb scale spots, were *B. cinerea* lesion formation was restricted (Dmitriev et al., 1990). Only little accumulation of cyclopentane phytoalexins was observed in onion bulb scales during infection by the specialized pathogen *B. allii* (Dmitriev et al., 1990). *B. allii* seems to either suppress tsibulin accumulation; analogous to the interaction of *B. narcissicola* and its host narcissus of actively degrade tsibulins, as discussed for other antifungal compounds above.

Isothiocyanates derive from glucosinolates in cells of plants of the Cruciferae, or mustard family (cabbage, kohirabi, Brussels sprout, cauliflower, broccoli, kale, horseradish, mustard, turnips, rutabaga) are potent antifungal and antimicrobial agents (Davidson, 1997). These compounds are formed from the action of the enzyme myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1) on the glucosinolates when plant tissues are injured or

disrupted. Common isothiocyanates include allyl, ethyl, methyl, benzyl and phenyl. The compounds are inhibitory to fungi, yeasts and bacteria in the range from 0.016 to 0.062 μg $mL^{-1}$ in the vapour phase or 10 to 600 μg $mL^{-1}$ in liquid media (Davidson, 1997). The mechanism by which isothiocyanates inhibit cells may involve enzymes by direct reaction with disulfide bond or through thiocyanate anion reaction to inactive sulphydryl enzymes. The isothiocyanates may act as uncouplers of oxidative phosphorylation (Davidson, 1997).

Purified metabolic extract of carrots and found it to be antifungal activity against the yeast *Candida lambica* at concentrations ranging from 55 to 220 mg $mL^{-1}$ and control some bacteria as well (Davidson, 1997). This inhibition was due to the compounds dodecanoic (monolaurin) and pentadanoic acids. An extract of carrots inhibited sporulation and aflatoxin production by *Aspergillus parasiticus* (Davidson, 1997). The inhibitor was not identified but was determined not to be 6-methoxymellein, *p*-hydroxybenzoic acid, or falcarindiol but rather to be part of the volatile carrot seed oil, a mixture of terpenoid compounds (Davidson, 1997).

Extracts of onions (Allium cepa L.) and garlic (Allium sativum L.) also contain volatile antimicrobial substances which inhibit Aspergillus flavus, A. parasiticus, Candida albicans, Cryptococcus, Penicillium, Rhodotorula, Saccharomyces, Torulopsis and Trichosporon species. The main compounds obtained from this extract is allicin (diallylthiosulfinate; thio-2-propene-1-sulfinic acid-S-allyl ester). This substance is produced by tissues when the cells are damaged and the enzyme (alliin-lyase; E.C. 4.4.1.4) act on the substrate (S-allyl-L-cysteine sulphoxide) because of enzyme and substrate are separated by cellular membranes inside the cells (Davidson, 1997). This has been suggested as a plant fungicide which undergoes thioldisulphide exchange reactions with free thiol groups in proteins and it is thought that this is the basis of its antimicrobial action. At 50 μg $mL^{-1}$, allicin in garlic juice inhibited the germination of sporangia and cysts and subsequent germ tube growth by Phytophthora infestans both in vitro and in vivo on the leaf surface. Similarly, in growth room experiments at concentrations from 50–1,000 μg $mL^{-1}$, allicin reduced the severity of cucumber downy mildew caused by Pseudoperonospora cubensis by approximately 50–100% (Davidson, 1997). The antimicrobial sulfur compounds of onions contain phenolic compounds protocatechuic acid and catechol, which contribute to their antimicrobial activities. Garlic oil inhibits ethanol production by Saccharomyces cerevisiae and delay sporulation of Hansenula anomala and Loderomyces elongisporus.

Resin from the flowers of the hop vine (*Humulus lupulus* L.) is used in the brewing industry for imparting a desirable bitter flavour to beer and about 3 to 12% of the resin is composed of α-bitter acids, including humulone (humulon), cohumulone and adhumulone and also β-bitter acids, including lupulone (lupulon), colupulone, xanthohumol and adlupulone (Davidson, 1997). Both types of bitter acids possess antimicrobial activity against gram-positive bacteria and fungi. These acids are considered to be the primary quality feature of hops, because they are a measure of the bitter potential and due to their antimicrobial and preservative properties.

### 4.1.2 Essential oils

Essential oils are highly volatile substances synthesized and stored in glandular trichomes of odiferous plants. The term essential oil refers not only to the complex oils isolated from the

plant, but also to their constituent compounds. Volatile components of essential oils are lipophilic molecules that volatilize at low temperatures.

Culinary aspects of many plants are based on essential oils, although other uses have been attributed (e.g., medicinal practices). Plants of the family *Laminaceae* (mints) present typically these oils in their composition (e.g., *Mentha* sp., *Monarda* sp., *Origanum* sp., *Rosmarinus* sp., *Thymus* sp.). Many of these essential oils isolated from mints are terpenes. However, other plants belonging to other families present essential oils as well, e.g. *Rutaceae*. They are mainly terpenes as well. The antimicrobial effects of two important plants: oregano (*Origanum vulgare* L.) and thyme (*Thymus* sp.) have been attributed to their essential oils which contain the terpenes carvacrol [2-methyl-5-(1-methylethyl) phenol] and thymol [5-methyl-2-(1-methylethyl)-phenol]. Firstly, the antimicrobial activities of oregano and thyme were proposed as antibacterial compounds. However, in recent works they have been proposed as antifungal properties as well. Essential oils obtained from thyme (*Thymus vulgaris* L.) and juniper (*Juniperus communis* L.) have been proved as fungicides against *P. digitatum* and *P. italicum* (Martínez & Hernández, 2007). They are actives against a great number of bacteria, moulds and yeasts.

In general, essential oils and plant extracts are sources of antifugal activity against a wide range of fungi (Grange & Ahmed, 1988). A rapid assay to determine antifungal activity in both plant extracts and essential oils have been shown by Barkai-Golan (2001) concluding that among 345 plant extracts analyzed, 13 showed high levels of activity against *B. cinerea*, which served as a test fungus. Garlic (*Allium* sp.) and pepper (*Capsicum* sp.) seem to be the major plants showing the highest persistent antifungal activity (Barkai-Golan, 2001). In addition, among the 49 essential oils tested, those of palmarosa (*Cymbopogon martinii* Roxb.) and red thyme (*Thymus zygis* L.) showed the greatest inhibitory effect on *B. cinerea* spore germination at the lowest concentration. The next best inhibitors were essential oils of clove buds (*Syzygium aromaticum* L.) and cinnamon leaf (*Cinnamomun zeylanicum* J Presl.). The most frequently occurring constituents in essential oils showing high antifungal activity were: *D*-limonene, cineole, α-pinene, β-pinene, β-myrcene and camphor. The fungicidal activity of the individual components, singly and in combination, is being studied (Barkai-Golan, 2001). Essential oil derived from another species of *Thymus*, *T. capitatus* L., markedly reduced development of *B. cinerea* in inoculated mandarin fruit when applied as a vapour. Scanning electron microscope observations indicated a direct damaging effect of the thyme oil on fungal hyphae (Arras & Piga, 1994).

In the family *Rutaceae*, several natural antifungal compounds have been isolated from lemon peel and have been identified. Among these are citral (lemonal), limetin, 5-geranosy-7-methoxycoumarin and isopimpeneyin. The antifungal activity of citral (terpenoids) has been exhibited against various fungi (Barkai-Golan, 2001). Introducing *Penicillum digitatum* spores into the oil glands of the peel of young lemon fruits revealed that citral is the main factor within the glands responsible for the inhibition of the pathogen growth (Barkai-Golan, 2001). In postharvest studies in lemon fruits it have been concluded that the changes in citral concentration in the lemon peel may determine the fruit sensitivity to postharvest decay. In

parallel with citral decline the flavedo of yellow lemon exhibited an increased level of neryl-acetate, which is a monoterpene ester which exhibits no inhibitory activity against *Penicillium digitatum*, and which, in low concentration (less than 500 ppm) may even stimulate development of the pathogen. The increase in the level of monoterpenes in the peel may explain, at least in part, the stimulatory effect on *P. digitatum* and *P. italicum* development, of the etheric oil derived from stored citrus fruit. The resistance of citrus peel to inoculations applied between the oil glands, found in early studies by Schiffmann-Nadel and Littauer (1956), was attributed to another factor, which does not change with fruit ripening and may be related to its chemical composition or its anatomic structure (Barkai-Golan, 2001).

Tulip bulbs and pistils contain high concentration of fungitoxic compounds, identified as lactones and termed tulipalins (van Baarlen et al., 2004). These compounds are sesquiterpenes, an important essential oils group. Tulipalin A is found in bulbs, whereas aerial plant parts contain mainly tulipalin B. Tulipalins A and B are stored in a glycosylates form, named tuliposides A and B. Tuliposides are less toxic to fungi than the corresponding unglycosylated lactones and are lactonized into tulipalins when the pH exceeds 5. All *Botrytis* species tested by Schönbeck & Schroeder, (1972) so far are sensitive to pure tulipalin A, except for the tulip-specific pathogen *B. tulipae*. Higher doses (7-10 μM, more than 30 μM for *B. tulipae*) are lethal to *Botrytis* spp. Conidia are at least three times more sensitive to tulipalin A than mycelium.

Among 22 naturally occurring monoterpenoids screened for activity against *Botrytis cinerea* and *Monilia fructicola* causing grey mould and brown rot diseases, the phenolic monoterpenoids carvacrol and thymol were the most effective in inhibiting spore germination and mycelial growth of the pathogens (Narayanasamy, 2006; Rong & Ting, 2000).

*Rhizoctonia solani* is sensitive to mint oils. Growth of *R. solani* was reduced when grown on culture medium amended with oils of *Monarda didyma* or when exposed to the vapours in a microatmosphere test (Gwinn et al., 2010). When dispersed in fungal culture media, oils from thyme (*Thymus vulgaris* L.), *Salvia fruticosa* Mill. and peppermint (*Mentha* x *piperita* L.), also reduced growth of *R. solani*. Growth of *R. solani* was completely suppressed by the phenolic monoterpene, carvacrol at a concentration of 100 mg $mL^{-1}$ and by its structural isomer, thymol, at a slightly higher concentration (150 mg $mL^{-1}$) (Gwinn et al., 2010). Carvacrol was the major constituent of essential oils collected from *Thymbra spicata* L., a member of the mint family that grows wild in the eastern Mediterranean; oil extracted from *T. spicata* completely suppressed growth of *R. solani*. Fungicidal activity of thyme oils has been correlated with thymol content (Gwinn et al., 2010).

Damping-off of seedlings caused by species of *Pythium* and *Rhizoctonia* can reduce number and quality of tomato seedlings. Losses due to *Pythium myriotylum* were reduced when monarda herbage (ground, dried leaves) from some varieties of *Monarda didyma* L., *M. clinopodia* L. and *M. menthifolia* L. were added to greenhouse growing medium at a rate of 10% (v/v) (Gwinn et al., 2010). Thymol was the major essential oil component of 'Croftway Pink', the most effective monarda herbage treatment for reducing losses due to *P. myriotylum*.

Bettiol et al. (2011) studied the effect of several essential and fixed oils (a mixture of not volatile esters of fatty acids) on the control *in vitro* and *in vivo* of green mould of oranges caused by *Penicillium digitatum*. They tested oils extracted from *Pogostemon cablin* Benth., *Mentha arvensis* L., *Cymbopogon citratus* Otto Stapf., *Ocimum basilicum* L., *Rosmarinus officinalis* L., *Lippia sidoides* Cham., *Zingiber officinale* Rosc., *Citrus aurantifolia* L., *Piper aduncum* L., *Allium sativum* L., *Copaifera langsdorffii* Desf., *Eucaliptus* spp. and *Azadirachta indica* A. Juss. The oils at 10,000 and 100,000 µL $L^{-1}$ controlled green mould and inhibited spores germination and mycelial growth in a similar level as compared to the fungicide treatment. However, treatment with oil in concentrations higher than 10,000 µL $L^{-1}$ caused ring damage and changed fruits flavour, which makes its implementation impractical in high concentrations.

### 4.1.3 Phenolic compounds

Phenolic compounds are those substances that possess an aromatic ring with one or more hydroxyl groups and can include functional derivates. Some of the important phenolic compounds include alkyl esters of parabens, phenolic antioxidants (e.g., BHA and TBHQ) and certain of the terpene fraction of the essential oil (e.g., thymol, carvacrol, eugenol and vanillin). Sesquiterpenes, with monoterpenes, are an important constituent of essential oils in plants. Simple phenolic compounds include monophenols (e.g.., cresol), diphenols (e.g., hydroquinone) and triphenols (e.g., gallic acid). Gallic acid occurs in plants as quinic acid esters or hydrolyzable tannins (tannic acid) (Davidson, 1997). These compounds are naturally present in plants. They have antibacterial properties but some of them have antifungal properties as well, for example against *Penicillium* sp., *Rhizopus* sp. and *Geotrichum candidum*.

The mechanism of phenolic compounds centres on their effects on cellular membranes. Simple phenols disrupt the cytoplasmic membrane and cause leakage of cells. Phenolics may also inhibit cellular proteins directly. However, some researchers have concluded that phenolic compounds may have a great many of mechanisms of action and that there may be several targets which lead to inhibition of microorganisms (Davidson, 1997).

Phenolic compounds have long been implicated in disease resistance in many horticultural crops (Barkai-Golan, 2001). Some occur constitutively and are considered to function as preformed or passive inhibitors, while others are formed in response to the ingress of pathogen and their appearance is considered as part of an active defence response (Barkai-Golan, 2001). They contribute to resistance through their antimicrobial properties; with elicit direct effects on the pathogen, or by affecting pathogenicity factors of the pathogen. However, they may also enhance resistance by contributing to the healing of wounds via lignifications of cell walls around wound zones. The cells surrounding the wound can produce and deposit lignin and suberin in their walls (Eckert, 1978). This compound protects the host from pathogen penetration or from the action of cell-wall degrading enzyme produced by the pathogen. As a result of wounding, the production of antimicrobial polyphenolic compounds can also contribute to wound protection. Phytoalexins are other toxic compounds can be formed at the wound area following inducement by initial infection. In this way, the inoculation of potatoes tubers with *Fusarium sambicinum*, the fungal pathogen of potato dry rot, resulted in an increase in phenolic acids suggesting that phenolic acid biosynthesis was induced. Following such inducement free

phenolic acids are removed as they are converted into lignin or are joined onto cell walls (Barkai-Golan, 2001).

Studies with cultured carrot cells indicated that phenolic compounds with low molecular weight, which are a link in lignin biosynthesis and free radicals produced during its polymerization may take part in resistance inducement by damaging fungal cell membranes, fungal enzymes or toxins (Barkai-Golan, 2001). Accumulation of phenolic compounds and callose deposition in cell walls of young tomato fruits, following inoculation with *B. cinerea*, were found to arrest fungal development thus retarding or preventing decay (Barkai-Golan, 2001). The mechanism by which phenolic compounds accumulate in the host is not yet clear, but research carried out with wheat leaves suggested that the chitin in the fungal cell walls acts as a stimulator to lignifications in the leaves (Pearce & Ride, 1982, as cited in Barkai-Golan, 2001).

*In vitro* assays have shown that the phenolic compounds, chlorogenic acid and ferulic acid directly inhibited *Fusarium oxysporum* and *Sclerotinia sclerotiorum* respectively. Benzoic acid derivatives have been shown to be the best inhibitors of some of the major postharvest pathogen, such as *Alternaria* spp., *B. cinerea*, *Penicillium digitatum*, *S. sclerotiorum* and *F. oxysporum* (Latanzio et al., 1995, as cited in Barkai-Golan, 2001).

The principal phenol in the pear fruit epidermis and subtending cell layers are chlorogenic and caffeic acids. The concentration of these phenols decline as fruit mature, with a corresponding increase in fruit susceptibility to the brown rot fungus (*Monilinia fructicola*). In fact, fungal spore germination or mycelial growth were not inhibited by concentrations similar to or exceeding those that occur in the tissue of inmature, resistant fruit.

Tannins, which are polyphenols, have been described by Byrde et al. (1973, as cited in Barkai-Golan, 2001) as inhibitors of polygalacturonase (PG) activity of *Sclerotinia fructicola* (anamorph of *Monilinia fructicola*) in apples and other pathogen/host combinations. Tannins of young banana fruits (Green & Morales, 1967, as cited in Barkai-Golan, 2001) and benzylisothiocyanate in unripe papaya fruits (Patil et al., 1973, as cited in Barkai-Golan, 2001) are additional examples of in-fruit toxic compounds.

Proanthocyanidins are widely distributed in the plant Kingdom and are constitutive components in a number of discrete tissues in most plant organs. The chemical structure and composition of proanthocyanidins vary among plant species, organs and also with the stage of organ development. A special type of tannins, proanthocyanidins (condensed tannins) are polymeric flavonoids that results from the condensations of two or more derivates of flavan-3,4-diol. Plant proanthocyanidins maintain *B. cinerea* in a quiescent stage, leading to delayed development of symptoms. The transition from quiescence into expansion is triggered during host senescence or ripening and occurs at a less senescent or ripe stage in susceptible varieties. Prolonging the quiescence of *B. cinerea* infections by increasing the proanthocyanidin content would reduce losses to grey mould, especially after harvest. However, proanthocyanidin levels are constitutive and are not known to be subject to modulation by external elicitors. Moreover, knowledge is lacking on the genes and enzymes involved in the subtle modifications of proanthocyanidin structure that affect their biological activity. The use of proanthocyanidin content as an indicator of grey mould resistance for the selection of cultivars with improved shelf-life has been suggested for grape and strawberry (van Baarlen et al., 2004).

Oleuropein is another phenolic compound found in olive leaf from the olive tree. This substance inhibits *Rhizopus* sp. and *Geotrichum candidum* (Davidson, 1997).

Another class of inhibitors of cell wall-degrading enzymes comprises PG-inhibitory proteins present in both infected and uninfected plant tissue (Barkai-Golan, 2001). Research carried out with pepper fruit has shown that cell wall protein of the host inhibited pectolytic enzyme production by *Glomerella cingulata*, whereas the pectolytic activity of *Botrytis cinerea* was much less affected by these proteins (Brown & Adikaram, 1983, as cited in Barkai-Golan, 2001). The fact that *B. cinerea* can rot an immature pepper fruit whereas *G. cingulata* can attack only the ripened fruit, suggested that protein inhibitors might play a role in the quiescent infection of pepper fruit by *Glomerella*.

A new protein inhibitor that may be involved in the inhibition of enzymes necessary for microbial development was isolated from cabbages (Lorito et al., 1994, as cited in Barkai-Golan, 2001); it significantly inhibited the growth of *B. cinerea* by blocking chitin synthesis, so causing cytoplasmic leakage. Several studies supported the theory that natural protein compounds within the plant tissue may act as inhibitors of pathogen enzymes and that these inhibitors may be responsible for the low levels of PG and PL found in infected tissue (Barkai-Golan, 2001). Recent studies show a close correlation between the changes in the level of epicatechin in the peel of avocado fruit and the inhibition of pectolytic enzyme activity of *Colletotrichum gloesporioides* (Wattad et al., 1994, as cited in Barkai-Golan, 2001).

### 4.1.4 Hydroxycinnamic acids

Hydroxycinnamic acids can be considered as phenolic compounds and are a class of polyphenols which are hydroxyl derivates of cinnamic acid and include caffeic, chlorogenic, *p*-coumaric, ferulic and sinapinic acids. They occur frequently as esters and less often as glucosides. Many of the studies with hydroxycinnamic acids have involved their antifungal properties. It has been reported that 500 µg $mL^{-1}$ of caffeic acid and 1,000 µg $mL^{-1}$ of chlorogenic acid inhibit some species of *Fusarium* (Davidson, 1997). It has been shown that ferulic acid at 5.0 mg 26 $mL^{-1}$ inhibits aflatoxin $B_1$ and $G_1$ production of *Aspergillus flavus* by approximately 50% and that of *A. parasiticus* by 75%. Salicylic and *trans*-cinnamic acids totally inhibit aflatoxin production at the same 5.0 mg 26 $mL^{-1}$ (Davidson, 1997). After a study of the effects of caffeic, chlorogenic, *p*-coumaric and ferulic acids at pH 3.5 on the growth of *Saccharomyces cerevisiae* was concluded that caffeic and chlorogenic acid had little effect on the organism at 1,000 µg $mL^{-1}$ (Davidson, 1997). In the presence of *p*-coumaric acid, however, the organism was completely inhibited by the same concentration. Ferulic acid was the most effective growth inhibitor tested. At 50 µg $mL^{-1}$; this compound extended the lag phase of *S. cerevisiae* and at 250 µg $mL^{-1}$, growth of the organism was completely inhibited. The degree of inhibition was inversely related to the polarity of the compounds.

### 4.1.5 Flavonoids

Flavonoids are a special group of phenolic compounds and some aspects of this group have been described above. The flavonoids consist of catechins and flavons, flavonols and their glycosides. Proanthocyanidins or condensed tannins are polymers of favan-3-ol and are found in apples, grapes, strawberries, plums, sorghum and barley (Davidson, 1997).

Benzoic acid, proanthocyanidins and flavonols account for 66% of cranberry microbial inhibition against the yeast *Saccharomyces bayanus*, with the latter two being the most important.

### 4.1.6 Plant growth substances and regulators

At the moment, some differences between plant growth substances and plant growth regulators can be considered (Arteca, 1996). Plant growth substances (PGS) or commonly called phytohormones are synthesized by plants whereas plant growth regulators (PGR) are those organic compounds other than nutrients (materials which supply either energy or essential mineral elements), which in small amounts promote, inhibit, or otherwise modify any physiological process in plants. Arteca (1996) used the term PGR to designate synthetic compounds and the term PGS for naturally occurring compounds produced by the plant.

Plant growth substances and regulators seem to be a group of important substances which they control the growth of plants and can have antifungal properties as well. It is very well known that phytohormones control all the physiological process in plants growth and development; therefore, they interfere with the influence of pathogens to attack plants. However, some of PGS and PGR have directly effect as fungicides. In this way, Martínez et al. (2011) reported that 100 mg of indole-3-acetic acid (IAA) delayed the *in vitro* mycelial growth of several *Botrytis cinerea* isolates obtained from potted plants in an isolate-dependent manner (Fig. 1).

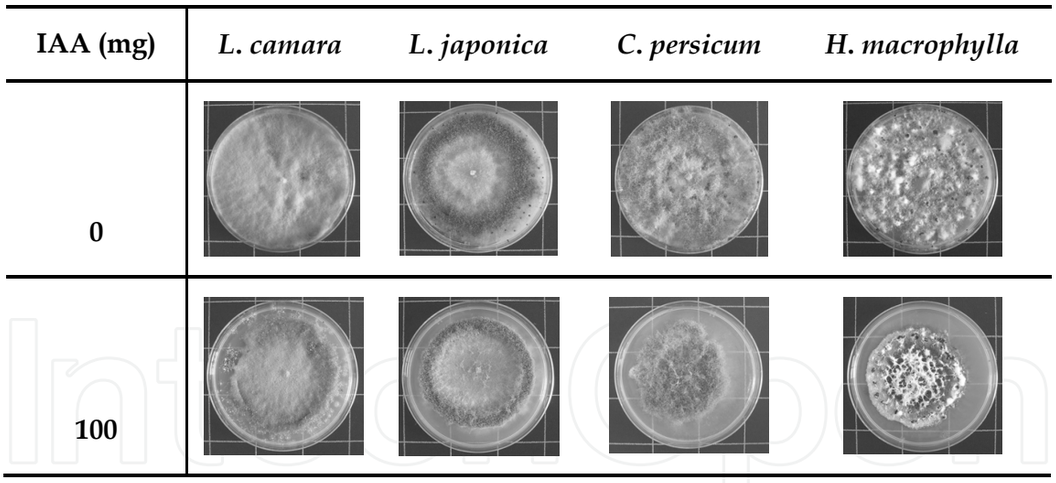


Fig. 1. Mycelia of four isolates of *B. cinerea* obtained from different potted plants (*Lantana camara*, *Lonicera japonica*, *Cyclamen persicum*, and *Hydrangea macrophylla*) grown *in vitro* on potato dextrose agar (PDA) at 26ºC after 35 days with 0 mg or 100 mg of IAA (adapted from Martínez et al., 2011)

The synthesis of plant growth substances in many fungi has been demonstrated, but now, the synthesis pathways have been only established in a few cases. Moreover, new substances seem to be important effect as growth regulator in fungi. Until now, indol-3-acetic acid, gibberellic acid, abscisic acid and ethylene, important hormones in plants, have been discovered in fungi like *Botrytis cinerea* (Sharon et al., 2004).

Jasmonic acid and its derivates, mainly methyl-jasmonate are present in most of the plants playing a plant growth substance role (Arteca, 1996). Jasmonic acid and the corresponding methyl ester are fragrant constituents of the essential oils of *Jasminum* sp. as well as other perfumery plants. These plant growth substances are now under study for evaluating their effects on citrus fruit decay and the decrease of chilling injury in postharvest. These substances applied as vapour, drencher or bath in citrus packinghouse at very low concentrations could be considered as an alternative to control decay in citrus industry in Spain (Monterde et al., 2002). Droby et al. (1999) found that methyl-jasmonate had antifungal activity against *Penicillium digitatum*, the principal fungus causing decay in citrus fruits.

The mode of action consists of that in response to wounding or pathogen attack, fatty acids of the jasmonate cascade are formed from membrane-bound linolenic acid by lipoxygenase-mediated peroxidation (Vick & Zimmerman, 1984). Linolenic acid is thought to participate in a lipid-based signalling system where jasmonates induce the synthesis of a family of wound-inducible defensive proteinase inhibitors (Farmer & Ryan, 1992) and low and high molecular weight phytoalexins such as flavonoids, alkaloids and terpenoids.

In relation with other PGS, treatment of celery prior to storage with gibberellic acid ($GA_3$) in juvenile plant tissue resulted in decay suppression during 1 month of storage at 2ºC, although $GA_3$ does not have any effect on fungal growth *in vitro* (Barkai-Golan, 2001). It was suggested that the phytohormone retards celery decay during storage by slowing down the conversion of (+) marmesin to psoralens, thereby maintaining the high level of (+) marmesin and low levels of psoralens and, thus increasing celery resistance to storage pathogens (Barkai-Golan, 2001). Martínez & Bañón, (2007) and Martínez et al. (2007) demonstrated that $GA_3$ has some effects on growth and development of fungal structures of the *Botrytis cinerea* isolates obtained from potted plants, but this phytohormone either increased the fungal development or had no effect on the growth, depending on the isolate.

### 4.1.7 Acetaldehyde and other volatile compounds

Acetaldehyde is a natural volatile compound produced by various plant organs and accumulates in fruits during ripening. It has shown fungicidal properties against various postharvest pathogens (Barkai-Golan, 2001). It is capable of inhibiting both spore germination and mycelial growth of common storage fungi and the development of yeast species responsible for spoilage of concentrated fruit juices. It has been reported to inactivate ribonuclease and to bind other proteins but the mechanism of aldehyde toxicity to fungal spores is still unknown (Barkai-Golan, 2001).

Fumigation apples, strawberries with acetaldehyde reduced decay cause by *Penicillium expansum*, *Rhizopus stolonifer* and *Botrytis cinerea* (Barkai-Golan, 2001). Avvisar & Pesis (1991 as cited in Barkai-Golan, 2001) showed that 0.05% acetaldehyde applied for 24 h suppressed decay caused by *B. cinerea*, *R. stolonifer* and *Aspergillus niger*.

Injuries resulting from acetaldehyde vapours have been reported for various products, such as cultivars of apples, strawberries, grapes, lettuce and carrot tissue cultures (Barkai-Golan, 2001). The efficacy of acetaldehyde vapours and of a number of other aliphatic aldehydes, produced naturally by sweet cherry cv. Bing, was evaluated in *P. expansum* inoculated fruits

(Mattheis & Roberts, 1993, as cited in Barkai-Golan, 2001). High concentrations of acetaldehyde, propanal and butanal suppressed conidial germination but resulted in extensive stem browning and fruit phytoxicity, which increased with the aldehyde concentration. On the other hand, stem quality is less of a concern for fruits intended for processing and for this purpose aldehyde fumigation may present an alternative to the use of synthetic fungicides.

Various volatiles (benzaldehyde, methyl salicylate and ethyl benzoate) have been recorded as growth suppressors. Nine out of 16 volatile compounds occurring naturally in peach and plum fruits greatly inhibited spore germination of *B. cinerea* and *Monilinia fructicola* (Barkai-Golan, 2001). The volatiles most effective in inhibiting spore germination were benzaldehyde, benzyl alcohol, γ-caprolactone and γ-valerolactone. Of these, benzaldehyde was active at the lowest concentrations tested and completely inhibited germination of *B. cinerea* spores at concentrations of 25 μL $L^{-1}$ and germination of *M. fructicola* spores at 125 μL $L^{-1}$. Ethyl benzoate was fungicidal against *Monilinia* sp. and fungistatic against *Botrytis* sp.

### 4.1.8 Ethanol

Ethanol is a substance produced in fruits (Barkai-Golan, 2001). It has been tested for control of brown rot and Rhizopus rot in peach fruits with varying degrees of success. Recently, the effects of ethanol solutions, at concentration of 10-20%, were evaluated for the control of postharvest decay of citrus fruits, peaches and nectarines (Barkai-Golan, 2001).

### 4.1.9 Hinokitiol

Hinokitiol is a natural volatile extracted from the root of trunk of Japanese cypress (*Hiba arborvitae*) with outstanding antifungal properties (Barkai-Golan, 2001). Hinokitiol reduced spore germination in *Monilia fructicola*, *Rhizopus oryzae* and *Botrytis cinerea*. In parallel, this volatile prevented decay of commercially harvested peaches in which more than 40% of the treated fruit developed brown rot caused by *M. fructicola* (Sholberg & Shimizu, 1991, as cited in Barkai-Golan, 2001).

Today melons are coated with wax containing imazalil (200 ppm) and under the above conditions, this leaves fungicidal residue above the level approved in some countries (0.5 ppm). Introducing hinokitiol into wax was also found to control decay during cold storage, caused mainly by *Alternaria alternata* and *Fusarium* spp. without any phytotoxic effects.

### 4.1.10 Glucosinolates

Glucosinolates are a large class of compounds that are derived from glucose and an amino acid. They occur as secondary metabolites of almost all plants of the order Brassicales, especially are present in Cruciferae's family. The studies carry out at the moment have been done with encouraging results (Barkai-Golan, 2001). When cells of plant tissues that metabolize glucosinolates are damaged, these compounds come into contact with the enzyme myrosinase, which catalyzes hydrolysis. The antifungal activity of six isothiocyanates has been tested on several postharvest pathogens *in vitro* and *in vivo* on artificially inoculated pears with encouraging results.

### 4.1.11 Latex

Latex is a stable dispersion of naturally occurring polymer microparticles in an aqueous medium. It is found in 10% of all angiosperms. This complex emulsion consisting of alkaloids, starches, sugars, oils, tannins, resins and gums that coagulates on exposure to air. It is also rich in enzymes like proteases, glucosidases, chitinases and lipases. It has been demonstrated that this substance is a source of natural fungicides (Barkai-Golan, 2001) which is regarded as both safe and effective against various diseases of banana, papaya and other fruits. The water-soluble fraction of papaya latex can completely digest the conidia of many fungi, including important postharvest pathogens (Indrakeerthi & Adikaram, 1996). Other latex extracted from several plants showed a strong antifungal activity against *Botrytis cinerea*, *Fusarium* sp. and *Trichoderma* sp. (Barkai-Golan, 2001).

### 4.1.12 Steroids

Steroids are terpenes with a particular ring structure composed by a specific arrangement of four cycloalkane ring that are joined to each other. Saponins are plant steroids, often glycosylated.

The saponin, α-tomatine, is a secondary metabolite produced in tomato leaves unripe fruits (Friedman, 2002, as cited in van Baarlen et al., 2004). It is also present in high concentration in the peel of green tomatoes. It is a potent antifungal and insecticidal compound that interacts with sterols in membranes (van Baarlen et al., 2004), It Inhibit mycelial growth of *B. cinerea* while not affecting germination of conidia. This substance also affects other fungal pathogens and its involvement in the development of quiescent infection has been suggested (Verhoeff & Liem, 1975, as cited in van Baarlen et al., 2004). Tomatine presumably is toxic due to its ability to bind to 3-β-hydroxy sterols in fungal membranes (Steel & Drysdale, 1988, as cited in Barkai-Golan, 2001). Most tomato pathogens, on the other hand, can specifically degrade tomatine and detoxify its effects through the activity of tomatinase (Barkai-Golan, 2001).

## 4.2 Inducible preformed compounds (inducible preformed resistance in plants)

Over the last two decades several studies have indicated that preformed antifungal compounds, which are normally present in healthy plant tissues, can be further induce in the host in response to pathogen attack or presence, as well as to other stresses. Induction of existing preformed compounds can take place in the tissue in which they are already present, or in a different tissue (Prusky & Keen, 1995).

These inducible preformed compounds can be induced due to infection, after association with surface plant or under an abiotic stress. This abiotic stress can be induced with storage techniques of fruits and vegetables. For example, heating is a postharvest fruit technique which can be used to inactivate senescence enzymes for prolonging the shelf-life by controlling high temperature during some periods of time as a type of physical treatment. At the same time, heating allows to maintain or to prolong the fungicide activity of compounds present in citrus peel like citral or certain proteins like quitinase and β-1,3-glucanase (Barkai-Golan, 2001). It can also induce phytoalexins in superficial wounds inoculated with the pathogen. In the other hand, heating can also catalyze biosynthesis of lignin and other analogues compounds in wounds which act on as a physical barrier against hyphae penetration of pathogen.

Antifungal substances isolated from unripe avocado fruit peel include monoene and diene compounds, of which diene is the more important (Prusky & Keen, 1993). Diene compound (1-acetoxi-2-hydroxy-oxo-heneicosa 12,15-diene) which it is a hydrocarbon, inhibits spore germination and mycelial growth of *Colletotrichum gloeosporioides*, at concentrations lower than those present in the peel. Prusky et al. (1990, as cited in Barkai-Golan, 2001) found that inoculation of unharvested or freshly harvested avocado fruit with *C. gloeosporioides*, but not with the stem-end fungus *Diplodia natalensis*, resulted in a temporarily enhanced level of these compounds. The response to this challenge doubled the amount of the preformed diene after 1 day and the effect persisted for 3 days, suggesting persistence of the elicitor. On the other hand, wounding of freshly harvested fruit resulted in a temporarily enhanced diene accumulation in the fruit, inducement did not occur in fruit 3-4 days after harvest (Barkai-Golan, 2001).

γ irradiation is another abiotic factor capable of inducing diene accumulation and $CO_2$ treatment also increase it. An inducement of antifungal diene also followed a high-$CO_2$ application. Exposing freshly harvested avocado fruit to 30% $CO_2$ resulted in increased concentration of the diene upon removal from the controlled atmosphere storage.

Resorcinolic compounds (resorcinols) are also considered as inducible preformed compounds. These compounds have been described in mango fruit. A mixture of resorcinolic compounds normally occurs in fungitoxic concentrations (154-232 μg $mL^{-1}$ fresh weight) in the peel of unripe mangoes whereas only very low concentrations are present in the fresh of the fruit (Droby et al., 1986). These fungitoxic compounds showed antifungal activity against *Alternaria alternata*, the causal agent of black spot in citrus fruit. This enhancement was accompanied by an increase in fruit resistance to fungal attack.

Exposure of the fruit to a controlled atmosphere containing up to 75% $CO_2$ was found to enhance of level of resorcinols in the peel itself were they are normally present; this enhancement was accompanied by decay retardation, as indicated by a delay in the appearance of the symptoms of *Alternaria alternata* infection (Barkai-Golan, 2001).

Other compounds that increase the level after infection are the bioactive polyacetylenes, falcarinol and falcarindiol, present in carrots, celery, celeriac and other umbeliferous vegetables.

In carrot roots, high concentrations of the antifungal polyacetylene compound falcarindiol, were recorded. This compound is found in extracellular oil droplets within the root periderm and the pericyclic areas (Garrod & Lewis, 1979). The high concentrations of the antifungal compound were suggested to result from the continuous contact of the carrot with organisms in rhizosphere or with various pathogens. One of the important antifungal compounds in carrot roots is the polyacetylenic compound, falcarinol.

### 4.3 Phytoalexins – Induced inhibitory compounds

Phytolalexins are low-molecular-weight toxic compounds produced in the host tissue in response to initial infection by microorganisms, or to an attempt at infection. In other words, in other to overcome an attack by the pathogen, the host is induced by the pathogen to produce antifungal compounds that would prevent pathogen development. However, the accumulation of phytoalexins does not depend on infection only. Such compounds may be elicited by fungal bacterial or viral metabolites, by mechanical damage, by plant constituents

released after injury, by a wide diversity of chemical compounds, or by low temperature irradiation and other stress conditions. Phytoalexins are thus considered to be general stress-response compounds, produced after biotic or abiotic stress. The most available evidence on the role of phytoalexins shows that disruption of cell membranes is a central factor in their toxicity (Barkai-Golan, 2001) and that the mechanism is consistent with the lipophilic properties of most phytoalexins.

The chemical composition of phytoalexins is elevated. Most important phytoalexins are terpenes and sesquiterpenes. The effects of these sesquiterpenoids – phytoalexins as well as non-phytoalexins were found to be much lower than the effect of the fungicide metalaxyl. In general, phytoalexins are not considered to be as potent as antibiotic compounds (Barkai-Golan, 2001).

An example of induction of phytoalexins by abiotic stress was reported by Kuc' (1972), as cited in Barkai-Golan, 2001) who observed that fruit peeling resulted in browning of the fresh accompanied by enhanced activity of phenylalanine ammonia lyase (PAL). There are indications that PAL activity is connected with productions of phytoalexins and other compounds involved in the defence mechanism of the plant. Radiation is also a cause of phytoalexins production; several studies with citrus fruits have also described γ-irradiation as a stress factor leading to the induction of antifungal phytoalexinic compounds in the treated fruit tissues.

Biosynthesis of toxic compounds as a result of wounding or other stress conditions is a ubiquitous phenomenon in various plant tissues. An example of such a synthesis is the production of the toxic compound 6-methoxymellein in carrot root in response to wounding or to ethylene application (Barkai-Golan, 2001); the application of *Botrytis cinerea* conidia and other fungal spores to the wounded area was found to stimulate the formation of this compound. A similar result is also achieved by the application of fungal produced pectinase, in spite of the fact that this enzyme does not affect cell vitality (Barkai-Golan, 2001). This toxic compound probably has an important role in the resistance of fresh carrots to infection. Carrots that have been stored for a long period at a low temperature lose the ability to produce this compound and, in parallel their susceptibility to pathogen increases. Enhanced resistance of carrots can also be induced by application of dead spores; carrot discs treated with *B. cinerea* spores which had previously been killed by heating developed a market resistance to living spores of the fungus, which was much greater than that of the control discs. The most effective inhibitor found in the tissues after the induction of resistance, as well as in the control tissue, were methoxymellein, *p*-hydroxybenzoic acid and polyacetylene falcarinol (Harding & Heale, 1980).

A sesquiterpenoid compound, rishitin, produced in potato tubers following infection by *Phythophthora infectans*, was first isolated by Tomiyama et al. (1968) from resistant potatoes that have been inoculated with the fungus. Rishitin and solavetivone have also been found to be induced in potato tuber discs 24 h after inoculation with *Fusarium sambucinum*, which causes dry rot in stored potatoes (Ray & Hammerschmidt, 1998, as cited in Barkai-Golan, 2001) and *Erwinia carotovora* (Coxon et al., 1974, as cited in Barkai-Golan, 2001). Other sesquiterpenoids that have been found in potatoes may also play a role in tubers disease resistance; they include rishitinol, lubimin, oxylubimin and others. The terpenoid phituberin was found to be constitutively present in tuber tissues at low levels, but it was further induced after inoculation with *F. sambucinum*. The phytoalexins,

phytuberol and lubimin appeared in potato discs by 48 h after inoculation, while solavetivone was produced in very low quantities. At least eight additional terpenoid compounds were induced in potato tubers in response to inoculation with pathogenic strains of *F. sambucinum* and they appeared 48-70 h after inoculation. Rishitin suppressed mycelial growth of the potato pathogen *Phythophthora infectans* on a defined medium (Engström et al., 1999, as cited in Barkai-Golan, 2001). A similar effect, however, was recorded for the naturally occurring plant sesquiterpenoids abscisic acid, cedrol and farnesol, although these compounds are found in healthy in plant tissue and are not associated with post-infection responses.

Other phytoalexins compounds are next listed: several phytoalexinic compounds, such as umbelliferone, scopoletin and sculetin, are produced in sweet potato roots infected by de fungus *Ceratocistis fimbriata* (Minamikawa et al., 1963). In addition, the resistance of celery petioles to pathogens has been attributed over the years to psoralens, linear furanocoumarins with are considered to be phytoalexins. Another phytoalexin found in celery tissue columbianetin, which probably also plays a more important role than psoralens in celery resistance to decay (Barkai-Golan, 2001). On the other hand, the phytoalexin capsidiol is a sesquiterpenoid compound produced by pepper fruit in response to infection with arrange of fungi.

Benzoic acid is a phytoalexin produced in apples as a result of infection by *Nectria galligena* and other pathogens. This acid has proved to be toxic only as the undissociated molecule and it is expressed only at low pH values such as can be found in unripe apples were the initial development of the fungus was indeed halted. With ripening and the decline in fruit tissue acidity, in conjunction with increasing sugar levels, the benzoic acid is the composed by the pathogen, ultimately to $CO_2$ and the fungus can resume active growth (Swinburne, 1983, as cited in Barkai-Golan, 2001). The elicitor of benzoic acid synthesis was found to be a protease produced by the pathogen (Swinburne, 1975, as cited in Barkai-Golan, 2001). This protease a non-specific elicitor and a number of proteases from several sources may elicit the same response. On the other hand, *Penicillium expansum*, *B. cinerea*, *Sclerotinia fructigena*, *Aspergillus niger*, which do not produce protease in the infected tissue and do not induce the accumulation of benzoic acid, can rot immature fruit (Barkai-Golan, 2001).

Inoculating lemon fruit with *Penicillium digitatum*, the pathogen specific to citrus fruits, results in the accumulation of phytoalexin scoparone (6,7-dimethyloxycoumarin). The induced compound has a greater toxic effect than that of the preformed antifungal compound naturally found in the fruit tissue, such as citral and limetin, as indicated by the inhibition of *P. digitatum* spore germination (Ben-Yehosua et al., 1992). Scoparone production can also be induced in the peel of various citrus fruits by ultraviolet (UV) illumination (Rodov et al., 1992).

Stilbenoids (stilbenes) are other phytoalexins group. They are a group of secondary products of heartwood formation in trees. Plants, especially grapes, can produce resveratrol, that act directly in their defence by inhibiting pathogen proliferation, or indirectly by disrupting chemical signal processes related to growth and development of pathogens or herbivores (Wedge & Camper, 2000). *Trans*-resveratrol (3,5,4′-trihydroxystibene) is one of the simplest stilbenes. It is a product of the plant secondary phenolic metabolism by the action of resveratrol synthase on *p*-coumaroyl-CoA and malonyl-CoA. It occurs in unrelated groups of angiosperms (Morales et al., 2000, as cited in van Baarlen et al., 2004). Besides

*trans*-resveratrol, numerous other stilbenes have been characterized in grapevine. These include a 3-*O*-β-glucoside of resveratrol called piceid that is formed by the action of a glycosyl transferase on reverastrol (van Baarlen et al., 2004) and a dimethylated derivate of reverastrol (3,5-dimethosy-4'hydroxystibene) named pterostilbene. This substance has the highest antifungal activity, but its concentration is less than 5 $\mu g\ g^{-1}$ in leaves and fruit of various grapevine cultivars (van Baarlen et al., 2004). The potency of pterostilbene increases in the presence of glycolic acid and organic acid that accumulates to high concentration in mature grape berries. Pterostilbenes may thus act as constitutive defence component in berries (van Baarlen et al., 2004).

An early study on the antifungal activity of stilbenes revealed that they rapidly inhibit the respiration of fungal cells, probably by acting as uncoupling agents and by forming protein-phenol complexes (Hart, 1981, as cited in van Baarlen et al., 2004). Based on the structural similarity of hydroxystilbenes and aromatic hydrocarbons, it was inferred that their mode of action may involve lipid peroxidation by blocking cytochrome c reductase and monooxygenases (Pezet & Pont, 1995, as cited in van Baarlen et al., 2004).

Analogues of stilbenes, the hydroxystilbenes, are other potent phytoalexins. The most active on fungal respiration were pterostilbene and ε-viniferin with respective $EC_{50}$ values of 20 $\mu g\ mL^{-1}$, and 37 $\mu g\ mL^{-1}$ (van Baarlen et al., 2004).

### 4.4 Pathogenic-related proteins, active oxygen species and lectins

Other substances that are induced by several factors and have antifungal properties are pathogenic-related proteins (PR), active oxygen species (AOS) and lectins. They are briefly described below.

Pathogenic–related proteins (PR proteins) represent a large array of proteins code by the host plants that are co-ordinately expressed under pathological or related situations. They have been characterized in over 70 plant species and 13 plant families including mono- and dicotyledonous plants. They are extremely diverse in terms of enzymatic and biological activity and have been grouped into 13 protein families based on primary structure and serological relationships. They primarily accumulate in plant cell walls and vacuoles. At least, *B. cinerea* infection leads to PR protein induction in many plants (van Baarlen et al., 2004).

Peroxidases are the most important group of PR proteins whose activity has been correlated with plant resistance against pathogens. Plant peroxidases, which are glycoproteins that catalyze the oxidation by peroxide of many organic and inorganic substrates, have been implicated in a wide range of physiological processes, such as ethylene biosynthesis, auxin metabolism, respiration, lignin formation, suberization, growth and senescence.

The importance of peroxidase lies in the fact that the host cell wall constitutes one of the first lines of defence against pathogen and peroxidase is a key enzyme in the world-building processes. Such processes include the accumulation of lignin and phenolic compounds and suberization. However, the resistance against pathogen may also been related to the highly reactive oxygen species such as $H_2O_2$ or oxygenase, which are likely to be toxic to pathogens and which are formed by peroxidase activity during the deposition of cell wall compounds (Goodman & Novacky, 1994).

Several glucanohydrolases found in plants, such as chitinase and β-1,3-glucanase, have received considerable attention as they are considered to play a major role in constitutive and inducible resistance against pathogens (El Ghaouth, 1994, as cited in Barkai-Golan, 2001). These enzymes are low-molecular weight proteins, frequently referred to as pathogenesis related (PR) proteins. They hydrolyze the major components of fungal cell walls which results in the inhibition of fungal growth (Schlumbaum et al., 1986). The chitinases, which are ubiquitous enzymes of bacteria, fungi, plants and animals hydrolyze the β-1,4-linkage between the N-acetylglucosamine residues of chitin, a polyssacharide of the cell wall of many fungi (Neuhaus, 1999). The glucanases, which are abundant, highly regulated enzymes, widely distributed in cell-plant species, are able to catalyze endo-type hydrolytic cleavage of glucosidic linkages in β-1,3-glucans (Barkai-Golan, 2001). The chitinases and β-1,3-glucanases are stimulated by infection and in response to elicitors. It was thus suggested that the deliberate stimulation and activation of PR proteins in the fruit tissue might lead to disease suppression by enhancing host resistance to infection.

Postharvest treatment with this elicitor has been found to activate antifungal hydrolases in several fruits: treatments of strawberries, bell peppers and tomato fruits with chitosan induces the production of hydrolases, which remained elevated for up to 14 days after treatment and reduced lesion development by *Botrytis cinerea* (Barkai-Golan, 2001). When applied as a stem scar treatment to bell peppers, chitosan stimulated the activities of chitinase, chitosanase and β-1,3-glucanase. Being capable of degrading fungal cell walls, this antifungal hydrolases are considered to play a major role in disease resistance (Barkai-Golan, 2001).

Many of several PR protein families display some toxicity towards *B. cinerea in vitro*. For some of them, this may be caused by their potential to degrade chitin and β-glucan fragments of *B. cinerea* cell walls (van Baarlen et al., 2004). A grape PR-like protein (chitinase) has one of the highest botryticidal activities. It inhibits germination of conidia with an $EC_{50}$ value of 7.5 μg $mL^{-1}$ (Derckel et al., 1998, as cited in van Baarlen et al., 2004) and it restricts the elongation of hyphae. Despite their anti-microbial activity *in vitro*, there is little evidence to support a potential role of PR proteins in effective plant disease resistance to *B. cinerea*.

The commercial potential of plants exhibiting higher levels of PR proteins will be hampered by the fact that PR proteins are associated with several undesirable effects such as the formation of haze in grape juices (Waters et al., 1996, as cited in van Baarlen et al., 2004) and allergenic reactions (van Baarlen et al., 2004).

Active oxygen, produced by plant cells during interactions with potential pathogens and in response to elicitors, has recently been suggested to be involved in pathogenesis. In response to pathogens, plants are generally able to mount a spectrum of defence responses, often coinciding with an oxidative burst involving active oxygen species (AOS) that commonly confers resistance to a wide range of pathogens. Active oxygen species, including superoxide, hydrogen peroxide and hydroxyl radical, can affect many cellular processes involved in plant-pathogen interactions (Baker & Orlandi, 1995). The direct antimicrobial effect of active oxygen species has not yet been clarified, but they are considered to play a role in various defence mechanisms, including lignin production, lipid peroxidation, phytoalexin production and hypersensitive responses. However, active oxygen can be difficult to monitor in plant cells because many of the active oxygen species are short lived and are suggest to cellular antioxidant mechanisms such as superoxide dismutases, peroxidases, catalase and other factors (Baker & Orlandi, 1995).

A first report on the production of active oxygen in potato tubers undergoing a hypersensitive response was given by Doke (1983) who demonstrated that $O_2$ production occurred in potato tissues upon inoculation with and incompatible race of *Phytophthora infestans* (i.e.; a race causing a hypersensitive response).

Beno-Moualen & Prusky (2000) found that the level of reactive oxygen species in freshly harvested unripe avocado fruit, which is resistant to infection, was higher than that in the susceptible ripe fruit.

Lectins are a class of sugar-binding proteins that are widely distributed in nature and their occurrence in plants has been known since the end of the 20th century. However, the role of plant lectins is still not well defined and understood (Barkai-Golan, 2001). Now, it is considered that lectins act as recognition determinants in the formation of symbiotic relations between leguminous plants and nitrogen-fixing bacteria and, moreover, they can play a role in the defence of plants against various animals, as well as phytopathogenic fungi (Sharon, 1997), such as *Trichoderma viride*, *Phytophthora citrophthora*, *Geotrichum candidum*, *Botrytis cinerea*, *Furarium moniliforme* and other pathogens (Barkai-Golan, 2001)

## 5. Using plant fungicides for commercial purposes

The optimization of plant natural compounds fungicides against fungal diseases for agriculture is an important research because it would permit to search some important alternatives to the use of synthetic fungicides. At the same time, the study of the role of these compounds that they play in plant metabolism will permit to contribute to the knowledge of plant's metabolism. Most of these compounds present a weak antifungal activity, so additional studies are necessary in order to optimize the use of these compounds as fungicides.

A large number of fungicides are already available to the farmer; the Pesticide Manual (Tomlin, 1994) contains 158 different fungicidal active ingredients in current use. Nevertheless, further industrial research aimed at the discovery and development of new compounds is extremely intensive and this is due to a number of important factors. Firstly, the development of fungicides with novel modes of action remains an important strategy in the search for ways to overcome problems associated with resistance to established products. Secondly, it is becoming increasingly desirable (some would say essential) to replace certain existing products with compounds of lower toxicity to non-target species and acceptable levels of persistence in the environment. Finally, in the increasingly competitive world, agrochemical companies are forced to look for new compounds which show marketable technical advantages over their own and their competitors' products (Clough & Godfrey, 1998).

In principle, fungicidal natural products can either be used as fungicides in their own right, or may be exploited as leads for the design of other novel synthetic materials. In the former approach, purified natural products constitute the active ingredient of a formulated mixture, or are used in mixture with a synthetic material. However, the use of natural products by themselves as fungicides has not been particularly successful for a number of reasons. Firstly, natural products possessing marketable levels of activity against a broad spectrum of commercially important diseases have proved to be very hard to find. Furthermore, they are often inherently unstable (for example, to sunlight) and consequently are not sufficiently

persistent in the field to deliver a useful effect. In addition, some lack selectivity of action and this can manifest itself in the form of toxicity to plants or mammals. Finally, many natural products derived from fermentation broths are present in low concentrations and are difficult to purify on a large scale. Some of these limitations can be overcome by making semisynthetic derivatives, but this inevitably adds to the overall cost (Clough & Godfrey, 1998).

For the reasons above, the agrochemical industry has largely focused on the second approach: the design of novel, fully synthetic compounds from a consideration of the structure of appropriate natural product leads. These synthetic compounds ideally possess optimized biological, physical and environmental properties and are often simpler in structure than their naturally occurring progenitors (Clough and Godfrey, 1998).

In spite of the arguments above expressed and the difficulties to obtain a natural substance which can have antifungal activity and stability at the same time, the researchers are continually searching new substances naturally occurring in nature with antifungal properties so that in the future and after optimization could be used as commercial proposes. Some of these formulates are being already commercialized and some of them are briefly exposed below.

Among these natural compounds, the biocides are extracted from plants and some of them are used as additives in food industry. They present different formulates according to their application mode (Wilson & Wisniewski, 1994). These natural biocides present a wide mode of action and, in general, they are composed by plant extracts, like citrus extracts which are neither toxic nor corrosive. Moreover, they are not irritate and are biodegradable with a good antimicrobial activity and fungicide properties. That is the case of CitroBio, produced in Florida (USA), in which the active ingredient is made from citric seeds. It is only contains 100% citric natural extract with a wide antimicrobial action. Other natural extract is P3-Tsunami which is considered like a product with a high effect against fungi which cause fruit and vegetable decay and also is used to control bacteria growth in cut-produces (Monterde et al., 2002).

Several plant and bacterial natural products have novel applications as plant protectants through the induction of systemic acquired resistance (SAR) processes. Commercial products that appear to induce SAR include Messenger® (EDEN Biosciences, Inc., Bothell, WA) and the bioprotectant fungicides Serenade® (AgraQuest, Davis, CA), Sonata® (AgraQuest, Davis, CA) and Milsana® (KHH BioSci, Inc., Raleigh, NC). Messenger is a harpin protein which switches on natural plant defences in response to bacterial leaf spot and fungal diseases such as *Botrytis* brunch rot and powdery mildew. Serenade is a microbial-protectant derived from *Bacillus subtilis,* with SAR activity that controls *Botrytis*, powdery and downy mildews, early blight and bacterial spot. Sonata is also a microbial-biopesticide with activity against *Botrytis*, downy and powdery mildews, rusts, *Sclerotinia* blight and rots. Milsana® is an extract from *Reynoutria sachalinensis* (giant knotweed) that induces phytoalexins able to confer resistance to powdery mildew and other diseases such as by *Botrytis*. However, elicitors with no innate antifungal activity will not appear active in most current screening high throughput screening systems. Many experimental approaches have been used to screen compounds and extracts from plants and microorganisms in order to discover new antifungal compounds.

Mints oils are well-known antifungal treatments that have been developed as natural fungicides. A mixture of mint oil and citric acid commercially available as Fungastop is a broad spectrum fungicide that reduced postharvest decay of lettuce (Martínez-Romero et al., 2008).

Although new fungicides based on natural plant extracts are continually developing, more research is necessary for optimizing applications and become a safe alternative for eliminating the chemical fungicides from agriculture. Meantime these types of plant fungicides are safe under some conditions and applied together with synthetic fungicides in order to reduce residues in an IPM strategy.

## 6. Acknowledgment

Thanks are due to my wife Olga and my daughter Paula for helping me to write this chapter.

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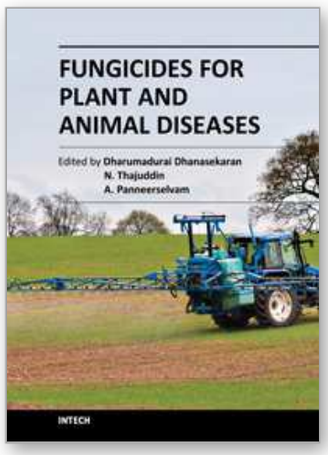


**Fungicides for Plant and Animal Diseases**

Edited by Dr. Dharumadurai Dhanasekaran

ISBN 978-953-307-804-5
Hard cover, 298 pages
**Publisher** InTech
**Published online** 13, January, 2012
**Published in print edition** January, 2012

A fungicide is a chemical pesticide compound that kills or inhibits the growth of fungi. In agriculture, fungicide is used to control fungi that threaten to destroy or compromise crops. Fungicides for Plant and Animal Diseases is a book that has been written to present the most significant advances in disciplines related to fungicides. This book comprises of 14 chapters considering the application of fungicides in the control and management of fungal diseases, which will be very helpful to the undergraduate and postgraduate students, researchers, teachers of microbiology, biotechnology, agriculture and horticulture.

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