



## REVIEW

# Use of Plant Extracts in the Control of Post-Harvest Fungal Rots in Apples

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### ABSTRACT

Different fungi alter apples in the post-harvest period, causing considerable economic losses and risks to consumer health due to the mycotoxins that some of these fungi produce. The control of these fungal alterations in apples is mainly dependent on the use of chemical fungicides, the effectiveness of which has been well proven. However, this use is subject to restrictions due to growing concerns about risks to human health and the environment and the continued development of pathogen resistance to commonly used fungicides. A new approach to control post-harvest fungi has been implemented through the application of plant extract. It is estimated that there are more than 250,000 higher plant species on Earth that can be evaluated for their antimicrobial bioactive chemical compounds. In recent decades, researchers have evaluated plant extracts and essential oils against fungi responsible for post-harvest apple rot. Interesting results have been obtained. The purpose of this project is to summarize and discuss the results of in vitro and in situ experiments of different literatures concerning the effects of compounds derived from plants on the control of fungi responsible for rotting apples in storage.

## 1. Introduction

With a cultivation area of nearly 5 million ha<sup>[90]</sup> and a production of 81 MTn<sup>[60]</sup>, apple growing holds an important share among the fruit sectors in the world.

However, apples are subject to several constraints that hinder their marketing and storage time. Among these constraints are, in the foreground, those of a sanitary and phytosanitary nature that have emerged since the mid-1990s<sup>[60]</sup>. Mainly, post-harvest fungal rots that cause considerable economic losses, up to 25% of the total harvest, even in developed countries, where storage technologies are most advanced<sup>[45]</sup>.

Several species of fungi are responsible for these rots.

They belong to different genera including *Penicillium*, *Botrytis*, *Alternaria*, *Gleosporium*, *Mucor*, *Rhizopus*, *Fusarium*, *Monilinia* and *Aspergillus*<sup>[15,59]</sup>. But the most important losses are caused, mainly, by the following species:- *Botrytis cinerea* agent of grey rot, *Penicillium expansum* agent of blue rot and the group of *Gloeosporioides* agents of lenticella rot. Some of these species also present a potential risk to human health due to the production of mycotoxins. *Penicillium expansum*, for example, can produce several mycotoxins including patulin, citrinin and chaetoglobosins that are carcinogenic<sup>[11]</sup>.

The practice of controlling these different apple rotting agents in the post-harvest period consists essentially in the application of synthetic fungicides in the pre-harvest period or immediately after harvest. However, this control practice

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is currently being challenged because of the emergence of pathogen strains resistant to the main registered active ingredients and also because of the residues that remain in the fruit and that greatly harm human health, not to mention the risk that such a practice presents to the environment.

Faced with all these risks, there was an obligation to look for alternative control practices that were less harmful to human health and more respectful of the environment.

Plant extracts derived mainly from medicinal plants and aromatic plants have thus been implemented as preventive methods for the development of post-harvest rots and have shown encouraging results both in vivo and in vitro<sup>[8,14,70,74]</sup>.

Many reports have approved the effectiveness of natural plant products (extracts and essential oils) in controlling fungal growth and mycotoxin production<sup>[110]</sup>, for example: cinnamon, clove, oregano, palmarosa and citronella oils<sup>[66]</sup>, tea tree oils<sup>[44]</sup>, thyme, cinnamon leaf and anise oils<sup>[25]</sup>.

The use of plant extracts as potential indigenous fungicides or in combination with other control measures is rather promising due to their antifungal activity, low phytotoxicity to the environment, biodegradability, systemic modes of action<sup>[13,97]</sup>. All these attributes make plant extracts a good, safe and effective alternative to systemic fungicides in the control of post-harvest diseases.

Our study consists of a detailed bibliographic analysis on the use of plant extracts in the biocontrol of post-harvest apple rot. More specifically, it aims to shed light on the progress made over the past two decades by examining the extracts used, their nature, their source plants, their target pathogens, their modes of action and their efficacy. The current state of the science on the use of plant extracts in the control of post-harvest fungal rots in apples is elucidated and discussed.

## 2. The Main Post-Harvest Fungal Diseases Of Apples

Apple storage diseases, mainly of fungal origin, are an important cause of apple downgrading after storage. Losses caused by these diseases can exceed 50% in developing countries<sup>[43]</sup>. Considerable losses in apple production occur after harvesting and during storage of fruits, a large part of which, which can exceed a quarter of production, is caused by parasitic diseases of fungal origin<sup>[2]</sup>. Parasitic fungi, penetrate fruits through wounds and/or lenticels and evolve more or less quickly on them, causing their alteration<sup>[23]</sup>.

According to Jijakli<sup>[45]</sup>, the main species of fungi infecting apples in storage are *Botrytis cinerea* Pers. grey rot agent, *Penicillium expansum* Link. blue rot agent and the group of Gloeosporioides lenticular rot agents usually called gloeosporiasis. *Alternaria*, has also been reported as

one of the main agents of apple rot both in the orchard and in storage by Attrassi et al.<sup>[16]</sup>.

### 2.1 *Botrytis cinerea* Pers.

*B. cinerea* is a fungus of the Ascomycota division, Leotiomycetes class, order Eurotiales, Family Sclerotiniaceae. *B. cinerea* is an injury parasite, like *Penicillium*, it contaminates fruits in orchards and fruit stations. In the orchard, *B. cinerea* penetrates the fruit via the senescent petals, there is formation of a dry rot of the eye characterized by browning and drying that can extend into the apple tissue<sup>[63]</sup>. In post-harvest, *B. cinerea* infects fruits through wounds and causes wet rots characterized by alteration of the flesh, with sharp and irregular contours. The flesh acquires a moist or soft consistency and turns a light to medium brown colour on the surface and light brown at depth<sup>[83]</sup>. In wet conditions, a dense grey felt formed by fruiting bodies develops on the surface of infected tissues exposed to light. However, in the dark, an abundant white mycelium appears that can contaminate neighbouring fruits<sup>[23]</sup>.

The fungus is preserved during winter as sclerotia in the soil or in plant debris<sup>[64]</sup>. Sclerotia are not directly infectious but they produce mycelium which will carry conidiophores and conidia<sup>[3]</sup>. The latter are transported in the storage crates via soil debris or any other organic matter. They are also present in the water used to treat fruit before storage.

### 2.2 *Penicillium expansum* Link

*P. expansum* belongs to the division Ascomycota, class Eurotiomycetes, order Eurotiales, family Trichoconaceae. *Penicillium* is a wound parasite. But can also penetrate fruit through lenticels. Infection occurs mainly in fruit stations, with inoculum being largely present in the atmosphere of cold rooms. The disease manifests itself as rots that appear within two months of harvest. This fungus not only causes rotting but can also produce a mycotoxin that is dangerous to human health called patulin<sup>[84]</sup>.

Typical symptoms of rot caused by *P. expansum* are wet lesions that are generally circular in shape with a clean, light brown outline. The infected tissue is soft and juicy, it can be easily detached from healthy tissue. The surface of old rots can be covered by fruiting bodies whose colour varies with their age: young, they are white; adults, they become bluish green, hence the name blue rot, given<sup>[83]</sup> to the disease. As they age, these fruiting bodies become dull and appear greyish. This rot develops rapidly on ripe fruits and is slower on those that have not yet reached climacteric peak. It is generally distinguished by a musty odor<sup>[42]</sup>. *P. expansum* spores can be stored seasonally in storage crates

where the fungus can grow and multiply. Contamination of these by spores can be done by different sources, namely the soil of orchards, infested fruits or via the air<sup>[42,85]</sup>. Spores produced on fruit rot lesions can be spread in storage chambers by refrigeration fans and can contaminate new fruit<sup>[85]</sup>.

### 2.3 The *Alternaria*

*Alternaria* can be a parasite of weakness. Contamination takes place in the orchard. *Alternaria* also causes lentil, eye and heart rots in apples and can be stored on leaves and twigs. Contaminated fruits have irregularly shaped spots, dark brown to black, with sharp contours that evolve slowly. In a humid atmosphere, an olive-brown felt appears on the surface of the fruit<sup>[38]</sup>.

### 2.4 The Group of Gloeosporioides Agents of Gloeosporiosis

Diseases of lenticella spots, commonly known as "gloeosporiosis", are important conservation diseases of pome fruits. This term "gloeosporiosis" includes diseases caused by fungi belonging to the genera *Neofabraea* (formerly *Pezizula*), including *N. alba* and *N. perennans*; *Glomerella*, (its asexual form is called *Colletotrichum*), including *C. acutatum* and *Nectria* or *Neonectria*, whose *N. galligena* and its asexual form called *Cylindrocarpon mali*<sup>[23,39]</sup>.

Infection occurs in the orchard, mainly in the last month before harvest, during rainy periods. But early contamination can also occur from 60 days before harvest. The spores attach themselves to the lenticels and enter the latent phase. Necrosis only appears after 4 or 5 months of storage, when the fruit begins to mature<sup>[39]</sup>.

## 3. Use of Plant Extracts in The Control of Apple Fungal Rots in Post-Harvest Conditions

A significant number of scientific publications address the antimicrobial effects of medicinal and aromatic plants using a variety of organic or green solvents with different extraction techniques, either traditional, modern or green<sup>[4,87,95]</sup>. Studies have shown that some plant-produced secondary metabolites, such as essential oils and volatile compounds, can have a biocidal action against post-harvest pathogens<sup>[30,35,104]</sup>. Volatile aromatic compounds, produced from fruits during maturation such as acetaldehyde, may also have fungicidal or fungistatic activities<sup>[65]</sup>. Much work has been done over the past thirty years to evaluate the antimicrobial effectiveness of various medicinal plant extracts against plant fungi. It has been reported that they play an important role in controlling plant diseases caused by these fungi<sup>[1,47,72,113,80]</sup>.

In 2015, it was possible to identify more than 400,000

plant species, the vast majority of which are flowering plants (369,000 listed species) and each year nearly 2,000 others are discovered<sup>[17]</sup>. These produce a wide range of chemical substances of various structures. Among these substances, a distinction is traditionally made between primary and secondary metabolites.

Plant extracts are preparations obtained, by the extractive action of a suitable solvent, on a plant or part of a plant, most often dry and ground. They contain the plant compounds solubilized by the solvent used. There are different extraction methods, including simple maceration of plant material in water, extraction with organic solvents of different polarities, extraction of supercritical fluids and various distillation methods<sup>[32]</sup>.

Extracts obtained from many plants have recently gained popularity and scientific interest for their antibacterial and antifungal properties<sup>[57,99,86]</sup>.

The use of plant extracts could be a useful alternative to synthetic fungicides in the control of rot fungi when handling fruits and vegetables after harvest<sup>[37]</sup>.

### 3.1 The Active Ingredients of Plant Extracts

Many studies have attributed the antifungal activity of plant extracts to the presence of polyphenols<sup>[88]</sup>. Many results have been reported on the antimicrobial properties of plant extracts containing different classes of phenolic compounds<sup>[7,81]</sup>. Phenolic compounds represent a rich source of biocides and preservatives that have long been explored as an alternative post-harvest control method<sup>[55,89]</sup>. In particular, many studies have shown the antimicrobial efficacy of certain classes of phenolic compounds, such as hydroxybenzoic acid derivatives<sup>[9,37,54,98]</sup>, coumaric acid and caffeic acid derivatives<sup>[50,103,112]</sup>, flavonoids and coumarins<sup>[71,73,87]</sup>, catechin, epicatechin, proanthocyanidins and tannins<sup>[31,34,77,96,107]</sup>. In addition, some authors have studied the relationship between the molecular structure and antimicrobial activity of certain phenolic compounds<sup>[9,21,53]</sup>.

The efficacy of seven phenolic compounds (esculetin, ferulic acid, quercetin, resveratrol, scopoletin, scoparone and umbelliferone) in controlling *P. expansum* growth and patulin production was evaluated by in vitro and in vivo studies by Sanzani et al<sup>[87]</sup>. In vitro screening showed that only quercetin significantly reduced the growth of *P. expansum* 14 days after inoculation (DAI), compared to the control. The remaining compounds showed no effect on fungal growth at either 8 or 14 DAI. Umbelliferone, quercetin and ferulic acid were found to be the most effective compounds to reduce patulin accumulation (34-48%) at 8 DAI, while only umbelliferone significantly reduced patulin accumulation at 14 DAI. The researchers also tested Quercetin and Umbelliferone (alone and combined) on

apples of the Granny Smith and Golden Delicious varieties. The effectiveness of *Penicillium* growth control was better expressed on Golden Delicious apples than on Granny Smith apples, with quercetin providing better control of the incidence of rot and disease severity compared to umbelliferone. But both compounds exert considerable control over the accumulation of patulin on both apple cultivars.

In a study by Sanzani et al.<sup>[87]</sup>, the efficacy of seven phenolic compounds (esculetin, ferulic acid, quercetin, resveratrol, scopoletin, scoparone and umbelliferone), in the control of *P. expansum* growth and patulin accumulation, was evaluated in vitro and in vivo. In vitro screening showed that quercetin and umbelliferone were the most effective compounds for controlling the growth of *P. expansum* and patulin accumulation, respectively. Quercetin and umbelliferone, which have also been shown to be effective in in vivo screening, were tested (alone or in combination) on Granny Smith and Golden Delicious apples. The efficacy in controlling *Penicillium* growth was better expressed on Golden Delicious than on Granny Smith, with quercetin providing better control of the incidence of rot and disease severity compared to umbelliferone. Both compounds exercised considerable control over patulin accumulation on both apple cultivars. Quercetin and umbelliferone can be considered as natural compounds to be used as an alternative strategy to chemical fungicides in post-treatment.

Many research laboratories are currently focusing on the antifungal activity of salicylic acid<sup>[105]</sup>. Salicylic acid is a natural phenolic hormone produced in plants by the route of phenylpropanoids as a signalling molecule that plays an essential role in the defence of plants against a number of fungi and other pathogens<sup>[99]</sup>. The antifungal activity of salicylic acid has been proven against several post-harvest pathogens, including *P. expansum*<sup>[26,27,109]</sup>, *B. cinerea*<sup>[102,111]</sup>, *Fusarium oxysporum*<sup>[61]</sup>, and *R. stolonifer*<sup>[75,79]</sup>. The above-mentioned fungal strains are harmful to storage in apples, but also in other fruits and vegetables.

Da Rocha Neto et al.<sup>[27]</sup> determined the antimicrobial effect of salicylic acid against *P. expansum* both in vitro and in situ. Their study showed that Salicylic acid, at a dose of 2.5 mM, inhibited 100% fungal germination in vitro and also controlled blue mould in situ when applied curatively to apples stored at temperatures of 25°C and 4°C. In addition, this compound preserved the physico-chemical characteristics of apples (weight and soluble solids content) after harvest and was found to be non-persistent in apples by HPLC analysis. Alilou<sup>[6]</sup> studied the effect of extracts from leaves and flowers of *Asteriscus graveolens subsp odorus* in three solvents of increasing polarity: petroleum ether, ethyl acetate and methanol. Photochemical tests of the leaves and flowers of *Asteriscus graveolens subsp.*

*odorus* and *Asteriscus imbricatus* showed the presence of alkaloids, flavonoids, catechic tannins, terpenes, coumarins and cyanogenic compounds. Saponins and quinones were also detected but only in flowers. The author was able to show the presence of caffeic acid, nevadensin, luteolin and artemetin in the leaves of *Asteriscus graveolens subsp. odorus*. Caffeic acid allowed complete inhibition of *P. expansum* at 1000 and 2000 ppm concentrations and *B. cinerea* at all concentrations tested. Flavones (nevadensin, luteolin and artemetin) showed a very significant antifungal effect on the species *P. expansum* and *B. cinerea*. The latter has shown a very high sensitivity to nevadensin. In addition to phenolic compounds, Terpenes and steroids have also been studied for their antifungal activity against deterioration agents in apples during storage. Jasmonates, for example (jasmonic acid and methyl jasmonates), can be used after harvest to improve natural resistance and reduce fruit rot<sup>[97]</sup>.

In a study by Bompeix et al.<sup>[22]</sup>, the use of carvone (mint extract) or eugenol (clove extract) by dipping harvested apples in water controlled the development of pathogenic fungi after harvest on these fruits.

### 3.2 Plant Extracts in Different Solvents

Gatto et al.<sup>[37]</sup> evaluated the in vitro and in vivo activity of extracts of nine wild edible herbaceous species (*Borago officinalis*, *Orobancha crenata*, *Plantago coronopus*, *P. lanceolata*, *Sanguisorba minor*, *Silene vulgaris*, *Sonchus asper*, *Sonchus oleraceus* and *Taraxacum officinale*) against *Botrytis cinerea*, *P. expansum* and other post harvest pathogens. The extracts were made in 80% aqueous methanol from a powder of the plant material. The phenolic composition of all extracts in summer evaluated by HPLC. Several derivatives of caffeic acid, apigenin and luteolin flavones, and kaempferol and quercetin flavonols have been identified. Extracts of *S. minor* and *O. crenata* and *P. coronopus* showed the highest efficacy. *O. Crenata* extract showed a smaller but still significant reduction in conidia germination in the fungi tested. In particular, *S. minor* significantly reduced in vitro the germination of conidia of *B. cinerea* and *P. expansum* (the % inhibition was 93%, 47%, respectively); extracts of both species were also effective in reducing germ tube elongation of these two pathogens even when a slight inhibition of conidial germination was observed (% inhibition reached 93% and 90% for *B. cinerea* and *P. expansum* respectively). These authors also showed a dose effect with an increase in antifungal activity as phenol concentration increased.

Alilou<sup>[6]</sup> studied the effect of extracts from leaves and flowers of *Asteriscus graveolens subsp odorus* in three solvents of increasing polarity: petroleum ether, ethyl acetate and methanol. For leaf extracts of *A. graveolens subsp.*

Odorus, the type of extraction solvent did not have a significant effect on their fungal activity against *P. expansum*. The extracts obtained by the three types of solvents all significantly reduced the growth rate of the fungus compared to the control. Petroleum ether extract is significantly more effective when obtained from flowers. Ethyl acetate and methanol extracts, on the other hand, were rather more effective when they were derived from the leaves.

Embaby et al. [33] studied the effect of propolis ethanol extract (PEE), In Vitro and In Vivo, against *A. alternata*, *A. niger*, *Fusarium sp.* and *P. expansum* rotting agents on apples, showing significant antifungal activity against the linear growth and dry weight of mycelium of all these pathogens and at all concentrations tested with respect to control. Propolis extract also reduced spore viability. These researchers also found that inhibition increased with increasing concentration of extracts. In situ control of apple decomposition with (PEE) revealed that the latter significantly inhibited the growth of rot on apples compared to the control. Lima et al. [58] also reported the inhibitory effect of propolis on post-harvest apple pathogens, *B. cinerea* and *P. expansum*.

Sharma et Raj [92] studied the inhibitory effect of plant extracts and their botanical formulations (BF1 water-based and BF2 cow urine) against *Botryosphaeria dothidea*, responsible for white rot in apple trees (*Malus domestica*) during storage. The technique of poisoned food has been carried out to evaluate the efficacy of different plant extracts and their botanical formulations. *Ocimum sanctum* leaf extract was the most effective of all treatments with an average inhibition of 54.07% of mycelium growth under in vitro conditions against the white rot pathogen. Out of twelve plants evaluated for their efficacy, six effective plants were selected: Karu (*Roylea elegans*), Artemisia (*Artemisia roxburghiana*), Neem (*Azadirachta indica*), Bana (*Vitex negundo*), Tulsi (*Ocimum sanctum*) and Darek (*Melia azedarach*). Among the botanical formulations, BF2 inhibited by 72.70% the mycelial growth of the white rot pathogen and BF1 by 66.37% at a concentration of 100%.

Kalidindi et al. [46] studied the in vitro antifungal properties of hydrosol extract. *Calendula arvensis* and *Calendula caeruleus*. At concentrations of 0.4 mg/L, hydrolate extracts of these two species had more pronounced antifungal activity against *P. expansum* and *P. digitatum* than essential oils. *C. caeruleus* had inhibition percentages greater than 65% for *P. digitatum* and *P. expansum* respectively.

*Annona* is the second largest genus of plants in the family Annonaceae, qualitative phytochemical analysis has revealed the presence of glycosides, flavonoids, phenols, tannins, saponins, alkaloids, carbohydrates and steroids in various extracts. The results of this study revealed that the

leaves of *A. squamosa* have potential antifungal activity against several pathogenic fungal strains among them *A. alternata*. Antifungal activity was determined using the agar well diffusion method. The results indicate that chloroform and methanol extracts have almost similar effects against *A. alternata*, with a percentage inhibition of 79.10% at 1 mg/mL and 83.58% at 2 mg/mL of chloroform extract, and 74.63% at 2 mg/mL of methanol.

Pontes et al. [78] showed that dichloromethane extracts from mature leaves of *Myrcia splendens*, a tree species present in the Brazilian Cerrado, have an inhibitory effect (10.2% after 19 day) on the mycelial growth of *A. alternata*.

In another study, the antifungal properties of hydrolate extracts from the aerial parts of five Asteraceae; *Calendula arvensis*, *Carthamus caeruleus*, *Echinops spinosus*, *Carlina vulgaris* and *Atractylis gummifera*, were tested In vitro against *P. expansum* by Belabbes [20]. The extracts of *C. carvensis* and *C. caeruleus* at a concentration of 0.4 mg/L, had shown very interesting antifungal activity against *P. expansum* by inducing a percentage of mycelial growth inhibition of 65.2% at a volume of 300  $\mu$ L by *C. carvensis* and 56.55% at a volume of 1000  $\mu$ L by *C. caeruleus*. Belabbes also studied the In vivo effect of hydrosol extract from the roots of *C. vulgaris* on the protection of apples against infection by *P. expansum*. The study showed that the severity of the disease caused by *P. expansum* was significantly reduced ( $P \leq 0.05$ ) by this extract, although this reduction was less than that obtained by the EOs from the roots of the same plant.

### 3.3 Essential Oils

The antifungal properties of EOs and their constituents have been the subject of several research studies [12,76]. Most of them are due to the inhibition of fungal mycelial growth in vitro. Several researchers have reported that mono- and sesquiterpenes, as major components of various essential oils, have enormous potential to strongly inhibit the growth of microbial pathogenic fungi. The antifungal activity of these compounds can be attributed to their interference with certain enzymatic reactions developed in cell wall synthesis [97,106].

Post-harvest conditions are, in addition, from a practical point of view, a privileged field of application for the use of EOs insofar as the sites of their application are limited to harvested fruits and environmental conditions, which usually alter the quality of EOs, are well controlled in the storage rooms. One of the successful uses of EOs as effective post-harvest fungicides has been reported in citrus fruits [94].

In the case of post-harvest apples, plant EOs from several families, mainly Lamiaceae and Asteraceae, but also from

other families (Myrtaceae, Rutaceae, Apiaceae Lauraceae, Poaceae, Illiaceae and Brassicaceae), have been tested against the main fungal decay agents and have been found to be effective against these agents (Table).

The table represents the result of our bibliographic research on the use of plant EOs against fungal rot in apples

in storage over the past twenty years. It was possible to list the results of 29 searches. They were classified according to the botanical family of the active EO source, plant species and for each research presented: the source plant, the target pathogen, the major components of EO. The type of tests performed (In vitro or in situ) and the main results obtained.

**Table 1.** Use of plant EO against fungal rots in apples in storage

	EO source plant	Target Agent	Type of test	The major component	Results	References
<b>Family of the Meliaceae</b>	Azadirachta indica	<i>A. alternata</i> , <i>Trichothecium roseum</i> , <i>Monilinia fructigena</i> , <i>Aspergillus niger</i> and <i>P. expansum</i>	In vivo		skin coating with 1% neem oil provided complete control (100%) of all the fungal decays under study.	Kumari et al. [51]
<b>Family of the lamiaceae</b>	• <i>Thymus vulgaris</i> • <i>Satureja montana</i> L	<i>B. cinerea</i>	In vitro In vivo	thymol, carvacrol p-cymène	• <i>thyme its EO at a concentration of 1% showed the highest efficacy</i>	• Banani et al. [19]
	• <i>Mentha pulegium</i> L	<i>A. alternata</i> <i>B. cinerea</i> <i>P. expansum</i>	in vitro	pulégone Menthol	• <i>The concentration of 300 µl/l was sufficient to cause total inhibition</i>	• Hmiri et al. [41]
	• <i>Lavandula Dentata</i> Spp. <i>Dentata</i> • <i>Lavandula Pedunculata</i> Spp. <i>Pedunculata</i>	<i>P. expansum</i> <i>R. stolonifer</i>	in vitro	anthocyanes flavones catéchols C-hétérosides O-hétérosides	• <i>inhibition of P. expansum is observed from 0.5 µl/ml R. stolonifer almost total at the concentration 0.5 µl/ml</i>	• Bachiri et al. [18]
	• <i>Lavandula multifida</i>	<i>Alternaria</i> sp. <i>P. expansum</i> <i>R. stolonifer</i>	In vitro	carvacrol	• <i>MIC was 0.125 µL/mL of air (microatmosphere method) for the three pathogens</i>	• A. Laghchimi et al. [52]
	• <i>Origanum compactum</i>	<i>B. cinerea</i>	In vitro	carvacrol thymol	• <i>inhibition of Botrytis cinerea was 100% at 100 ppm</i>	• Chebli et al. [24]
	• <i>Origanum vulgare</i>	<i>B. cinerea</i> <i>P. expansum</i>	In vitro	carvacrol thymol	• <i>MIC 5.6 µl / L for 5 min of EO treatment combined with hot air flow</i>	• Frankova et al. [36]
	• <i>Melissa officinalis</i>	<i>P. expansum</i> <i>R. stolonifer</i> <i>A. alternata</i> <i>B. cinerea</i>	In vitro	P-mentha1, 2,3-triol P-menth-3-en-8-ol oxyde de pipériténone Zoxyde de pipéritone	• <i>IP100% at 1 µL/mL, while exhibiting strong antifungal activity against B. cinerea, with a PI of 76.81% at 2 µL/mL</i>	• Y. El Ouadi et al., [108]
	• <i>Rosmarinus officinalis</i>	<i>P. expansum</i>	In vitro in vivo	Eucalyptol	• <i>EO at 100 and 1000 µl L-1 reduce the growth, number and viability of P. expansum spores.</i>	• Vieira et al. [101]
	• <i>thymus zygis</i>	<i>B. cinerea</i> <i>P. expansum</i>	In vitro	Thymol o-cymène	<i>Total inhibition</i>	• Gonçalves et al. [40]
	• <i>Thymus danensis</i> • <i>Thymus carmanicus</i>	<i>R. stolonifer</i> <i>B. cinerea</i>	In vivo	le thymol α-terpinene carvacrol p-cymène	• <i>an inhibitory effect 300 µl/L, against B. cinerea and R. stolonifer</i>	• Nabigol et al. [69]
	• <i>Thymus glandulosus</i>	<i>B. cinerea</i>	In vitro	carvacrol thymol	<i>Inhibition of the pathogen was 100% at 100 ppm and the IC50 was 79.2 ppm.</i>	• Chebli et al. [24]

Family of the asteraceae	<ul style="list-style-type: none"> <li>• <i>Asteriscus imbricatus</i></li> <li>• <i>Asteriscus graveolens</i> subsp. <i>Olorus</i></li> </ul>	<ul style="list-style-type: none"> <li><i>P. expansum</i></li> <li><i>B. cinerea</i></li> </ul>	<ul style="list-style-type: none"> <li>In vivo</li> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>thymol isobutyrate</li> <li>2,5-dimethoxy-p-cymène</li> <li>α-pinène</li> <li>cis-chrysanthényle acétal</li> <li>6-oxocyclonerolidol</li> </ul>	<ul style="list-style-type: none"> <li>• fungicidal effect on two fungi at a concentration of 2000 ppm with a IP of the mycelial growth of <i>P. expansum</i> and <i>B. cinerea</i> of 100% and 97.01%, respectively</li> </ul>	<ul style="list-style-type: none"> <li>• Alilou<sup>[6]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>Hélichrysum Italicum</i> d'Algérie</li> </ul>	<ul style="list-style-type: none"> <li><i>B. cinerea</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>Carvacrol</li> <li>linalol</li> </ul>	<ul style="list-style-type: none"> <li>• une complete inhibition of <i>B. cinerea</i> growth</li> </ul>	<ul style="list-style-type: none"> <li>• Romagnoli et al.<sup>[82]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>Pulicaria mauritani-ca coss</i></li> </ul>	<ul style="list-style-type: none"> <li><i>P. expansum</i></li> <li><i>Alternaria sp</i></li> <li><i>R. stolonifer</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>carvotanacetone 13</li> <li>linalol</li> <li>carvacrol16</li> </ul>	<ul style="list-style-type: none"> <li>• Inhibition rate reaches 62.70% for <i>Alternaria sp</i> and 85.52% for <i>P. expansum</i> at 0.25 ul/mL and 100% at 2 uL/mL. <i>R. stolonifer</i> at 40 μL / disc.</li> </ul>	<ul style="list-style-type: none"> <li>• Znini et al.<sup>[114]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>Carthamus Caeruleus</i></li> <li>• <i>Carlina vulgaris</i></li> <li>• <i>Atractylis gummifera</i></li> <li>• <i>Echinops Spinosus</i></li> <li>• <i>Calendula arvenisest</i></li> </ul>	<ul style="list-style-type: none"> <li><i>P.expansum</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> <li>in vivo</li> </ul>	<ul style="list-style-type: none"> <li>l'oxyde de carline ses- quiterpènes</li> </ul>	<ul style="list-style-type: none"> <li>• the concentrations used from 20 to 200 mg/L, had inhibition percentages from 53.33% to 100% in vivo the HE of <i>C. vulgaris</i> used at 0.02 mg/L air, has a protective effect on apples.</li> </ul>	<ul style="list-style-type: none"> <li>• Belabbes<sup>[20]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>Warionia saharae</i></li> </ul>	<ul style="list-style-type: none"> <li><i>Alternaria sp</i></li> <li><i>P. expansum</i></li> <li><i>R.stolonifer</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>β-eudesmol</li> <li>nerolidole</li> <li>linalol</li> </ul>	<ul style="list-style-type: none"> <li>• inhibition of mycelial growth of each strain significantly influenced by the HE concentration of 2 μL/mL of air.</li> </ul>	<ul style="list-style-type: none"> <li>• Znini.M et al.<sup>[114]</sup></li> </ul>
Family of the Rutaceae	<ul style="list-style-type: none"> <li>• <i>Citrus sinensis</i></li> </ul>	<ul style="list-style-type: none"> <li><i>P. expansum</i></li> <li><i>A. alternata</i></li> <li><i>B. cinerea</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>limonène</li> </ul>	<ul style="list-style-type: none"> <li>• Fungicide at 700 ppm and 1000 ppm, the oil was extremely toxic for spore germination.</li> </ul>	<ul style="list-style-type: none"> <li>• Sharma et al.<sup>[93]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>Citrus limon</i></li> <li>• <i>Citrus aurantifolia</i></li> </ul>	<ul style="list-style-type: none"> <li><i>B. cinerea</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>limonène</li> <li>γ-terpinène</li> </ul>	<ul style="list-style-type: none"> <li>• In direct contact method, <i>B. cinerea</i> was completely inhibited at 20°C, a mixture of lemon and lime EO did not inhibit the mycelial growth of <i>B. cinerea</i> at all concentrations tested.</li> </ul>	<ul style="list-style-type: none"> <li>• Mbili et al.<sup>[67]</sup></li> </ul>
Family of the Myrtaceae	<ul style="list-style-type: none"> <li>• <i>Syzigium aromaticum</i></li> </ul>	<ul style="list-style-type: none"> <li><i>P. expansum</i></li> <li><i>B. cinerea</i></li> <li><i>P. vagabunda</i></li> <li><i>M. fructigena</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> <li>In vivo</li> </ul>	<ul style="list-style-type: none"> <li>eugénol</li> </ul>	<ul style="list-style-type: none"> <li>• The mycelial growth of the four pathogens tested was completely inhibited when they were treated with 150 μl of EO at 20°C.</li> </ul>	<ul style="list-style-type: none"> <li>• Amiri et al.<sup>[10]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>Eucalyptus camaldulensis</i></li> </ul>	<ul style="list-style-type: none"> <li><i>A. alternata</i></li> <li><i>P. expansum</i></li> </ul>	<ul style="list-style-type: none"> <li>In vitro</li> </ul>	<ul style="list-style-type: none"> <li>1,8-cinéole</li> <li>α-pinène</li> </ul>	<ul style="list-style-type: none"> <li>• It took 30 μl of HE to completely inhibit the mycelial growth of <i>A. alternata</i> and <i>P. expansum</i>.</li> </ul>	<ul style="list-style-type: none"> <li>• Hmiri et al.<sup>[41]</sup></li> </ul>
	<ul style="list-style-type: none"> <li>• <i>melaleuca alternifolia</i></li> </ul>	<ul style="list-style-type: none"> <li><i>P. expansum</i></li> </ul>	<ul style="list-style-type: none"> <li>in vitro</li> </ul>	<ul style="list-style-type: none"> <li>terpinène-4-ol</li> </ul>	<ul style="list-style-type: none"> <li>• Total inhibition of in vitro germination of <i>P. expansum</i>, even at a lower concentration of 0.125 or 0.25 g L-1.</li> </ul>	<ul style="list-style-type: none"> <li>• da Rocha Neto et al.<sup>[28,29]</sup></li> </ul>

Family of the apiaceae	• <i>Ammodaucus leucotrichus</i> Coss et Dur	<i>B. cinerea</i> <i>P. expansum</i> <i>R. stolonifer</i>	in vitro	perillaldéhyde	• IP reached 65.23% for <i>B. cinerea</i> and 55.22% for <i>P. expansum</i> at 0.125 µL/mL, and a PI of 100% was observed at 0.5 µL/mL, <i>R. stolonifer</i> was totally inhibited at 0.25 µL/mL air.	• Mansouri et al. [62]
	• <i>Apium graveolens</i> L	<i>B. cinerea</i>	in vitro	limonène β-sélinène	• completely inhibit the mycelial growth of the fungal strain.	• Lboumhamdi et al. [56]
Family of the lauraceae	• <i>Cinnamomum zeyla-cium ou verum</i>	<i>P. expansum</i>	in vivo in vitro	eugénol	• EO at 100 and 1000 µl L-1 reduces the growth, number and viability of <i>P. expansum</i> spores 24 hours after germination initiation.	• Vieira et al., 2018 [101]
Family of the poaceae	• <i>Cymbopogon winterianus ou citratus</i>	<i>B. cinerea</i>	in vitro	citronellal neral	• the mycelial growth of <i>B. cinerea</i> was completely inhibited at 20 °C, in a regular atmosphere.	• Mbili et al., 2015 [67]
	• <i>Cymbopogon martini</i>	<i>P. expansum</i>	in vitro	géraniol géranium néral myrcène	• inhibited the in vitro germination of <i>P. expansum</i> , even at a lower concentration of 0.125 or 0.25 g L-1	• da Rocha Neto et al. [28]
Family of the illiaceae	• <i>Illicium verum</i>	<i>P. expansum</i>	in vitro	L'anéthol Limonène	• in vitro of <i>P. expansum</i> , inhibited even at a lower concentration of 0.125 or 0.25 g L-1	• da Rocha Neto et al. [29]
Family of the Brassicaceae	• <i>Brassica nigra</i>	<i>B. cinerea</i>	in vivo in vitro	isothiocyanate d'allyle	• the minimum inhibitory concentration (MIC) in vitro and in vivo of mustard essential oil was 15.42 µl / Lair	• Aguilar-González et al. [5]

**Abbreviations** : PI: pourcentage inhibition; EO: essentiel oil; MCI: the minimum inhibitory concentration

The analysis of this table allowed us to make the following observations :

(1) A significant part of this research (12 out of 29) was carried out by Moroccan researchers. This can be explained by, among other things : (A) Morocco's status in the international apple market as it is one of the major producers of this fruit and (B) by the fact that Morocco has a large reservoir of medicinal and aromatic plants.

(2) 43 plant species from 9 different families were tested for the effectiveness of their EOs against apple rot agents in storage.

(3) Scientific research has mainly concerned plants of the Lamiaceae family (6 genera, 14 species and 10 scientific publications) and that of Asteraceae (11 genera, 15 species and 7 scientific publications)

(4) 50% of the Lamiaceae species included in our study belong to the genus *Thymus*, which ranks first among all the species used in these studies.

(5) The target species against which the EOs have been tested are dominated by the species *P. expansum* (tested 21 times) followed by *B. cinerea* (15 times) then *R. stolonifer* (7 times) and the species *Alternaria*, *Alternaria* sp and *A. alternata* (8 times) and finally *N. alba* (1 time).

(6) Tests on the antifungal activity of EOs against apple

rot are mostly performed in vitro. Of the 30 scientific studies involved in our study, only 8 have tested EOs in situ on stored apples.

(7) Thymol and carvacrol are the major active components of most of the HEs involved in our study.

(8) The results of scientific research conducted over the past 20 years, although variable from one study to another, clearly show that plant EOs are effective against post-harvest apple fungal rot agents. Some EOs totally inhibit mycelial growth and pathogen spore germination at low concentrations and significantly reduce lesions on apples treated with these EOs.

#### 4. Discussion and Conclusion

In search of other alternatives to chemical control in the control of plant pathogenic fungi, a new biological control method was developed in the 1980s, consisting of the application of natural plant products and since then and until now, several studies have been carried out on plant extracts and their effectiveness in controlling fungal plant diseases. All these studies have shown the effectiveness of these products in the management of plant fungal diseases and their ability to be safely incorporated as appropriate alternatives to synthetic fungicides.



Over the past two decades, this new biological control method has been tested by several researchers for its effectiveness in biocontrol of apple fungal rots in post-harvest conditions. The results obtained were interesting and very encouraging. The aim of our project was to establish a state of the art in science on this subject. The analysis of the results of this listed scientific research allowed us to draw the following conclusions:

(1) In all the research studies included in our project, plant extracts (essential oils and solvent extracts) have shown great efficacy against the main post-harvest apple rot agents by expressing fungicidal or fungistatic activity against them. Some extracts have allowed a total inhibition of the growth of pathogenic fungi, such as the EO of *W. saharae* against *Alternaria* sp<sup>[114]</sup>; the EO of *Asteriscus imbricatus* against *P. expansum*<sup>[6]</sup>, *Melissa officinalis* EO against *P. expansum*<sup>[108]</sup> and salicylic acid against *P. expansum*<sup>[27]</sup>. The latter not only protected the apples from rotting agents but was also able to preserve important physiological characteristics of the fruit and extended the shelf life of the treated apples.

(2) Many reports have approved the effectiveness of natural plant products in controlling the production of mycotoxins, for example: cinnamon, clove, oregano, palmarosa and citronella oils<sup>[66]</sup>, tea tree oils<sup>[44]</sup>, thyme, cinnamon leaf and anise oils<sup>[25]</sup>, sweet basil, neem, eucalyptus, datura, garlic and oleander extracts (Nashwa et Abo-Elyousr, 2012).

(3) The most important part of the studies carried out on the use of plant extracts against apple fungal decay agents has been dedicated to essential oils. Although the latter only concern 10% of plants, known as "aromatic" plants (Bruneton, 1999; Degryse et al., 2008). This is certainly due to the functional and physico-chemical biological qualities of these compounds.

(4) Most of the work carried out has been carried out in vitro and little work has been done on the in situ effects of plant extracts, particularly EOs, on fruits after harvesting.

(5) In the few studies where extracts were tested in situ, a minor effect was generally observed, especially with EOs indicating the existence of different interactions of the latter with the environment. By virtue of their biological, functional and physico-chemical properties, essential oils are chemically unstable and susceptible to degradation. Their composition can easily be modified as a result of oxidation, chemical interactions or volatilization. One of the ways to counter these problems is to microencapsulate these oils before use. This, has been tested by Kupaei et Garmakhany (2014) on mangoes in conservation and the results are very satisfactory: The microencapsulation of EOs has not only improved their chemical,

oxidative and thermal stability but has also increased their shelf life, biological activity, functional activity, controlled release, physicochemical properties and overall quality.

(6) Most of the work has not gone beyond the stage of tests carried out in vitro and/or in situ on the antifungal activity of these plant extracts against post-harvest apple rot agents. This type of test is, in fact, only the first link in a long chain leading to the development of biopesticide in formulations that are appropriate, safe for human health and the environment, and can be marketed.

(7) It is very difficult to compare the results of all the work done on the control of apple fungal rots by plant extracts or to reproduce them because several factors influence the results of this work: (A) the technique used in the tests (by poisonous foods or by microatmosphere<sup>[20,28,29,33,114]</sup>); (B) the type of solvent used and its extraction capacity<sup>[6]</sup>; (C) the dose of the extract or EO<sup>[20,28,29,33,114]</sup>; (D) the synergy between the components of the EO or extract<sup>[29,114]</sup>, knowing that this composition very variable according to the origin of HE or the extract<sup>[48,68]</sup> and depending on the plant organ uses the physiological stage of the plant and many other factors; (E). The target pathogen, (F) the treated apple (variety, maturity, etc.); (G) packaging conditions and many other factors<sup>[6]</sup>.

(8) Few researchers have investigated the effects of EOs on the physiology of treated apples and the interaction of these EOs with packaging materials.

(9) The mechanisms of action of plant extracts and EOs against post-harvest apple rot agents are not fully understood. Given that plant extracts are made up of a mixture of different compounds, it is easier to speculate that the antifungal activity should be the result of a plethora of possible modes of action. In the majority of the studies carried out, the researchers did not identify the mode of action of plant extracts and EOs on the agents tested.

(10) In the studies conducted on EOs, the majority of authors did not identify the molecule responsible for the fungicidal effect they only speculated that the majority component of each EO was the active ingredient responsible for this fungicidal effect.

(11) The majority of plant extracts studied for their fungicidal effect have not been marketed. Their commercial application can facilitate the overall management of post-harvest rot and minimize risks to human health due to the high use of chemical compounds by the apple industry.

(12) Some post-harvest apple rotting agents may have developed resistance to some plant extracts, *B. cinerea* has been able to develop resistance to  $\alpha$ -tomatine a fungicidally active plant extract according to Verhoeff et Liem<sup>[100]</sup>.

(13) There are no studies that show the effect of these

extracts on apples in the long term.

(14) The majority of post-harvest apple rot agents that were tested by the authors were not extracted from apples.

(15) Very few studies have tested the combined use of plant extracts and EOs with other biological control methods or the effect of a mixture of several types of extracts things that would be of great interest.

In conclusion, it should be noted that coordinated and continuous research into natural products can provide a safer alternative for the control of post-harvest apple rot. Research efforts should aim to establish the mode of action of products in such a way as to provide important indications for their application. Natural products that have been shown to be effective in in vitro and in vivo studies should be considered for commercial application. Emphasis should be placed on the development of products that can be easily used by end users. Therefore, it seems that more research is needed to formulate botanical products for commercial purposes as bio-fungicides against post-harvest apple diseases.

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