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# ECO-FRIENDLY MANAGEMENT OF POSTHARVEST DISEASES OF APPLE FRUITS: AN OVERVIEW

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***Abstract***

*Apples (Malus × domestica Borkh.) are the most economically important fruits over the world due to their nutritional and bioactive constituents, with significant value for human healthy. Maintaining these attributes during the post-harvest period is a major concern of all those involved in the field, including researchers. In the present synthesis, the results obtained in the last period of time are reviewed, regarding the sustainable management of post-harvest apples diseases, considering the eco-friendly approaches applied both in the pre harvest period and after the fruit harvest. Promising results were obtained regarding: (i) improving plants growing environmental conditions and pre harvest practices using fungicides and the application of complementary, environmentally friendly measures; (ii) exploring the epiphytic and endophytic flora with a view to some microorganisms exploitation as biological control agents; (iii) nanomaterials using and of plant extracts, as well as of some resistance chemical inducers; (iv) genetic engineering, omics-based approaches and machine learning algorithms. With a view to the successful implementation of the most appropriate innovative strategies for apples fruits diseases post-harvest management it is necessary to continue such studies through a close collaboration between researchers and practitioners.*

***Key words***: *apples, post-harvest, pathogens, eco-friendly control*

## INTRODUCTION

Apples (*Malus* × *domestica* Borkh.) are the most economically important fruits over the world due to their nutritional and bioactive constituents with significant value for human healthy [57]. Therefore, the maintaining of these attributes during storage and the entire supply chain are challenges of interest for all implicated actors [54]. In a close link with several Sustainable Development Goals of the 2030 Agenda, United Nation General Assembly designated 2021 the International Year of Fruits and Vegetables [24].

It is estimated that about 45% to 55% of global fruit and vegetable production is lost, respectively [59]. Post-harvest losses of apples can be caused by many pre harvest and post harvest factors. From the first category can be mentioned: environmental factors variation during the growing period, harvest time and handling practices, qualitative indicators of the fruit, fruit health related to crop protection programs, calcium content, volatile terpenes (such as α- farnesane production) etc. [75, 30, 41, 72, 69, 12, 9, 29, 33]. Not unimportant are also the post-harvest practices, including various treatments based on fungicides to control pathogens [61, 41], which sometimes do not have effectiveness due to the appearance of fungicide resistance [78].

As [7] reviewed, in addition to the quantitative losses, there are also qualitative ones caused by the installation of fungi. From the economically point of view, *Penicillium expansum* determines the most significant post-harvest disease on fruits. It macerates the host tissue, with simultaneously secretion of toxic substances (e.g. D-gluconic acid and mycotoxins, as is patulin). *Alternaria* toxins have been identified as well, both in the case of fresh fruits and in products derived from them [58]. Therefore, specific, and rapid fungus detection, such as loop-mediated isothermal amplification (LAMP) assay developed by [25] before storage, as well as the optimization of apple storage conditions proved to be the ways to reduce the mycotoxins accumulation [7, 83, 4].

During post-harvest period various pathogens, have been identified and studied in detail and different means of their control have been (and are) applied [12]. Early detection of possible new pathogens, novel knowledge on fruit-fungus interaction and development new tools to do this are on researcher's agenda [53, 41]. Thus, the behavior of infected fruits with *Neofabraea* spp. and *Cadophora* spp. (identified by TaqMan PCR assays) during the growing season and their subsequent evolution after several months of storage, when symptoms occurred is absolutely necessary, in order to develop measures to prevent the risks of infection during the growing season [41]. It can be mentioned that *Neofabraea* spp. was also identified in Romania, in the case of post-harvest advanced cold storage [14].

In the Nordic countries, the increase of the demands for locally organic fruits imposed the need to consider adaptation to climatic conditions and disease resistance/tolerance, as the principal issues for apple breeding programs [55]. Alternative control approaches are also needed [19], including those that directed to reduce the losses in intelligent agriculture [62]. Additionally, the biological control, the bio-preservation approaches, and meta-omics techniques with a view to emerge new strategies to increase the shelf-life of fruits and vegetables are needed [46]. In the same vein, [15] noted that "the study of plants with traditional uses as - plant protectors - is essential for understand more about the inner value of flora" is still valid today, more than ever.

In view of the above, the purpose of this synthesis is to provide an overview of the concerns in recent years for the control of post-harvest apple fruits diseases, based on environmentally friendly, safe, and sustainable tools, such as:

(i) improving plants growing environmental conditions and pre harvest practices by the application of complementary, environmentally friendly measures;

(ii) exploring the epiphytic and endophytic flora with a view to some microorganisms' exploitation as biological control agents;

(iii) nanomaterials using and of plant extracts, as well as of some resistance chemical inducers;

(iv) genetic engineering, omics-based approaches and machine learning (ML) algorithms.

## MATERIALS AND METHODS

In order to achieve the proposed objectives and produce a critical review approach that meets the readers' expectations, we proceeded to an exhaustive search process based on relevant key words or phrases, as regard as the research issue (e.g. "eco-friendly pathogens control", "apples postharvest pathogens management", "biocontrol agents", "epiphytic flora", "endophytes", "nanomaterials"), to access appropriate and actually relevant articles.

Clarivate Analytics databases (Web of Science; Science Direct Freedom Collection, Elsevier; Scopus, Elsevier; CAB Abstract) have been accessed, also, an advanced search on Google has been done. With a view to include or exclude criteria, the search filters available in the platform were used.

The information organization was carried out on the basis of the most current results of scientific research, which can offer new study topics in the future, as well as possibilities for implementation in practice, in accordance with current trends concerning the new theoretical frameworks and emerging perspectives, which revolve around the sustainable agriculture general concept.

## RESULTS AND DISCUSSIONS

### Improving plants growing environmental conditions and pre harvest practices by the application of complementary, environmentally friendly measures

The post-harvest quality of apples and increased tolerance to the attack of pathogens depend, among other factors, on environmental conditions (such as rainfall, air humidity and temperature) at certain phenophases of fruit evolution [9], as well as on management of tree health and vigor during orchard establishment (especially with regard to soil-borne pathogens) [31]. As a matter of fact, climate change itself impacts the physiology of the response to different pathogens and pests [3]. Global warming has also contributed to the emergence of new pathogens, such as *Colletotrichum acutatum*, which causes apple anthracnose and whose incidence is increasing [29].

Pre harvest practices designed to ensure improved conditions (e.g. pruning, nutrition, irrigation or drainage) are of interest, as [9] showed. High rainfall during flowering and early fruit development were correlated with improved fruits quality. Also, a humidity higher than 77% at the beginning of June led to the obtaining of more tolerant fruits at the attack of the fungus *Botrytis cinerea*. Moreover, harvesting at the right maturity level and proper post-harvest handling reduced the incidence of bruising, while storage of "Golden Delicious" apples by 93.3%, to 37.7% in the absence of treatment (dipping in calcium and fungicide). In addition, no symptoms of rot were detected [72].

In the case of organic orchards, attention was paid also to pre harvest practices (orchard floor management, summer pruning), establishing representative indices for fruit maturity (such as starch hydrolysis), as well as the use of natural products (such as ethanol), for apples phytosanitary protection during storage [71]. Besides to the specific effects of differently colored selective films [ChromatiNets (R)] on physiological processes in the plant canopy, [65] also highlighted their impact on pests and diseases. Also, [12] pointed out that anti-hail photo selective netting provides apples protection against key emerging pests (such as *Halyomorpha halys* and *Drosophila suzukii*), It has a beneficial effect on arthropods, protects against pathogenic fungi, and positively influences fruit quality.

Although the number of new diseases in apples has increased, treatment technologies have been directed towards reducing the number of sprays during the growing season, towards the application of complementary, environmentally friendly measures such as the use of organic fungicides, then systemic or therapeutic ergosterol biosynthesis inhibitors (EBI) [73], as well as of some physical strategies [12]. But, still more than 10 years ago, [73] specified that in order to make control decisions well-founded from the point of view of environmental safety, as well as from the economic viewpoint, it is necessary to integrate, correlate, transmit information on the evolution of the pathogen, weather monitoring and disease forecasting. As [77] stated, a more accurate prediction of the severity for rot pathogen *Neofabraea perennans*, as well as the recently discovered minor rot *Phacidiopycnis washingtonensis*, need to be carried out. A more targeted application of pre harvest fungicides, as well as post-harvest physical treatments are of interest, too.

Studies highlighted the growth inhibitory effect on *B. cinerea* fungus when 'Fuji' apples were dipped in 300 μg/mL of chlorogenic acid, for 30 minutes and the authors indicated its role in preventing gray mold disease [77]. This effect was due to the increase in enzymatic activity relative to phenol metabolism, as well as increasing the content of total phenols, flavonoids and lignin.

Another phenolic compound that has been shown to be effective *in vitro* against *B. cinerea* is phlorizin [32]. In a dose of 1.0 g/L it significantly reduced the incidence of the disease and inhibited the lesions diameter spread. The application of a combined treatment (dipping in a solution of 5% calcium chloride $CaCl_2$+5 mM salicylic acid - SA for 10 min) has been shown to be effective in inhibiting the fungus *Colletotrichum gloeosporioides* [82]. In addition, it was noticed a preservation of the quality by reducing the decline in the total content of soluble substances, titratable acidity and firmness.

In order to reduce losses during the storage period, one of the primary conditions is the direction to store only healthy fruits. Otherwise, in the case of storage in a controlled atmosphere, the ability to suppress diseases is reduced. If we talk about traditional storage, the fruits are almost completely destroyed. In Romania, [35] noticed that after four months of organic fruits storage in cold conditions ($1^0$ C, 90% humidity), without any post-harvest treatment, the fruits damages were caused mainly by fungal pathogens (such as *Gloeosporium* sp. originated from field) and wounds infections caused by *Penicillium* sp. and *Fusarium* sp.

Last but not least, the development of molecular methods for the early detection of the most important pathogens and a precise diagnosis of apples diseases may be key factors. To these depends to make a correct decision with a view to assure an economic and sustainable growth of farmers, storage owners and managers [74, 68].

**Exploring the epiphytic and endophytic flora with a view to exploitation of some microorganisms as biological control agents**

In addition to the initial use of synthetic fungicides, which have been proven to cause some inconvenience, later on, researchers in the field resorted to the development of alternative methods, such as compounds generally recognized as safe (GRAS) [60], or so named good agricultural practices (GAP) [66]. Application of environment-friendly natural compounds (plants extracts, essential oils, and active compounds or secondary metabolites) [36] and using of antagonistic bacterial and fungal microorganisms are viable alternatives, along to refrigeration [80, 18, 10, 22, 84, 81].

The effectiveness of biological control methods was also demonstrated. Exploring fruit microbiomes and their association with their hosts have been addressed [8], when the yeast *Metschnikowia fructicola* was used as bio control agent during apples cold storage. It was shown that the yeast persisted in high abundance (>28% relative abundance) on the fruit surface and significantly reduced the richness and fungal microbiome, as regard as its composition and structure, relative to the control. Fungal pathogens (such as *Alternaria*, *Aspergillus*, *Comoclatris*, *Stemphylium*, *Nigrospora*, *Penicillium*, and *Podosphaera*) have been reduced as presence. Nowadays, DNA metabarcoding approach to explore the fungal and bacterial epiphytic microbiota changes is carried out [4, 5]. Also, the mechanism of action [11, 22] and effectiveness [38, 39] were analyzed. There was also used the DNA metabarcoding approach and characterized the fungal and bacterial community in three/four-year-old shoots (old bark) or one-year-old shoots (young bark) of 'Golden Delicious' and 'Gala' cultivars [5]. It was highlighted the microbiota dependence on apple tree age and genotype.

Besides, research are focused on microorganisms' isolation, their mechanisms of action, the application methods, the efficacy enhancement, products formulation and commercialization of biological control agents (BCAs) designed for post harvest control of fungal diseases of fruits and vegetables [10, 22].The characterization of the microbiome in *Malus triloba* [39] and its exploitation as a source of new BCAs on pathogens such as *B.cinerea* and *P.expansum* allowed the identification and molecular characterization of some genera (e.g. *Bosea*, *Microlunatus*, *Microbacterium*, *Mycetecola*, *Rhizobium* and *Paraphoma)*. Also, from 237 screened strains, 92 inhibited *P.expansum* (39%) and 87 strains inhibited *B.cinerea* (38%). Such results can be used in the future to develop new post-harvest formulations. Exploitation of the indigenous microbiota of cider-apples cv. "Bedan" conducted to promising results [4]. It exerts not only antifungal effect, but also anti-patulin activity in relation with *P.expansum*.

Lactic acid bacteria (e.g. *Lactobacillus plantarum* DSM 20174) has been shown to have antimicrobial activity against pathogens (*A. flavus*, *C. acutatum*, *C. gloeosporioides*, and *Fusarium avenaceum*), both *in vitro* and *in situ*, with a stronger inhibitory effect on spore germination (89.62%–97.61%), than on mycelia growth. *In situ,* necrosis inhibition ranged from 42.54% for *C. acutatum* to 54.47% for *A. flavus* [84]. Studies performed by [18] regarding the antagonistic effects of mycobiota of apple fruits (forty-nine isolates) against *C. acutatum* that cause bitter rot on fruit emphasized that eight isolates inhibited growth of *C. acutatum* by more than 50%, and 6 out of 8 fungal isolates prevented disease developing on the inoculated apples. *Pestalotiopsis guepinii* was not able to control bitter rot effectively. But, still an inoculation at 4x10(6) conidia/mL, together with a 0.1-mL conidial suspension of *C. acutatum* (1x10(6) conidia/mL) inhibited bitter rot by 39.5%.

Regarding the mode of action of *Rhodotorula glutinis*, [47] highlighted and explained the ability of the yeast to attach to spores and hyphae of *B. cinerea*, due to some protein components located on the yeast cell surface and which might contain glycosylation modification. In addition, [64] showed the inhibitory effect on *P.expansum* of three antagonistic yeasts (Y33, Y29 and Y24) of *Metschnikowia pulcherrima*, as well as their patulin degradation capacity. The most effective was proven to be Y29. Exploitation of epiphytic microflora characteristics was also demonstrated by [38] following the selection of 60 yeasts, of which 10 were tested *in vitro* against *B. cinerea* fungus. The isolates antagonist action properties were tested *in vivo*, too. Three of them (L7 of *Aureobasidium pullulans*, L2 of *Citeromyces matritensis*, and L10 of *Cryptococcus flavescens*) proved to be effective as potential biological control agents.

As early as about 10 years ago, [43] emphasized the urgent need to develop appropriate formulations when using BCAs during the pre harvest period, based on the results of their own studies using the yeast *Pichia anomala* strain K, as an antagonist against the fungus *P. expansum*. A density threshold of $1\times10^4$ cfu $cm^{-2}$ of strain K on the apple surface seemed to be required just after harvest, for high protective activity, whatever the method and time of application. Pre harvest biological treatments may have remarkably effects on strain K population density and its efficacy, according to the variations in meteorological conditions. Later on, the same author [44] by *in vitro* and a semi-commercial large-scale trials demonstrated the antagonistic activity of some bacterial strains (*tAlcaligenes*, *Bacillus*, *Brevibacteriwn*, *Pantoea*, *Pseudomonas*, and *Serratia*) to control the brown rot caused by *Monilinia fructigena*, and *M. laxa*. The efficacy of these antagonists (ACBC1, SF14, SP10 and ACBP1) capable of producing lytic enzymes and lipopeptides was comparable to that of commercial products (e.g. *B. subtilis* Y1336 and *P. agglomerans* P10c), but slightly lower than that of the thiophanate-methyl fungicide.

In addition to these approaches, [70] directed research including the genetic characterization of BCAs. In the case of the *Candida oleophila* I - 182 yeast, a genome similar in size to that of the model yeast strain *Saccharomyces cerevisiae* S288c was shown to be present. Authors stated that such results may contribute to a better understanding of the properties of bio control at the molecular level. According to the results of [10] in the fruits treated with *C.oleophila* incidence of *P.expansum* and the diameter of the lesions were significantly reduced. A rapidly colonizing of apple peels and an increasing of the activity of some enzymes induced the disease resistance. Because freeze-drying is widely used for the preservation of microorganisms, [11] considered different possibilities for optimizing cryoprotective components for *C. oleophila*. The optimal formulation was represented by 15% trehalose, 2% sodium glutamate, and 10% skim milk powder, when a survival rate of 69.7% has been obtained. The effect on blue mold has been promising, too.

Promising results as BCAs of some plant root-associated rhizobacteria (RAB) have been also obtained [52, 40]. RAB isolated from legumes (such as *Pseudomonas* and *Serratia*) suppressed the growth of *P.expansum*, *B.cinerea* and *Mucor piriformis* during apples cold storage [52]. By studies performed *in vitro*, but also *in vivo* by using the antagonistic rhizobacterium *Paenibacillus polymyxa* (APEC128), against anthracnose caused by *Colletotrichum gloeosporioides* and *C. acutatum* it was emphasized that a suspension concentration of APEC128 ($1\times10^8$ colony forming units (cfu)/mL) assured a diseases suppression (by 83.6% and 79%, respectively) [40]. This effect has been explained by an increasing of protease and amylase, which might inhibit mycelial growth. Further, based on studies carried out by [44] on the ability of bacteria isolated from natural soil to produce lytic enzymes (amylase, cellulase and protease), hydrocyanic acid (HCN) and lipopeptides (bacillomycin, fengycin, iturin and surfactin) two bacterial isolates (*Bacillus amyloliquefaciens* B10W10 and *Pseudomonas* sp. B11W11) have been found to be most effective. These reduced the brown rot incidence caused by *M. fructigena*, as against to the synthetic fungicide, in a semi-commercial large-scale trial. It has also been shown that fengycin is a primary active compound of *B. amyloliquefaciens* against a broad range of foodborne pathogenic microorganisms [49].

The beneficial effects of the *Botryosphaeria dothidea fungus* control by endophytic bacterium *Bacillus velezensis* (strain P2-1) have also been recently demonstrated [81], without significantly affecting fruits qualitative characteristics. Its action is due to the ability to synthesize of antifungal lipopeptides and polyketides, as well as to enhance the expression levels of pathogenesis-related genes (*MdPR1* and *MdPR5*).To these effects is added the ability to be effective against *B. dothidea* fungicide-resistant forms.

**Nanomaterials using and of plant extracts, as well as of some resistance chemical inducers**

The use of nanotechnology within the agricultural system has proven to be beneficial and has led to an increase in agricultural yield. In particular, nano-phytopathology represents a new era in the early detection of plant pathogens, in monitoring the pathogens populations, as well as their interaction with the host plant. The transfer of genetic material between the pathogen and the host can be also done [2].

Nanoparticles (NPs) can assure a control of post-harvest decays in the case of various fruits, including apples [60]. In the case of climacteric fruits (such as apple fruits), beside zinc, silver and chitosan, the most effective to significantly delay ripening by reducing weight, moisture and fruit firmness losses were zinc NPs. Also, by their biopolymer-like features, a superior antibacterial, antifungal, and antiviral properties have been emphasized, as against the edible coatings [56].

It was recommended the introduction into the *P.expansum* management programs of chitosan NPs [1]. The exogenous applied as NPs or bulk form induced in apple (cv.Anna) a strong systemic acquired resistance (SAR) against the fungus. An over expression of the studied defense-associated genes (chitinase, peroxidase, β-1.3-glucanase, xyloglucan endotransglycosylase, pathogenesis-related protein - PR8, and phenylalanine ammonia lyase-1) was noticed. A potent, economical and environmentally friendly measure regarding the use of nano preservatives proved to be the use of green synthesis of nano silver (AgNPs) using black tea extract [50]. This prevented post-harvest damage caused by *M. fructigena* and increased the apple fruits shelf life. No adverse effects were detected to human health. In the case of the application of polyphenols (exposed to rapid decomposition under normal conditions of temperature, oxygen and light), their encapsulation or intercalation into NPs has proven effective. There was tested the antifungal effect on blue mold of the polyphenols of pomegranate fruit peel into silica NPs [6]. The inhibitory action was significantly higher as compared to NPs and extract alone, in agar well diffusion method. Moreover, the stability of the plant extract was improved.

Some authors remark that "the study of plants with traditional uses as - plant protectors - is essential for understand more about the inner value of flora" is still valid today, more than ever [15]. The use of essential organic oils (such as those extracted from *Thymus vulgaris*, *Lavandula angustifolia*, *Rosmarinus officinalis*) has been shown to be effective for the integrated control of pathogenic fungi, such as *Fusarium avenaceum, B. cinerea, P. expansum,* and *Neofabraea vagabunda*. At the same time, the potential to inhibit mycelium growth (especially by *T. vulgaris* and L. *angustifolia.* ) by applying it through dipping treatment can be correlated with their chemical composition [19].The treatment moment (curative or protective), as well as the mode of application are also important, as it was noticed by tests performed using of garlic extracts and clove oil directly or through volatile exposure, *in vivo,* against postharvest pathogens *B.cinerea*, *P.expansum* and *Neofabraea alba* [17]. When garlic extract was curative applied directly to the fruit, the postharvest decay caused by *B. cinerea* and *P. expansum* has been reduced.

The effectiveness of some resistance chemical inducers (such as chitosan, methyl jasmonate, salicylic acid, silicon, and thiabendazole) was also highlighted [42] in controlling the fungi *B. cinerea* and *P. expansum*. Thus, for *B. cinerea* the 80-96% reduction in incidence was due to silicon, while chitosan provided a 92-100% reduction for *P. expansum*. In addition, high doses of chitosan and silicon increased phenylalanine ammonia-lyase levels for *B. cinerea*. Chitosan (regardless of the dose) favored the activity of scopoletin and scoparin from fruits in the case of both pathogens. Added to this it was an increase in lignin content when the doses of the two inducers were high [42].

The pre-cooling of freshly harvested apples (cv. Royal Delicious) associated with their surface coating with neem (*Azadirachta indica*) oil and marigold flower (*Tagetes erectus*) extract highlighted the ability to effectively maintain the fruits physico-chemical and physiological characteristics, especially for the neem oil (1.5 - 2%). This also induced a significant reduction of diseases incidence [79]. Methanolic extract of mulberry leaves (4%) ensured the inhibition of mycelial growth of *P.expansum* (40.3% effectiveness), as well as reduced of sporulation [45]. Ethyl acetate extract (8%) was found to have similar effects to the chemical control (imazalil with 75.1% inhibition). Some color changes of the fruits have been produced by using the second solvent.

Tests performed *in vitro* showed the ability of essential oils extracted from lavender and oregano to inhibit mycelial growth in *B.cinerea* and *P.expansum*, by up to 90% for both pathogens [16]. The efficacy of neem extract (as compared with fennel, lavender, thyme, pennyroyal, salvia and asafetida extracts) both *in vitro* and *in vivo* studies, against *B. cinerea* was also emphasised [28]. During apples storage, a 25 % neem aqueous extract determined a decrease of disease severity by 89.11 % as against the control. Generally, plants extracts determined an increase of the activity of different enzymes (peroxidase, phenylalanine ammonia-lyase, β-1.3-glucanase and polyphenol oxidase) in apple fruits, when the pathogens have been present. On the other hand, cinnamon, pimento, and laurel extracts had a high *in vitro* antifungal activity against *B. cinerea*, but at that concentration the postharvest effect was not so good. Even for cinnamon extract (the most promising one), higher concentration should be applied to be effective *in vivo*, during fruits storage [63].

Promising results (e.g. high efficacy, low cost, and safety to human health) for a natural fungicides exploitation against the apple ring rot produced by *Botryosphaeria dothidea* have been obtained in China [48], by using the alkaloid berberine extracted from the *Coptis chinensis* (a medicinal plant). Its effect was a successful inhibition the hyphal growth, while the *B. dothidea* membrane permeability was increased, as well as the activity of some energy metabolism implicated enzymes (succinate dehydrogenase and malate dehydrogenase) has been repressed. The aqueous extract from the epicarp of pomegranate fruits applied *in vivo* at a concentration of 50 mg/mL (and proven to contain high amounts of phenols -2737.44 mg GAE/L and flavonoids -309 mg QE/L) was shown to reduce by 21.03 and respectively, by 42% the severity of the disease caused by *Monilia laxa* and *M. fructigena* [23].

**Genetic engineering, omics-based approaches and machine learning (ML) algorhitms**

The role of genetic manipulation was highlighted [21], regarding that the silencing of the APPLE VACUOLAR PROCESSING ENZYME 4 (MdVPE4) which causes a decrease in *B. dothidea* disease resistance. Its over expression has the opposite effect, increasing resistance and, respectively, influencing the genes involved in disease resistance in the case of fruits (APPLE POLYGALACTURONASE 1 (MdPG1), APPLE POLYGALACTURONASE INHIBITOR PROTEIN 1 (MdPGIP1), APPLE ENDOCHITINASE 1 (MdCHI1) and APPLE THAUMAINTIN-LIKE (MdCHI1) MdTHA1). APPLE CYSTEINE PROTEINASE INHIBITOR 1 (MdCPI1) was also found to be involved in the process of modulating MdVPE4 activity.This highlights the interaction between MdVPE4 and MdCPI1 in the framework of modulating fruit resistance to *B. dothidea* attack.

Genetic studies for the manipulation of terpenes have been in attention [37]. It is well known the production of the sesquiterpene (E,E) -α-farnesene, by apples fruits. There were identified four quantitative trait loci (QTLs) in a segregating 'Royal Gala' (RG) x 'Granny Smith' (GS) population and it was elucidated their implication in fungal pathogenesis of some post-harvest pathogens (*Colletotrichum acutatum*, *P. expansum* and *Neofabraea alba*).

On the same note, investigations carried out by [37] focused on understanding and controlling the virulence of the *P.expansum* fungus have led to the identification of the Blisteringl gene. This gene affects the internal and external processing of a protein (with a DnaJ domain) involving vesicle-mediated transport in a family of fungi with medical, commercial, and agricultural importance. The researchers' attention was also directed to understanding the action of *Bacillus amyloliquefaciens* (the function of fengycin) in *P.expansum* control. There were done the construction of BA-16-8 (a fenC gene deletion mutant), by PCR approaches and testing the inhibitory effect of the mutant via high-performance liquid chromatography, mass spectrometry, and *P.expansum* growth inhibition assay *in vitro* [26]. It has been shown that fengycin is the key component of *B. amyloliquefaciens* BA-16-8 in the control of blue mold disease. As [13] noticed, the development of genetic resources in order to investigate at the molecular level the mechanisms that characterize the bio control activity on *P.expansum* by the basidiomycetes yeast *Papiliotrema terrestris*, is an urgent need to promote biological (and integrated) means of control and to reduce the fungicides treatments.

In addition to genetic studies, the analysis of metabolic changes during *P.expansum* infection based on the ultra performance liquid chromatography and the quadrupole-time of flight mass spectrometry (UPLC-Q-TOF/MS) technique performed by [67] led to the identification of significant metabolomic differences between control and infected samples. The differences were especially as regard as to the secondary metabolite biosynthesis, ATP-binding cassette transporters, amino acids and carbon metabolism in early infection time.This information can be used to characterize the mechanism of the pathology and the discovery of biomarkers for quality control. The use of antifungal proteins (AFPs), such as those from *Penicillium chrysogenum* (PAF, PAFB and PAFC) and *Neosartorya fischeri* (NFAP2), has been shown to act in a species-specific manner, in controlling post harvest pathogens. PAFB was the most efficient against *P. digitatum*, *P. italicum* and *P. expansum*. PAFC and NFAP2 showed moderate antifungal activity, whereas PAF was the least active protein [27].

Besides different other researches which are performed in this field, digitization and digitalization are also important components of the agriculture development in the global economy [76]. Application of machine learning (ML) algorhitms (the state - of - the art technology) in agriculture domain, in each activity area (pre harvesting, harvesting and post harvesting) allows more efficient and precise farming, with less human manpower and with high quality production [51]. In addition, the application of ML-based techniques for forecasting, detection, and classification of diseases and pests proved to be of interest for a smart, precise and environmentally friendly agriculture. Furthermore, further developments are needed [20], advances that come in support of improving the quality and yield of crops, along with increasing the degree of tolerance to abiotic and biotic stress factors [34].

## CONCLUSIONS

Non-chemical treatments to sustainable control of post harvest apple fruits diseases include a large range of different approaches.

The most suitable cultivar must be chosen, to be cultivated in the environmental conditions corresponding to it. The appropriate techniques applied during the growing period, fruits harvesting at the corresponding moment, as well as their manipulation during the entire link between the farm and consumer must be taken into account.

It is necessary to improve plants growing environmental conditions and pre harvest practices by using small doses of fungicides and the application of complementary, environmentally friendly measures.

Exploring the epiphytic and endophytic flora with a view to exploitation of some microorganisms as biological control agents have been proved to give very good results. Nanomaterials using and of plant extracts, as well as of some resistance chemical inducers can reduce the costs, increase the efficiency and safety of applied treatments.

Genetic engineering, omics-based approaches and machine learning algorithms are nowadays promising innovative means that can be applied to early pathogens detection, as well as to better diseases management.

It is necessary to continue such studies, through a close collaboration between researchers and practitioner, with a view to the successful implementation of the most appropriate innovative strategies for post-harvest management of apples.

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