Chapter

Pomegranate: Postharvest Fungal Diseases and Control

Annamaria Mincuzzi and Antonio Ippolito

Abstract

Due to well-known nutraceutical properties, pomegranate (*Punica granatum* L.) cultivation is recently increasing in various areas of the world including Italy. Fungal diseases are the major causes of postharvest yield and economic losses. Most of the fungi infect pomegranates in the field during the blooming stage remaining latent until fruit ripening, others infect fruit during harvest and postharvest handling through rind injuries. Main postharvest fungal diseases of pomegranates are gray and blue molds caused by *Botrytis* spp. and *Penicillium* spp., respectively, black heart and black spot due to *Alternaria* spp., anthracnose related to species ascribable to *Colletotrichum* genus, and Coniella rot, due to *Coniella granati*. Few fungicides are allowed for pre- and postharvest treatments, making it extremely difficult to control fungal infections. In this scenario, especially in organic fruit production, alternative control means may be a desirable solution to reduce pomegranate losses during the production chain. This chapter focuses on the most important postharvest diseases of pomegranates and possible strategies and means to reduce spoilage.

Keywords: balausta, *Aspergillus*, *Talaromyces*, chitosan, seaweed extract, *Pilidiella*, minor crop, pomegranate loss, pomegranate market, fludioxonil

1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the order of Myrtales, but the family type is uncertain between Punicaceae and Lythraceae [1]. Regarding the binomial name, the genus *Punica* reflects the feminized Roman name of Chartage, instead the species epithet *granatum* referred to the seeded arils; the common English name "pomegranate" describe its anatomical features of "grainy apple" [2, 3]. The Transcaucasian-Caspian region [4] is the center of diffusion of pomegranate shrubs, which early spread in the Mediterranean basin and then gradually reach the New World. Being a heliophilous plant, pomegranates tolerate temperature until 45–48°C with hot wind, even though temperatures below −18°C are harmful [1]. Pomegranate cultivation is promoted by the cool winter and hot summer; these features match with Mediterranean climate, although these shrubs are fully spread in tropical and sub-tropical areas too [2].

1.1 An outline of botanical features

Some botanical features of pomegranate plants are relevant in plant pathology. First and foremost, flower biology. Being an andromonoecy shrub, it brings both hermaphroditic and functionally male flowers to the same plant [5]. Male flowers bloom earlier than bisexual ones and have a *vexillum* function attracting insects, and a selective advantage improving the genetic variability related to cross-pollination instead of self-pollination [5]. Fertile hermaphrodite flowers have well-developed *gynoecium* and *androecium*, urceolate vase-shaped calyx, and prominent U-shaped ovary; often they show big ovary and long style, the *stigma* emerges at the same height as the anthers or above them. On the other hand, male flowers are smaller, since the *androecium* is well-developed, but the *gynoecium* (including ovary and ovules) is rudimental: the style is shorter than anthers; the calyx is bell-shaped and the ovary is V-shaped. Furthermore, several intermediate flower forms exist that may be more or less close to the chief sexual forms showing different degrees of anatomical functionality [1, 4, 5]. Usually, hermaphroditic flowers (and occasionally a few intermediate forms) are fertilized [1, 5]. This is a delicate phase because latent fungal pathogens infect fruit during this stage passing through the open connection with the ovary [6]. According to the cultivar, between 5 and 8 months after fertilization and fruit set, pomegranate fruit, named *balausta*, ripen [4]. The most significant morphological feature of this berry-like and fleshy fruit is the persistence of the residues of the thick floral calyx [1] creating optimal ecological niche for fungal settlement and growth. In addition, the persistence of necrotic stamens acts as secondary source chiefly for wound fungal pathogens [6]. The leathery and woody rind and the richness in polyphenolic compounds guarantee physical and chemical protection from pathogenic fungi to a certain degree [1]. Internally, the carpel, made of light yellow and spongy tissue, as the mesocarp, acts as preferential way for latent fungi diffusion. Internally the *balausta* is reparteed in asymmetrical chambers, called "*locules,*" by membranes made of papery tissue [1, 2, 4]. Arils, attached to the carpel and wrapped by membranes, are the edible portion of pomegranates, constitute 40–60% of fruit weight, and are the most susceptible part of the fruit. Over time, pathogen diffusion happens chamber by chamber. Pomegranate is a non-climacteric fruit, so it must be harvested at the optimum maturity stage [4].

1.2 An outline of horticultural features and disorder prevention

Few horticultural characteristics are important to control disorders, improve disease resistance, and enhance healthy fruit production which is the key point for extended storage. Pomegranates could grow in different soils but prefer fertile and humus-rich ones with a medium-deep density and well-drained avoiding water stagnation [1]. During the dry season, especially in arid and semi-arid environments, pomegranate plants need a regular daily irrigation to prevent water stress; also considering reduction of groundwater resources, drip irrigation is one of the favorite techniques [7]. Furthermore, within 0.5% of soil mass, pomegranate is a saline-tolerant plant: sodium, chlorine, and potassium are accumulated in root tissues, preventing diffusion of toxic salts [1, 4]. Also, fertilization is important to prevent both pomegranate disorders, like cracking, and related diseases due to wound pathogens; nitrogen and calcium are the most involved chemical elements. Soil application of watery nitrogen enhances vegetative growth, fruit size, and yield; in addition, foliar application of potassium chloride, potassium sulfate, and microelements improves both yield and growth of pomegranate [4, 8]. Among foliar treatments, calcium application is the most significant; it induces physiological resistance and stabilization of the cell membrane, preventing low-quality production and supporting postharvest storage [9]; in addition, if applied at the blooming stage and 1 month later, it prevents fruit cracking [10]. Indeed, nitrogen and calcium are key chemical elements for disease prevention; also pruning practices may influence disorder and disease development. In some parts of the world, multiple trunk method is the traditional growing practice for pomegranates, from three to five main trunks are developed and branches are open-vase pruned. This favors the branch maintenance year-after-year and allows the replacement of diseased one [4]. The single stem method provides a single trunk of up to 30 cm in height, after which divided into three or four main branches; this way ensures better vase-shape maintenance and adequate penetration of light that is fundamental for pomegranate veraison [4]. However, sunrays surplus could cause sunburn damaging fruit, indeed the perfect light balance is noticeable [1].

1.3 An outline of pomegranate trade

In the last decades, pomegranate cultivation increased worldwide to face consumer requests; pomegranates display nutraceutical properties due to the high content of active secondary metabolites (i.e., alkaloids, terpenoids, and polyphenols). These compounds are well-known for both antimicrobial and antioxidant properties, and therapeutic ones that feed this trade [11]. Consequently, the request for fresh fruit and related processed products is rapidly growing reaching a global production of about 8.1 million tons [12]. In the world, the chief producers are India, China, and Iran which produced 70% of pomegranates [12], although official data are not available. Particularly, Indian production represents 41% of the global trade having 288,000 ha of pomegranate orchards and producing 3,256,000 tons of fruit gathered in the Maharashtra state and in the Solapur district [13]. In India, the most spread cultivars are Bhagwa and Arakta available throughout the year; these pomegranates support export to European, Middle East, and Asian countries, especially during the production gap (December, January, and March) [14]. Even though China is the second worldwide producer, 70% of produced soft-seeded 'Tunisia" pomegranates are headed to the national markets [14]. A similar scenario distinguishes the Iran market, which mainly aspires to internal commerce of "Malas Yazdi" pomegranates due to export troubles [14]. Regarding Europe, the main producers are Spain and Italy, detailing Spanish production aims at export, instead Italian is not enough to satisfy internal requests [12]; indeed, Italy imports 4% of the global production of pomegranates. Being high-quality, flavorful, and royalty-free cultivars [15], chiefly cultivated plants belong to the Israeli "Akko," the American "Wonderful" and the Spanish "Mollar de Elche," this last is featured by sweetness and herbaceous seeds. These cultivars, such as their wide-spread clones, sequentially ripening in September and October, so are available almost till January [4, 16]. Most updated Italian data referred to 2021 when pomegranate orchards are 1420 ha and have a total production of 192,485 [17]. As displayed in **Table 1**, comparing 2011 and 2019 production years, the present production is about 800- and 3600-fold increased, respectively. Almost the entire production happens in southern Italy, especially in Apulia and Sicily regions, where the Mediterranean climate is favorable. Pomegranate cultivation is promoted by the cool winter and hot summer, although these shrubs are spread in tropical and sub-tropical areas too [2]. Being a minor crop, worldwide analysis of

Measured parameters	2011	2016	2021
Total surface (ha)	62	622	1420
Productive surface (ha)	60	402	1249
Total production (q)	5131	46,343	192,485
Harvested production (q)	4968	45,717	186,972

Table 1.
Pomegranate Italian production. Pomegranate surface (ha) and fruit production (q) are compared in different years.

pomegranate market is lacking due to both the absence of consistent and updated data and grouping within a single Harmonized Code1 (HS code) [18].

1.4 An outline of yield and economic losses

Yield losses caused by fungal postharvest rots may significantly reduce pomegranate production [19] partially justifying differences between produced and harvested amount of fruit (**Table 1**). Yield losses are reflected in economic ones. Particularly, fruit losses are distributed among the field, wholesale, and retail sites referring to related transportation too; similar data are obtained for Indian [20, 21] and South African markets [22, 23]. In the field importance of injuries, cracking, and fungal infections is highlighted rather than secondary infections and physical damages (dehydration, overripening, etc.) mainly involved during the other phases [20, 21]. In India, 10% of yield losses occur in the field and wholesale and 15% are in retail [20, 21], whereas, in South Africa, the percentages involved are 18, 23, and 21%, respectively [22, 23].

2. Postharvest pomegranate diseases

A careful eye in presenting postharvest fungal diseases of pomegranates is needed, according to the mode of infection that gives rise to "latent" infections and "wound" ones. In the field, latent pathogens infect the future fruit during the blooming stage and remain latent until environmental and physiological optimal conditions let them to develop; usually, this happens during postharvest stages. Chief diseases ascribable to this group are gray mold, black heart and black rot, soft rot, and anthracnose, which represent around 65% of total infections [6]. The remaining percentage belongs to wound pathogens that penetrate pomegranate rind and infect fruit following traumatic events caused by bad handling (i.e., no close-cropped peduncle), pests (i.e., borers), weathering (i.e., hail and rain), and abiotic damages (i.e., cracking) occurring from the field till the retail. Blue mold and aspergillosis are the main diseases related to these events [6]. Both groups of pathogens may be responsible also for secondary infections, generally related to nesting or infected stamens. Treatments to control primary and secondary infections, as well as good agronomical practices (i.e., debris and mummy removal) are needed to reduce yield and economic losses [19]. To this aim, a reliable identification of the chief genera and species of fungi is needed to face them with specific/effective substances. Pathogen/disease characterization is primarily based on fruit symptom evaluation, followed by pathogen isolation and observation of their specific macroscopic/microscopic structures on a broad spectrum medium, such as potato dextrose agar (PDA). Finally, molecular analyses are applied to univocally confirm their identity.

2.1 Latent infections

2.1.1 Alternariosis

Alternaria genus is the etiological agent of black spot and black heart diseases, even though the latter is more hazardous to human health. Indeed, *Alternaria* spp. produce more than 30 mutagenic or genotoxic mycotoxins, among which the most relevant are alternariol, alternariol monomethyl ether, altenuene, altertoxins, tentoxin, and tenuazonic acid [24]. Distinguishable symptoms feature both diseases. Black spot symptoms are necrotic areas in pomegranate rind, which appear as small circular black blotches, reddish-black in the middle, and surrounded by yellowish-green halos. Internally fruit are healthy and edible (**Figure 1A**). On the other hand, pomegranates affected by black heart are apparently asymptomatic, but the inner part is rotted. Often, black-heart alternariosis is related to darker rind color and/or lighter weight due to dehydration and aril disintegration; occasionally fruit may appear asymmetric and irregularly shaped. Internally, brown and soft-rotted arils become grayish-black in color (**Figure 1B**,**C**). Infection symptoms spread from the calyx area (crown) to the entire fruit along the carpel via; this diffusion pathway is shared by all latent fungal pathogens. Alternariosis is one of the most widespread pomegranate diseases, and these have been reported all over the world: Italy, Israel,

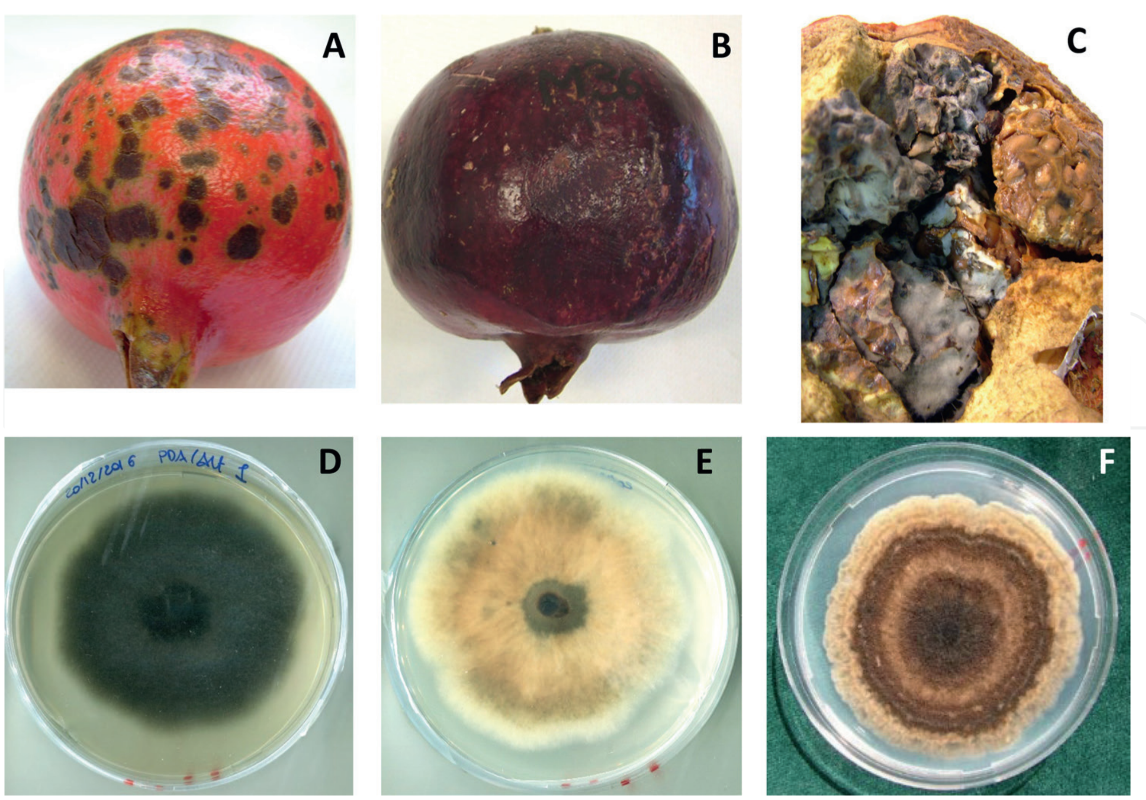

Figure 1.
External symptoms of (A) black spot and (B) black heart caused by Alternaria *spp. (C) Close-up on heart rot showing mycelium growth. Colonies on PDA of* Alternaria alternata*: (D) dark and (E) whitish strains. (F) Colony of* Alternaria arborescens *on PDA [6].*

Greece, California, Spain, and India. Species involved in Alternaria diseases belong to *A. alternata* and *A. arborescens* species-complex, their distribution is about overlapped; in addition, just in India is recorded *A. burnsii* [13, 25–29]. Although, within this genus conidial morphology is easily recognizable, the sporulation pattern is different among species and/or morphotypes, but intraspecific variability of colony morphology is high (**Figure 1C**-**E**). Colonies varied both in color (whitish, brown, deep green, and/or black) and texture (flat, fluffy, and/or wooly); sometimes *Alternaria* colonies grown on artificial media display typical patterns including different colors and textures. Within *A. alternata* complex, *alternata* is the most spread morphotype that has dark brown conidia (20 ± 10 μm in diameter), oval-ellipsoidal shaped, with 4 ± 1 transverse septa; conidia are arranged in branched chains. Colonies belonging to morphotype *tenuissima* are greenish with white margins; their conidia are elongated with a long-tapered beak. *A. alternata* morphotype *limoniasperae* is less common but observed as pomegranate pathogen too; colonies are flat, light brown, and granulated with undulating edges, while conidia are long, ellipsoidal, and display 1–3 transverse septa. The second *Alternaria* species recorded on pomegranate fruit is *A. arborescens* [26] whose colonies are greenish-gray or brown in color, characterized by a slow growth rate; conidia shape ranges from oval to ellipsoidal, with 1–4 transverse and 1–2 longitudinal septa. Being erect and straight, branched, bended, and geniculate, *A. burnsii* conidiophores are typical whereas conidia are ununiformly slight but featured by a short beak [30]. Due to morphological heterogeneity, molecular approach is useful to identify species and morphotypes, particularly it may be advantageous using OPA 1–3 or OPA 10–2 barcoding regions [31] and/or a multilocus approach as described by Aloi et al. [26].

2.1.2 Gray mold

Gray mold is among the main postharvest diseases of pomegranates all over the world [32–34]. As proved by Testempasis and colleagues [35], gray mold is caused by species belonging to *B. cinerea* complex as *B. cinerea s.s.*, *B. pseudocinerea,* and *B. cinerea* group *S*., being the former two the most spread etiological agents. The infection starts in the crown area showing small tan-colored spots, which rapidly spread to the whole fruit (**Figure 2A**-**C**). Developing, spots became darker and softer until causing rind collapse; finally, gray fluffy mycelium and black sclerotia grow (**Figure 2D**). Internally, softening and browning of the arils and the development of gray mycelium feature this infection. Aiming to control postharvest losses, nesting among contiguous fruit is important because this significantly enhances pathogen diffusion. Early, colonies are whitish (**Figure 2E**, **F**), then become brownish-gray, and finally covered by sclerotia circle-arranged. Lemon-like conidia measure 7.7 ± 2.4 × 6.8 ± 2.5 μm on average, while sclerotia are 2.9 ± 1.5 × 2.1 ± 0.6 mm. Morphological identification within the species complex is not possible, therefore a duplex PCR assay to evaluate indels in the *mrr1* gene is the optimal solution; suggested primer pairs are BcinN-in-F/BcinN-in-R and Mrr1-spez-F/Mrr1-spez-R [35].

2.1.3 Coniella rot

Considering symptoms, especially at early stage, Coniella fruit rot resembles gray mold. Hence, specific features must be considered. Coniella rot is caused by *Coniella granati* which is synonymized with *Pilidiella granati*; this host-specific fungus is well-known since the end of the nineteenth century when it was

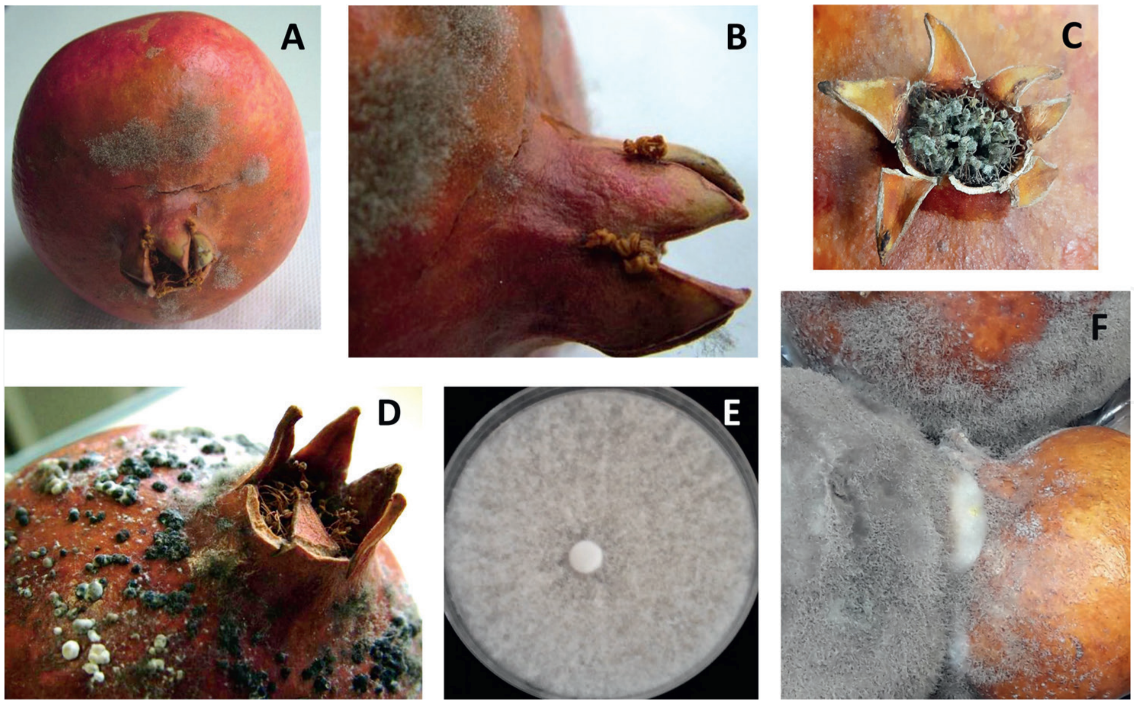

Figure 2.
Gray mold caused by Botrytis cinerea*. (A) Early stage of rot. (B) Close-up image of sporulated gray mycelium, (C) infected stamens, and (D) black sclerotia. (E)* B. cinerea *young colony on PDA. (F) Nesting secondary infection among fruit [6].*

isolated in Italy [36]. Nowadays, it is worldwide spread from Spain and Greece till Tunisia and South Africa causing both shoot blight and canker disease and the above-mentioned crown rot [37–40]. Crown rot symptoms consist of small circular rind spots, spread around the calyx area; these rapidly broaden and make lesions softer and darker till reaching brown color (**Figure 3A**). At maturity, a thin whitish mycelium with spherical pycnidia, ranging between dark brown and black in color, covers the lesions. Internally fruit decay is soft and brown and involves arils too (**Figure 3B**,**C**). Usually, due to softness, decayed part of each fruit splits. PDA colony ranges between white and creamy in color, texture is leathery and covered by abundant dark pycnidia (110 ± 30 μm in diameter, **Figure 3D**) with thin membranous walls. In addition, hyphae are septate and conidia (13.75 ± 3.750 × 3.5 ± 1.5 μm) are unicellular and hyaline, ellipsoid to fusiform in shape, straight or slightly curved. Even though morphological identification is relevant, molecular confirmation based on Internal Transcribed Spacer ITS5/ITS4 is useful [6].

2.1.4 Anthracnose

Anthracnose is increasingly widespread, especially in tropical and subtropical areas where rainfall and damp wind could enhance its dissemination when temperatures increase [41]; although climate change is favoring its diffusion in the Mediterranean region, it is retained as a minor disease. Species belonging to *Colletotrichum* species-complex are the etiological agents; usually, pomegranate fruit symptoms are superimposable among different species displaying typical anthracnose (**Figure 4A**). Both species-complex and species are differently distributed in the world, indeed in the southeastern United States *C. theobromicola*, *C. siamense*,

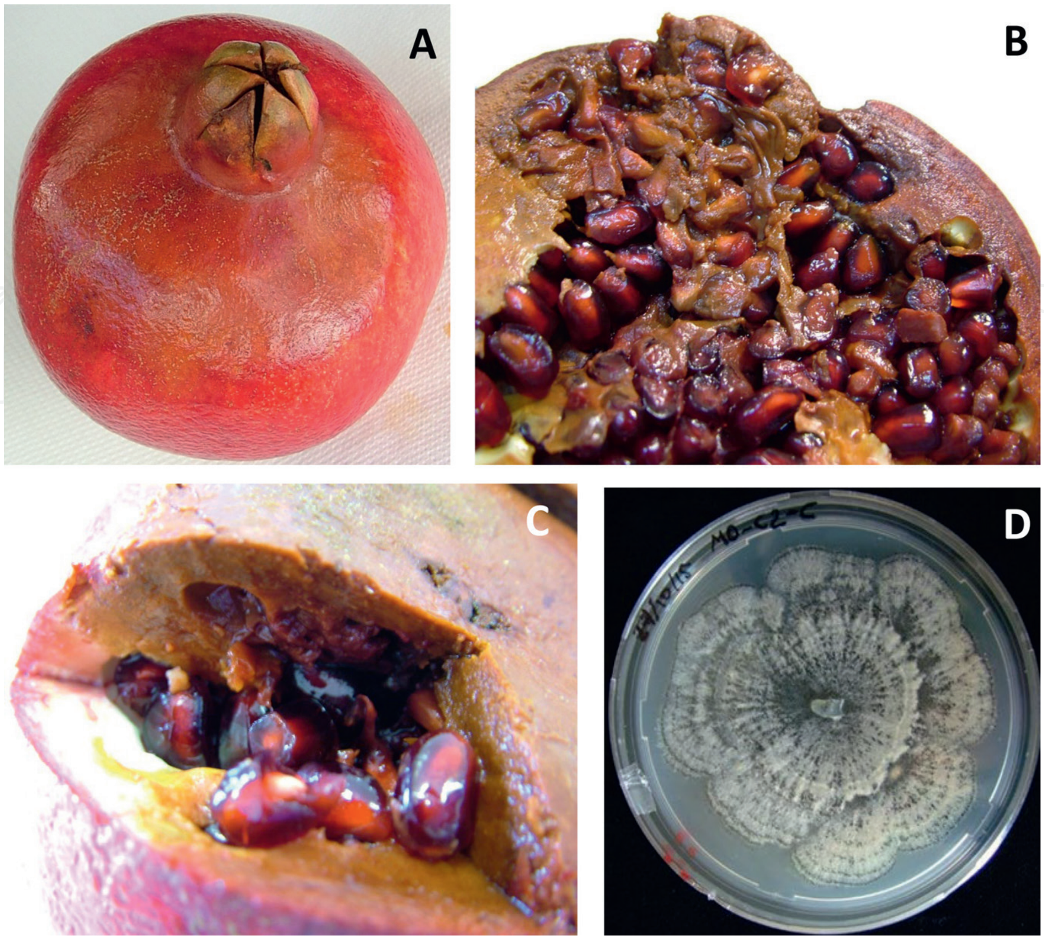

Figure 3.
Coniella granati rot. (A) External symptoms. (B) View of internal decay. (C) Close-up of the soft rot. (D) Colony on PDA plate [6].

C. gloeosporioides, *C. nymphaeae*, *C. fiorinae*, and *C. simmondsii* are common post-harvest pathogens of pomegranates [42]. Similarly, in Brazil, *C. theobromicola*, *C. siamense*, *C. gloeosporioides*, and *C. tropicale* are recorded [43, 44], instead in the old countries most widespread species belong to *C. acutatum* complex and *C. gloeosporioides* complex. For instance, *C. gloeosporioides* is recorded in Turkey and in Albania [44] and *C. acutatum* is registered in Italy [6]. Generally, anthracnose causes the typical soft sunken lesions, which merge as they grow; progressively, white mycelium develops onto and lesions become circular, concentric, and brown with darker spots (**Figure 4B**). On PDA, strains of *C. acutatum* appear fluffy, early white with a reverse ranging from salmon till grayish color, then peachy-pink with pinkish-salmon conidial masses (**Figure 4C**). On the other hand, mycelium of *C. gloeosporioides* displays a texture ranging from faintly aerial to dense cottony; color of colonies ranges from white to pale-olivaceous reaching gray. Acervuli are abundant, small dark-based with sprinkled setae. Meanly, conidia of *C. acutatum s.s.* and *C. gloeosporioides s.s.* measure 11.3 ± 2.8 × 4.2 ± 1.1 μm and 12 ± 2.9 × 4.9 ± 2.1 μm, respectively, although, generally, the size is host specific (**Figure 4C**). Both chief species are one-celled, but the first has elliptical-fusiform conidia, instead the last shows oval to oblong, end-pointed conidia. Being species-complex, morphological features are not enough to identify each species within the complex, therefore is needed a molecular multilocus approach according to species-complex they belong [45].

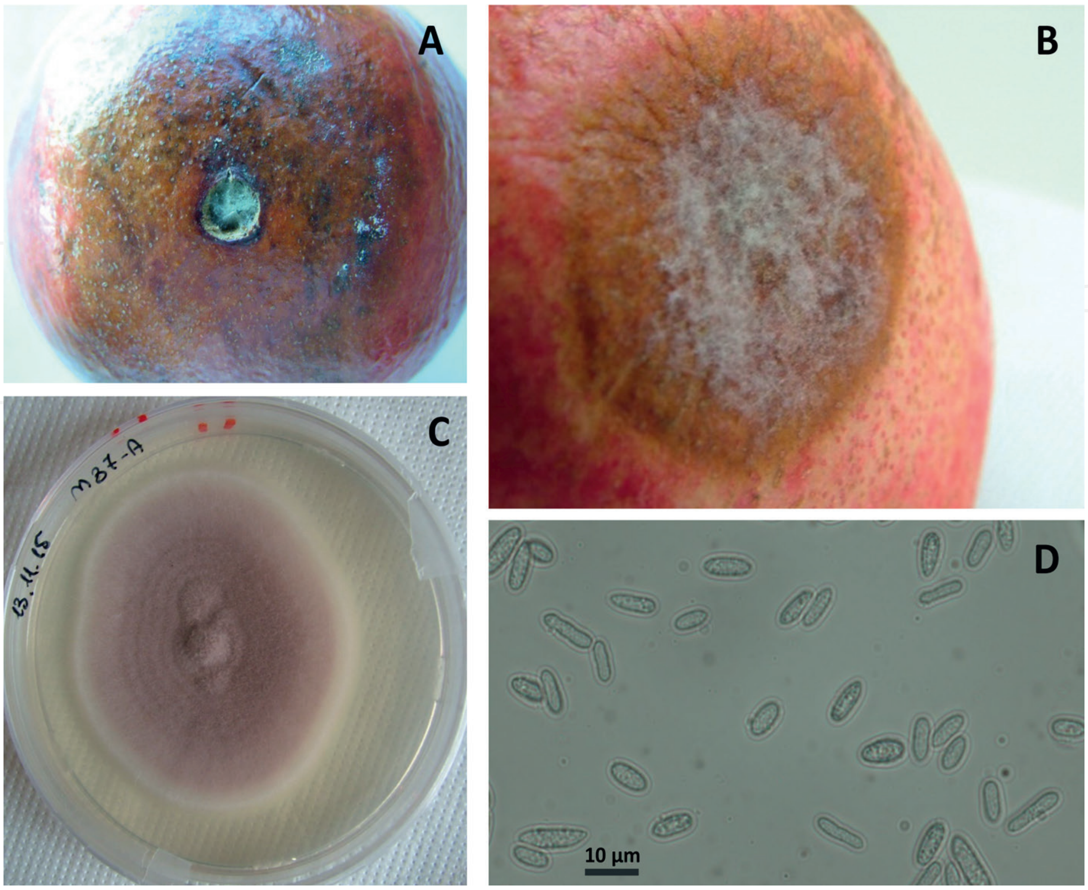

Figure 4.
Anthracnose caused by Colletotrichum acutatum s.s. *Artificial (A) and natural (B) infections. (C)* Colletotrichum acutatum s.s. *colony grown on PDA plate and (D) conidia [6].*

2.2 Wound infections

2.2.1 Blue-green mold

The main fungal group involved in pomegranate wound infections belong to *Penicillium sensu lato* (*s.l.*) that includes *Penicillium s.s.* and *Talaromyces* genera; within this last genus, just *T. albobivertillius* species is recorded, which results spread both in Italy and China [46, 47]. In *Penicillium s.s.* group, the most abundant species are *P. glabrum*, *P. adametzioides*, and *P. brevicompactum.* Minor species belonging to this genus are *P. jhonkrugii*, *P. pagulum*, and *P. citrinum*. Among these, being not pathogenic for pomegranate fruit, *P. brevicompactum* and *P. jhonkrugii* are not relevant; however, producing cytotoxic compounds named brevianamide A and mycophenolic acid [48], *P. brevicompactum* is putatively hazardous for human health. *P. glabrum* has been reported with high incidence also in Greece, Spain, Uzbekistan, and Slovak Republic [37, 49–52], while *P. adametzioides* has been isolated in Israel [53] too. Although in Italy is believed a minor species, *P. citrinum* (synonymized with *P. implicatum*) is spread in Slovak Republic [51] and Pakistan [53]; being widespread and a citrinin producer [54], its presence is relevant; indeed, citrinin is a nephrotoxic mycotoxin [55, 56]. According to most up-date literature, other *Penicillium* species involved as postharvest pathogens of pomegranate fruit are *P. expansum*, *P. sclerotiorum*, and *P. minioluteum*, the last being synonymized with *Talaromyces minioluteus* and re-arranged in *T. minioluteus*-complex, that includes eight species [57].

Usually, lesions due to these fungi appear as brownish and circular necrotic areas, which became darker, deeper, and occasionally irregularly shaped until reach and infect arils. Finally, blue-green sporification grow inside or onto infected pomegranates. Infected stamens can serve as source of secondary inoculum increasing fungal diffusion (**Figure 5A**-**D**). Cultured colonies of *Penicillium s.l.* display different textures, from powdery to crustose or velvety; in general, they are blue-green colored and rounded by concentric whitish margins of different thicknesses and/or roughness. The reverse side of each colony has different colors according to pigments and/or metabolites produced, although, often, shades range between white-yellow or red-brownish. Due to species-specificity of produced metabolites, the metabolomic profile is useful for species identification. Regarding micromorphology, features vary based on species, although typical brush-like conidiophores have spherical and unicellular conidia and are arranged as unbranching chains on the top of the phialides. Conidia diameter, which meanly ranges between 2.5 and 5 μm, and wall ornamentation should be useful for species identification. However, morphological identification of *Penicillium s.l.* species is complex; indeed, molecular confirmation by PCR is needed. Bt2a/Bt2b primer pair is used to identify *Penicillium s.l.* species amplifying a portion of the β-tubulin gene [48].

2.2.2 Aspergillosis

Often, aspergillosis of pomegranates is caused by species of *Aspergillus* belonging to the section *nigri*, also called "black aspergilli." Almost 27 species belong to this section, displaying high inter- and intra-specificity genomic variability; their taxonomy is really

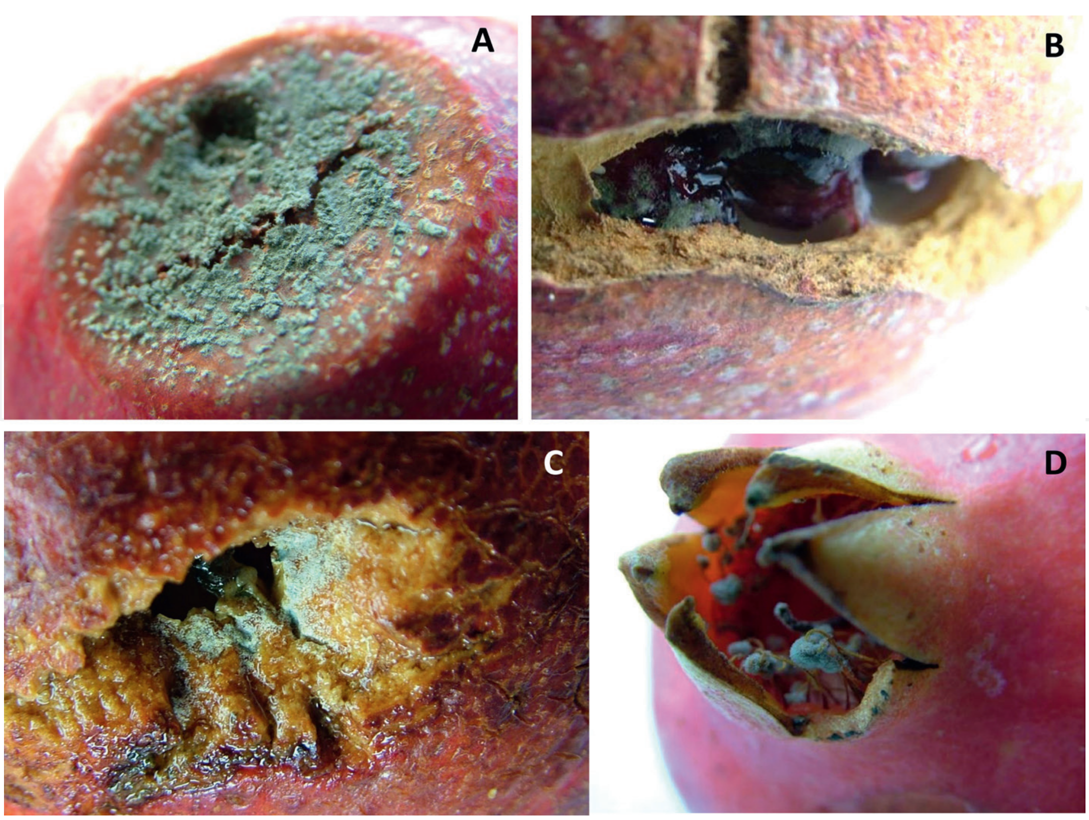

Figure 5.
Penicillium s.l. decay. (A) Necrotic lesion covered with blue-greenish sporification. (B) Cracking, (C) wound, and (D) infected stamens [6].

complicated and not completely solved [58, 59]. Species may be misidentified, and it is not easy evaluating the incidence of each species within the section [59]; furthermore, species identification is relevant especially from a sanitary point of view since some species are potential mycotoxin producers [59]. In general, black aspergilli are well-known as wound pathogens but they could cause internal decay too [6, 60]. *A. niger,* the most spread species within this genus, is recorded in Turkey, Greece, India, Pakistan, and China [61–64], but no evidence of its presence in Italy, where black aspergilli isolated from pomegranate belong to the species *A. tubingensis*, *A. welwitschiae*, *A. uvarum*, and *A. japonicus* [6], being the first identified also in China [65]. *A. niger* and its sister species *A. welwitschiae* are the most hazardous species since they are putative producers of ochratoxin A (OTA) and fumonisins. According to IARC classification, these last are nephrotoxic and hepatotoxic with potential carcinogenic effects on rat and mice; instead, OTA has nephrotoxic and teratogenic, carcinogenic, and immunotoxic properties in rats and possibly in humans [59]. Unfortunately, regulations still do not report limits for mycotoxin amount in fresh pomegranates and their derivatives. Regarding distinctive symptoms, early stage of infection is featured by rind concentric discoloration, from yellow to red-brownish colored (**Figure 6A**-**C**). Internally, pomegranates show a soft brownish-black rot of the arils that may be covered by black powdery sporulation (**Figure 6D**). Black aspergilli cultures appear cottony or velvet-like in texture (**Figure 6E**). Hyphae are septate and hyaline, and mycelium is whitish, but spore development leads to a black appearance; in general, yellow and white shades characterize colony reverse. Characteristic conidiophores are aspergillum-shaped; metulae maintain the phialides and related vesicles. Conidia (2–5 μm in diameter) are arranged in radial chains that give it the characteristic shape. As in the case of *Penicillium s.l.*,

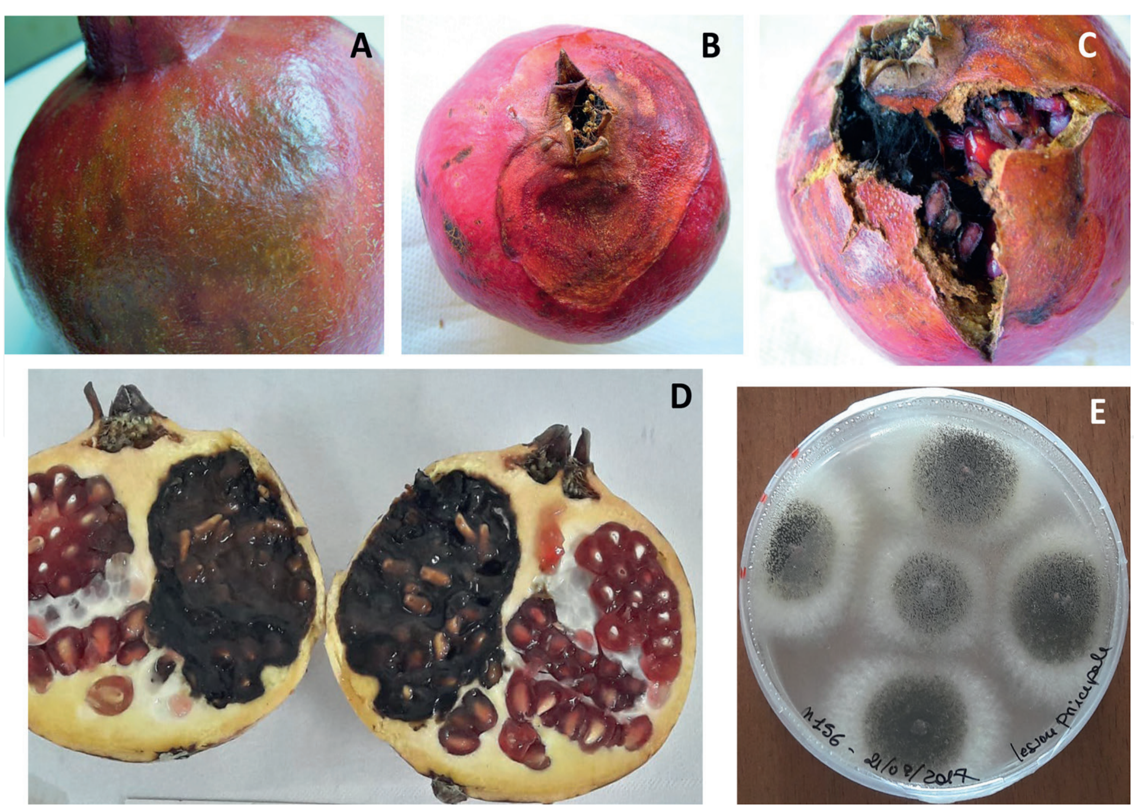

Figure 6.
Aspergillus sect. Nigri rot. (A) Early and (B) advanced stages of the disease. (C) Rind cracking of diseased fruit. (D) View of internal decay; significant is the soft texture of the tissues. (E) Front of a PDA plate of Aspergillus *spp. [6].*

species identification according to macro- and micromorphology is very difficult, requesting molecular confirmation by PCR amplification of a calmodulin portion with CMD5/CMD6 primer pair [48].

2.3 Other fungal diseases

Among fungi involved in pomegranate diseases, there is *Cytospora* spp., which can cause wood canker, branch dieback, and postharvest fruit rot. Although in Spain has been reported *C. annulata* [32], the most widespread species is *C. punicae*. This latter has been reported in various pomegranate cultivation areas such as United States, South Africa, Greece, Cyprus, and Italy [6, 66–69]. Postharvest fruit decay is identifiable by circular soft lesions of the rind, creamy-brownish colored, and centrally darker; related subcutaneous area shows a yellowish and corky appearance. In culture, colonies are whitish, then become olive green; at maturity, colonies are dark brown and covered by globose pycnidia (375 ± 125 μm). Allantoid, aseptate, and hyaline conidia meanly measure 5 ± 1 × 1.5 ± 0.5 μm. Finally, are occasionally identified fungal pathogens belonging to *Fusarium* genus in Egypt and Tunisia [70–72]; being a mycotoxigenic genus that potentially produces deoxynivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, nivalenol, fusarenon X, T-2 toxin, HT-2 toxin, neosolaniol, diacetoxyscirpenol; zearalenone, fumonisin B1, fumonisin B2, and fusaric acid [73], fusariosis presence needs to be monitored. Also, *Cladosporium* spp. is described as the etiological agent of fruit rots both in Spain and in China [32, 74].

3. Disease control

Obtaining healthy pomegranates is the early step to ensure an extended postharvest life, so fruit sorting during the production chain is relevant. Cold-storage temperature significantly influences the incidence of postharvest diseases and related storage life. Although optimal cold-storage temperature slightly changes among cultivars, in general, 6.5 ± 1°C is fine for most of them in presence of 90–95% relative humidity (RH). Weight loss and decay incidence are increased by higher temperatures, instead chilling injuries are enhanced by lower temperatures; similarly, higher RH favors fungal growth and lower one causes weight loss [75]. In addition, arranging pomegranates in microperforated plastic bags can reduce fruit dehydration allowing transpiration and minimizing condensation, indeed it is possible to further extend the storage life of fruit. These bags create a modified atmosphere [76].

Latent and wound pathogens display different modes of infection, so it is important to take action otherwise; particularly, to control postharvest latent pathogens is needed preharvest application of fungicides during the blooming stage; instead to reduce infections caused by wound pathogens during postharvest processing is useful to act on harvested pomegranates. However, good agronomical practices based on pruning, mummy and debris removal, adequate irrigation, and fertilization are key steps in fungal disease prevention. Nevertheless, in general, fruit are just postharvest treated [77], also because often are no chemical fungicides labeled for preharvest application, like happen in Florida and Italy [6, 77] except for temporary emergency registration. In agreement with the United Nations Priorities, disease prevention is basic to reduce food waste from 30 to 15% and discarded fruit by 20%; finally, UNO requests the reduction of postharvest pesticides by 20% by 2030. This “One Health” approach is useful to reduce fruit-waste and economic losses, defend human health by reducing exposure to chemical fungicides, and decrease fungal resistance to chemical molecules. Regardless of cultivar susceptibility and conventional/alternative nature of the treatment, fungicide application timing is significant to nip-in-the-bud infections.

Tested chemical fungicides aim to stroke main pomegranate postharvest fungal pathogens, such as *B. cinerea*, *A. alternata*, *C. granati,* and *C. gloeosporioides*. In addition, being a minor crop, no fungicides are fully registered in most of the producer countries, such as Italy, where regulations change year by year. As an example, in this country in 2019 and in 2020, to reduce gray mold incidence, fludioxonil postharvest application was allowed, and in 2021 preharvest employment of the beneficial microorganism *Bacillus amyloliquefaciens* sbs. *plantarum,* strain D747, was temporarily approved in addition to sulfur and copper. Particularly, since 2021 employment of copper, well-known for its broad-spectrum anti-bacterial properties, has been banned due to toxicity to the whole ecosystem (humans, animals, plants, and environment). Therefore, the already allowed microorganism, together with boscalid, fludioxonil, commercial formulates based on *Trichoderma asperellum* or *Trichoderma atroviridae* strain T11, and essential oils (geraniol, thymol, and eugenol) have been temporarily approved for 2022. Finally, for treatments on leaves, *Coniothyrium minitans* is temporarily allowed as well as dazomet, metam-potassium, and metam-sodium for treatment in soil.

Fludioxonil belongs to phenylpyrroles that originated from pyrrolnitrin antibiotic, which is produced by different species within the *Pseudomonas* genus [78]. It is a non-systemic fungicide, exploitable both pre- and postharvest, and broad-spectrum; it is effective against *B. cinerea* and other fungal pathogens, such as *Alternaria* spp. and *Aspergillus* spp. [75, 79]. Although its mode of action is not fully understood, fludioxonil inhibits spore germination, germ tube elongation, and mycelium growth of *B. cinerea*. Its mode of action involves the hyperactivation of the high osmolarity glycerol (HOG) signaling pathway through group III hybrid histidine kinases (HK). Being part of the multistep phosphorelay systems (MSP), HKs of group III are needed for the variability of cellular signal transduction in eukaryotic organisms. These signaling systems allow interaction and response between microorganisms and environments through cellular homeostasis regulation, but HOG is responsible for fungicide action also. In the HisKA domain, the histidine H736 is the putative signaling switch [80]. As argued by Xavier and colleagues [77] treating fungicide effectiveness, almost two treatments during the blooming stage are enough to significantly reduce gray mold incidence; in addition, he suggests to alternatively use different active molecules to avoid fungal resistance.

Concerning boscalid, it strikes different stages of fungal development from germ tube elongation and spore germination till appressoria formation or mycelial growth; in addition, during absorption through leaf surface, it is translaminarily and acropetally transported and distributed. Boscalid is a carboxamide fungicide that blocks fungal respiration inhibiting succinate dehydrogenase (SDH) activity. It acts by binding itself to the ubiquinone reduction site within complex II of the mitochondrial electron transport chain. Its site-specific mode of action makes boscalid a broad-spectrum fungicide, but this implies high risk to develop resistant fungal strains also [81]. Generally, these resistant strains are related to point mutations that reach to the substitution of histidine to arginine (H272R) or tyrosine (H272Y) within SDH.

The US Environment Protection Agency classified fludioxonil as reduced-risk compound, but some researchers described its potentially hazardous effects due to mitochondrial oxidative damages that are reflected in organ-specific responses and diseases acting as comorbid factors. Indeed, European Union (EU) set a maximum residue limit (MRL) of 3 ppm within 7 days after pomegranate treatment [82, 83]. However, fludioxonil residues half from the 7th till the 30th day after application [75] and, as observed by Usanmaz et al., fludioxonil concentration decreases below 0.25 ppm after 7 days from the application [79]. Similar considerations concern boscalid that is considered a new-generation fungicide; as other members belonging to SDH inhibitors, it is considered safe. Although, due to its brief lifetime (it is tuned in 2003), EU set 2 mg/kg as MRL value for pomegranate [84].

Hence, in this scenario, the balance of advantages and disadvantages should be considered when choosing the chemical fungicides employment. Therefore, alternative control means may be the safe solution to control postharvest losses caused by fungal pathogens, reducing putative mycotoxin contamination, and avoiding health hazardous compounds like chemical fungicide residues. As stated above, among approved and commercially available microorganisms, there is *B. amyloliquefaciens*. Its efficacy is related to competition for space and nutrients, induction of resistance, and antibiosis. Lipopeptides, such as iturines, fengycin, and surfactine, are produced by *B. amyloliquefaciens* to control gray mold [85]. During the blooming stage, in the field application of *B. amyloliquefaciens* significantly allows controlling postharvest losses caused by *Botrytis* spp.; treated pomegranates enhance the activity of enzymes involved in defense mechanisms, like polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, superoxide dismutase, and catalase (86). By these defense mechanisms, substances (i.e., quinones, reactive oxygen species) and lignin, which are active against fungi, are produced [86]. Furthermore, being elicitors of the induced systemic resistance, chitinase and β-1,3-glucanase are enhanced and boosted by iturin A and surfactine [87]. Repeated treatments during the blooming stage should improve *B. amyloliquefaciens* efficacy due to gradual physiological reduction of defense mechanisms during fruit ripening [88, 89].

Among EU-approved basic substances there is chitosan hydrochloride. Basic substances are "active substances, not predominantly used as plant protection products but which may be of value for plant protection and for which the economic interest of applying for approval may be limited" [https://www.efsa.europa.eu/en/supporting/pub/en-1900]. Chitosan is a D-glucosamine linear polymer available in several commercial formulates differing in composition. It acts in three different ways: it elicits host defense mechanisms, it discloses antimicrobial activity, and it has coating properties [90]. Its effectiveness changes based on the origin (it can be obtained by exoskeleton of crustaceans or insects or fungal mycelium), deacetylation degree (from 60 to 100%), molecular weight (between 3800 and 20,000 Da), production process (chemical or biological synthesis), environmental features (like pH and temperature), and not least sensitivity of fungal species [90–92]. Its water solubility, bio-adhesive properties, and related user-friendliness depend on just mentioned parameters and hydrochloride. Antifungal properties are also highly influenced by its composition [92], although its effectiveness in controlling postharvest rots of pomegranates is proven. Therefore, chitosan hydrochloride display both direct and undirect antifungal effects as described by Munhuweyi et al. [78]: halving of mycelial growth of *C. granati*, *Botrytis* sp., and *Penicillium* sp. is validated by *in vitro* trial; *in vivo* assay displays a reduction by 18–66% of rot incidence caused by the same fungi. Main postharvest fungal pathogens of pomegranates, such as *Botrytis* spp., *Penicillium* spp., and *C. granati*, are controlled by using chitosan concentration between 0.5 and 2.2 g/L, with an efficacy comparable to fludioxonil chemical control [78]. Chitosan could be applied as fruit coating also. It changes respiration and transpiration rate, so delaying ripening, reducing decay incidence, and maintaining qualitative parameters till 6 months [93]. However, its employment is more popular regarding fresh arils. Often chitosan effectiveness is improved by combination with essential oils for both whole fruit and arils [78, 94]. On the other hand, geraniol, thymol, and eugenol have recently been approved, indeed no reliable data are available, although preliminary *in vitro* trials regarding their efficacy is in progress [95].

Chitosan is also usable combined with sulfur nanoparticles, this element is historically known for its antifungal properties: Greek and Romans used it as drug and disinfectant [96, 97]. Its broad-spectrum efficacy contributed to sulfur success, which over time has been included in other fungicide formulations, such as nanoparticles and dots, often displaying stronger antimicrobial activity and greater environmental friendliness [96, 98, 99]. Sulfur success is related to wide efficacy, high sensitivity, and selectivity to fungi, in addition, fungal resistance due to its employment is limited. Based on low toxicity to humans and animals and rapid dissipation, elemental sulfur is safe enough also, but hydrogen sulfide and sulfur dioxide are hazardous to humans and animals [97]. Common sulfur mode of action is not clearly understood; the most popular theory suggests involvement of sulfur permeability of fungal cell walls due to ergosterol content. Hence, sulfur passes into the cell cytoplasm and damages both cytochrome *b* and *c* and the proteic and non-proteic sulfhydryl groups implicated in the mitochondrial electron transport chain [97].

4. Conclusions

Although in recent years pomegranate is worldwide spreading due to high market value and nutraceutical properties, it is still considered a minor crop, so conventional and alternative fungicides registered for this crop are scarce. This entails important postharvest yield and economic losses mainly related to fungal diseases. Albeit losses are evident in the post-harvest phase, most of the infections start in the field during the blooming stage, so preharvest treatments are needed to control them. Remaining infections are caused by wound pathogens that attack fruit through injuries; these infections being reparteed along the whole processing chain imply a particular care of fruit from harvest to the retail. The effects of good agronomical practices, preharvest treatments during the blooming stage, fruit sorting along the production chain, optimal storage conditions, and good hygiene conditions decrease the incidence of postharvest pomegranate rots and extend commercial pomegranate availability. Alternative control means deeply supporting the reduction of postharvest pomegranate disease incidence defending human and animal health by both fungicide residues and fungal mycotoxins and ensuring a One Health approach to saving food waste production.

Acknowledgements

This work was conducted within PRIMA StopMedWaste “Innovative Sustainable technologies to extend the shelf-life of Perishable Mediterranean fresh fruit, vegetables, and aromatic plants and to reduce WASTE,” which is funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA), Project ID: 1556, a program supported by the European Union, and Euphresco Basics “Basic substances as an environmentally friendly alternative to synthetic pesticides for plant protection” projects. Authors aknowledge “Masseria Fruttirossi Srl” (Castellaneta, Taranto, Italy), for their collaboration in the research activities.

Appendices and nomenclature

EU	European Union
HK	hybrid histidine kinases
HOG	high osmolarity glycerol
HS code	Harmonized Code
IARC	International Agency for Research on Cancer
MRL	maximum residue limit
MSP	multistep phosphorelay systems
OTA	ochratoxin A
PDA	potato dextrose agar
RH	relative humidity
UNO	United Nations Organization

Author details

Annamaria Mincuzzi* and Antonio Ippolito
Department of Soil, Plant, and Food Sciences, University of Bari Aldo Moro, Bari, Italy

*Address all correspondence to: annamaria.mincuzzi@uniba.it

References

[1] Teixeira da Silva JA, Rana TS, Narzary D, Verma N, Meshram DT, Ranade SA. Pomegranate biology and biotechnology: A review. Scientia Horticulturae. 2013;**160**:85-107

[2] Stover E, Mercure EW. The pomegranate: A new look at the fruit of paradise. HortScience. 2007;**42**(5):1088-1092

[3] Chandra R, Babu KD, Jadhav VT, Jaime A, Silva TD. Origin, history and domestication of pomegranate. Fruit, Vegetable and Cereal Science and Biotechnology. 2010;**4**(2):1-6

[4] Holland D, Hatib K, Bar-Ya'akov I. Pomegranate: Botany, horticulture, breeding. In: Janick J, editor. curatore. Horticultural Reviews [Internet]. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2009. pp. 127-191

[5] Wetzstein HY, Ravid N, Wilkins E, Martinelli AP. A morphological and histological characterization of bisexual and male flower types in pomegranate. Journal of the American Society for Horticultural Science. 2011;**136**(2):83-92

[6] Mincuzzi A, Sanzani SM, Palou L, Ragni M, Ippolito A. Postharvest rot of pomegranate fruit in Southern Italy: Characterization of the Main Pathogens. Journal of Fungi. 2022;**8**(5):475

[7] Parvizi H, Sepaskhah AR, Ahmadi SH. Effect of drip irrigation and fertilizer regimes on fruit yields and water productivity of a pomegranate (*Punica granatum* (L.) cv. Rabab) orchard. Agricultural Water Management. 2014;**146**:45-56

[8] Dhillon WS, Gill PPS, Singh NP. Effect of nitrogen, phosphorus and potassium fertilization on growth, yield and quality of pomegranate 'Kandhari'. Acta Horticulturae. 2011;**890**(890):327-332

[9] Korkmaz N, Aşkın MA. Effects of calcium and boron foliar application on pomegranate (*Punica granatum* L.) fruit quality, yield, and seasonal changes of leaf mineral nutrition. Acta Horticulturae. 2015;**1089**:413-422

[10] Davarpanah S, Tehranifar A, Abadía J, Val J, Davarynejad G, Aran M, et al. Foliar calcium fertilization reduces fruit cracking in pomegranate (*Punica granatum* cv. Ardestani). Scientia Horticulturae. 2018;**230**:86-91

[11] Wink M. Current understanding of modes of action of multicomponent bioactive phytochemicals: Potential for nutraceuticals and antimicrobials. Annual Review of Food Science and Technology. 2022;**13**(1):337-359

[12] Pienaar L. The Economic Contribution of South Africa's Pomegranate Industry [Internet]. Stellenbosch, South Africa: Elsenburg: WCDOA; 2021. Available from: https://www.sapomegranate.co.za/wp-content/uploads/2021/05/PomegranteReport_2021F.pdf [Accessed: 2022-09-20]

[13] Manjunatha N, Sharma J, Pokhare SS, Agarrwal R, Patil PG, Sirsat JD, et al. Characterization of *Alternaria* and *Colletotrichum* Species Associated with Pomegranate (*Punica granatum* L.) in Maharashtra State of India. Journal of Fungi. 2022;**8**(10):1040

[14] Kahramanoglu İ. Trends in pomegranate sector: Production, postharvest handling and marketing. International Journal of Agriculture

Forestry and Life Sciences. 2019;**3**(2):239-246

[15] Özgüven AI, Gültekİn U, GözlekçİS, YİlmazI, YİlmazC, Küçük E, et al. A review of the economics and the marketing of the pomegranate industry in Turkey. Acta Horticulturae. 2015;**1089**:221-228

[16] Stiletto A, Trestini S. Factors behind consumers' choices for healthy fruits: a review of pomegranate and its food derivatives. Agricultural Economics. 2021;**9**(1):31

[17] Istat.it. [Internet]. Available from: https://www.istat.it/ [Accessed: 2022-11-13]

[18] Pienaar L, Vanessa B-J. The economic contribution of South Africa's pomegranate industry. Ağrı. 2021;**18**(4):57-64

[19] Mincuzzi A. personal communication

[20] Murthy DS, Gajanana TM, Sudha M, Dakshinamoorthy V. Marketing and post-harvest losses in fruits: Its implications on availability and economy. Indian Journal of Agricultural Economics. 2009;**64**(2):259-275

[21] Shanabhoga MB, Suresha SV, Varadaraju GM, Dechamma S. Marketing of pomegranate by the growers using public and private extension service. Research Journal of Agricultural Sciences. 2017;**8**:705-707

[22] Opara IK, Fawole OA, Kelly C, Opara UL. Quantification of on-farm pomegranate fruit postharvest losses and waste, and implications on sustainability indicators: South African case study. Sustainability. 2021;**13**(9):5168

[23] Opara IK, Fawole OA, Opara UL. Postharvest losses of pomegranate fruit at the packhouse and implications for sustainability indicators. Sustainability. 2021;**13**(9):5187

[24] Myresiotis CK, Testempasis S, Vryzas Z, Karaoglanidis GS, Papadopoulou-MourkidouE.Determination of mycotoxins in pomegranate fruits and juices using a QuEChERS-based method. Food Chemistry. 2015;**182**:81-88

[25] Gat T, Liarzi O, Skovorodnikova Y, Ezra D. Characterization of alternaria alternata causing black spot disease of pomegranate in Israel using a molecular marker. Plant Disease. 2012;**96**(10):1513-1518

[26] Aloi F, Riolo M, Sanzani SM, Mincuzzi A, Ippolito A, Siciliano I, et al. Characterization of *Alternaria* species associated with heart rot of pomegranate fruit. Journal of Fungi. 2021;**7**(3):172

[27] Tziros GT, Lagopodi AL, Tzavella-Klonari K. *Alternaria alternata* fruit rot of pomegranate (*Punica granatum*) in Greece. Plant Pathology. 2008;**57**(2):379

[28] Luo Y, Hou L, Förster H, Pryor B, Adaskaveg JE. Identification of *Alternaria* species causing heart rot of pomegranates in California. Plant Disease. 2017;**101**(3):421-427

[29] Berbegal M, López-Cortés I, Salazar D, Gramaje D, Pérez-Sierra A, Garcia-Jimenez J, et al. First report of *Alternaria* black spot of pomegranate caused by Alternaria alternata in Spain. Plant Disease. 2014;**98**(5):689-689

[30] Singh NK, Patel DB, Chaudhari SR, Morad BG, Rabari SM, Chattopadhyay A, et al. Characterization of the plant pathogenic isolates of *Alternaria burnsii*. National Academy of Agricultural Science. 2016;**34**(6):1461-1465

[31] Garganese F, Schena L, Siciliano I, Prigigallo MI, Spadaro D, De Grassi A,

et al. Characterization of citrus-associated *Alternaria* species in Mediterranean areas. PLoS One. 2016;**11**(9):e0163255

[32] Palou L, Taberner V, Guardado A, Del Río MÁ, Montesinos-Herrero C. Incidence and etiology of postharvest fungal diseases of pomegranate (*Punica granatum* cv Mollar de Elche) in Spain. Phytopathologia Mediterranea. 2013;**52**(3):478-489

[33] Bardas GA, Tzelepis GD, Lotos L, Karaoglanidis GS. First report of *Botrytis cinerea* causing gray mold of pomegranate (*Punica granatum*) in Greece. Plant Disease. 2009;**93**(12):1346-1346

[34] Tedford EC, Adaskaveg JE, Ott AJ. Impact of Scholar (a new postharvest fungicide) on the California pomegranate industry. Phytopathology News. 2005;**39**(2):19

[35] Testempasis S, Puckett RD, Michailides TJ, Karaoglanidis GS. Genetic structure and fungicide resistance profile of *Botrytis* spp. populations causing postharvest gray mold of pomegranate fruit in Greece and California. Postharvest Biology and Technology. 2020;**170**:111319

[36] Alvarez LV, Groenewald JZ, Crous PW. Revising the Schizoparmaceae: *Coniella* and its synonyms *Pilidiella* and *Schizoparme*. Studies in Mycology. 2016;**85**(1):1-34

[37] Palou L, Guardado A, Montesinos-Herrero C. First report of *Penicillium* spp. and *Pilidiella granati* causing postharvest fruit rot of pomegranate in Spain. New Disease Reports. 2010;**22**(21):2044-0588

[38] Thomidis T. Pathogenicity and characterization of *Pilidiella granati* causing pomegranate diseases in Greece. European Journal of Plant Pathology. 2015;**141**(1):45-50

[39] Jabnoun-Khiareddine H, Ibrahim N, Aydi Ben Abdallah R, Mars M, Daami-Remadi M. Response of Tunisian pomegranate (*Punica granatum* L.) cultivars and several plant hosts to *Coniella granati* (Saccardo). The Horticulture Journal. 2018;**5**:2376-0354

[40] Lennox CL, Mostert L, Venter E, Laubscher W, Meitz-Hopkins JC. First report of *Coniella granati* fruit rot and dieback on pomegranate in the western cape of South Africa. Plant Disease. 2018;**102**(4):821

[41] Joshi MS, Sawant DM, Gaikwad AP. Isolate variations in *Colletotrichum gloeosporioides* infecting pomegranate. The Journal of Plant Protection Sciences. 2014;**6**(1):21-26

[42] Silva-Cabral JRA, Batista LRL, de Costa JO, de Ferro MM, Silva SJC, Lima de GA, et al. First report of *Colletotrichum tropicale* causing anthracnose on pomegranate in Brazil. Plant Disease. 2019;**103**(3):583-583

[43] Silva-Cabral JRA, Batista LRL, de Oliveira Costa JF, da Rocha Amorim EP, de Andrade Lima GS, Assunção IP. Three species of *Colletotrichum* are associated with anthracnose of pomegranate in Brazil. Comunicata Scientiae. 2020;**11**:e3126-e3126

[44] Cara M, Mincuzzi A, Merkuri J, Vrapi H, Cara O, Ippolito A, et al. *Colletotrichum gloeosporioides sensu stricto* as causal agent of anthracnose on pomegranate fruit in Albania. Crop protection. 2020;**137**:105291

[45] dos Santos Vieira WA, Bezerra PA, da Silva AC, Veloso JS, Câmara MPS, Doyle VP. Optimal markers for the identification of Colletotrichum species. Molecular Phylogenetics and Evolution. 2020;**143**:106694

[46] Huang S, Li T, Yang T, Zheng X, Yang D, Fu G, et al. First report of pulp rot in externally asymptomatic pomegranate fruit caused by *Talaromyces albobiverticillius* in Henan Province, China. Plant Disease. 2022;**106**(7):1989

[47] Mincuzzi A, Ippolito A, Montemurro C, Sanzani SM. Characterization of *Penicillium s.s.* and *Aspergillus* sect. *nigri* causing postharvest rots of pomegranate fruit in Southern Italy. International Journal of Food Microbiology. 2020;**314**:108389

[48] Rand TG, Giles S, Flemming J, Miller JD, Puniani E. Inflammatory and cytotoxic responses in mouse lungs exposed to purified toxins from building isolated *Penicillium brevicompactum* Dierckx and *P. chrysogenum* Thom. Toxicological Sciences. 2005;**87**(1):213-222

[49] Bardas GA, Tzelepis GD, Lotos L, Karaoglanidis GS. First report of *Penicillium glabrum* causing fruit rot of pomegranate (*Punica granatum*) in Greece. Plant Disease. 2009;**93**:1347

[50] Hammerschmidt L, Wray V, Lin W, Kamilova E, Proksch P, Aly AH. New styrylpyrones from the fungal endophyte *Penicillium glabrum* isolated from *Punica granatum*. Phytochemistry Letters. 2012;**5**(3):600-603

[51] Labuda R, Hudec K, Piecková E, Mezey J, Bohovič R, Mátéová S, et al. *Penicillium implicatum* causes a destructive rot of pomegranate fruits. Mycopathologia. 2004;**157**(2):217-223

[52] Ezra D, Kirshner B, Hershcovich M, Shtienberg D, Kosto I. Heart rot of pomegranate: Disease etiology and the events leading to development of symptoms. Plant Disease. 2015;**99**(4):496-501

[53] Khokhar I, Bajwa R, Nasim G. New report of *Penicillium implicatum* causing a postharvest rot of pomegranate fruit in Pakistan. Australasian Plant Disease Notes. 2013;**8**(1):39-41

[54] Houbraken J, Frisvad JC, Samson RA. Taxonomy of *Penicillium* section *citrina*. Studies in mycology. 2011;**70**:53-138

[55] Pfohl-Leszkowicz A, Manderville RA. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Molecular nutrition & food research. 2007;**51**(1):61-99

[56] Pascual-Ahuir A, Vanacloig-Pedros E, Proft M. Toxicity mechanisms of the food contaminant citrinin: Application of a quantitative yeast model. Nutrients. 2014;**6**(5):2077-2087

[57] Pyrri I, Visagie CM, Soccio P, Houbraken J. Re-evaluation of the taxonomy of *Talaromyces minioluteus*. Journal of Fungi. 2021;**7**(11):993

[58] Vesth TC, Nybo JL, Theobald S, Frisvad JC, Larsen TO, Nielsen KF, et al. Investigation of inter- and intraspecies variation through genome sequencing of *Aspergillus* section *Nigri*. Nature Genetics. 2018;**50**(12):1688-1695

[59] Perrone G, Gallo A. *Aspergillus* species and their associated mycotoxins. In: Moretti A, Susca A, editors. Mycotoxigenic Fungi [Internet]. New York, NY: Springer; 2017. pp. 33-49. (Methods in Molecular Biology; Vol. 1542 Available: http://link.springer.com/10.1007/978-1-4939-6707-0_3

[60] Yehia HM. Heart rot caused by *Aspergillus niger* through splitting in leathery skin of pomegranate fruit. African Journal of Microbiology Research. 2013;**7**:834-837

[61] Pala H, Tatli A, Yilmaz C, Özgüven AI. Important diseases of pomegranate fruit and control possibilities

in Turkey. In: I International Symposium on Pomegranate and Minor Mediterranean Fruits 818. 2006. pp. 285-290

[62] Thomidis T. Fruit rots of pomegranate (cv. wonderful) in Greece. Australasian Plant Pathology. 2014;**43**(5):583-588

[63] Munhuweyi K, Lennox CL, Meitz-Hopkins JC, Caleb OJ, Opara UL. Major diseases of pomegranate (*Punica granatum* L.), their causes and management—A review. Scientia Horticulturae. 2016;**211**:126-139

[64] Jatoi GH, Muhammad S, Metlo WA, Al-Ani LKT, Haseenullah Abro MA, Gadhi MA, et al. Efficacy of different essential oils, fungicides and biocontrol agents against *Aspergillus niger* the causal agent of fruit rot in Pomegranate International. Journal of Biosciences. 2020;**16**(3):51-65

[65] Guo MJ, Wang QT, Cheng YH, Hou CL. Identification of *Aspergillus tubingensis* causing pomegranate fruit rot in China. Australasian Plant Pathology. 2021;**50**(2):233-240

[66] Peduto Hand F, Choudhury RA, Gubler WD. First report of *Cytospora punicae* causing wood canker and branch dieback of pomegranate (*Punica granatum*) in the United States. Plant Disease. 2014;**98**(6):853-853

[67] Venter E, Lennox CL, Meitz-Hopkins JC. First report of *Cytospora punicae* causing post-harvest fruit rot on pomegranate in South Africa. Plant Disease. 2017;**101**(4):631-631

[68] Palavouzis SC, Tzamos S, Paplomatas E, Thomidis T. First report of *Cytospora punicae* isolated from pomegranate plants with symptom of collar rot in northern Greece. Journal of Plant Pathology. 2015;**97**(1):216

[69] Samouel S, Kanetis L. First report of *Cytospora punicae* causing trunk canker of pomegranate (*Punica granatum*) in Cyprus. Plant Disease. 2016;**100**(1):222-222

[70] Ammar MI, El-Naggar MA. Screening and characterization of fungi and their associated mycotoxins in some fruit crops. International Journal of Advanced Research. 2014;**2**(4):1216-1227

[71] Benfradj N, Mannai S, Fathallah A, Boughalleb-M'Hamdi N. First report of *Fusarium oxysporum* and *Globisporangium ultimum* as causal agents of pomegranate fruit rot in Tunisia. New Disease Reports. 2022;**45**(1):e12055

[72] Shi W, Tan Y, Wang S, Gardiner D, De Saeger S, Liao Y, et al. Mycotoxigenic potentials of *Fusarium* species in various culture matrices revealed by mycotoxin profiling. Toxins. 2016;**9**(1):6

[73] Zhou Y, Yang Y, Guo J, Bai J, Hu X. Black spot disease of pomegranate caused by *Cladosporium cladosporioides* in China. IOP Conference Series: Earth and Environmental Science. 2018;**170**:022131

[74] Kahramanoğlu İ, Aktaş M, Gündüz Ş. Effects of fludioxonil, propolis and black seed oil application on the postharvest quality of "Wonderful" pomegranate. PLoS One. 2018;**13**(5):e0198411

[75] Lufu R, Ambaw A, Opara UL. The influence of internal packaging (Liners) on moisture dynamics and physical and physiological quality of pomegranate fruit during cold storage. Food. 2021;**10**(6):1388

[76] Xavier KV, Kc AN, Vallad GE. Fungicide application timing essential for the management of leaf spot and fruit rot on pomegranate (*Punica granatum* L.) in Florida. Plant Disease. 2020;**104**(6):1629-1637

[77] Wang W, Fang Y, Imran M, Hu Z, Zhang S, Huang Z, et al. Characterization of the field fludioxonil resistance and its molecular basis in *Botrytis cinerea* from Shanghai Province in China. Microorganisms. 2021;**9**(2):266

[78] Munhuweyi K, Lennox CL, Meitz-Hopkins JC, Caleb OJ, Sigge GO, Opara UL. Investigating the effects of crab shell chitosan on fungal mycelial growth and postharvest quality attributes of pomegranate whole fruit and arils. Scientia Horticulturae. 2017;**220**:78-89

[79] Usanmaz S, Alas T. Degradation of postharvest applied fludioxonil in pomegranate fruit cv. Wonderful and efficacy on the gray mould. Journal of Stored Products and Postharvest Research. 2019;**10**(2):20-23

[80] Bersching K, Jacob S. The molecular mechanism of fludioxonil action is different to osmotic stress sensing. Journal of Fungi. 2021;**7**(5):393

[81] Hu S, Zhang J, Zhang Y, He S, Zhu F. Baseline sensitivity and toxic actions of boscalid against *Sclerotinia sclerotiorum*. Crop Protection. 2018;**110**:83-90

[82] EU Pesticides Database - European Commission [Internet]. Available from: https://ec.europa.eu› screen › mrls [Accessed: 2022-11-13]

[83] Brandhorst TT, Klein BS. Uncertainty surrounding the mechanism and safety of the post-harvest fungicide fludioxonil. Food and Chemical Toxicology. 2019;**123**:561-565

[84] European Food Safety Authority (EFSA). National summary reports on pesticide residue analysis performed in 2018. EFS3 [Internet]. 2020;**17**(4):1-228. Available from: https://data.europa.eu/doi/10.2903/sp.efsa.2020.EN-1814

[85] Calvo H, Mendiara I, Arias E, Blanco D, Venturini ME. The role of iturin A from *B. amyloliquefaciens* BUZ-14 in the inhibition of the most common postharvest fruit rots. Food Microbiology. 2019;**82**:62-69

[86] Elanchezhiyan K, Keerthana U, Nagendran K, Prabhukarthikeyan SR, Prabakar K, Raguchander T, et al. Multifaceted benefits of *Bacillus amyloliquefaciens* strain FBZ24 in the management of wilt disease in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Physiological and Molecular Plant Pathology. 2018;**103**:92-101

[87] Yamamoto S, Shiraishi S, Suzuki S. Are cyclic lipopeptides produced by *Bacillus amyloliquefaciens* S13-3 responsible for the plant defence response in strawberry against *Colletotrichum gloeosporioides*? Letters in Applied Microbiology. 2015;**60**(4):379-386

[88] Purrington CB. Costs of resistance. Current opinion in plant biology. 2000;**3**(4):305-308

[89] Bekir J, Bouajila J, Mars M. The effect of cultivar and ripening on antioxidant system and PAL activity of pomegranate (*Punica Granatum* L.) grown in Tunisia. Journal of Food Biochemistry. 2015;**39**(5):575-584

[90] Romanazzi G, Moumni M. Chitosan and other edible coatings to extend shelf life, manage postharvest decay, and reduce loss and waste of fresh fruits and vegetables. Current Opinion in Biotechnology. 2022;**78**:102834

[91] Velásquez CL, Avelizapa LR. A Review on the physicochemical and biological aspects of the chitosan antifungal activity in agricultural applications. Journal of Research Updates in Polymer Science. 2020;**9**:70-79

[92] Shahrajabian MH, Chaski C, Polyzos N, Tzortzakis N,

Petropoulos SA. Sustainable agriculture systems in vegetable production using chitin and chitosan as plant biostimulants. Biomolecules. 2021;**11**(6):819

[93] Candir E, Ozdemir AE, Aksoy MC. Effects of chitosan coating and modified atmosphere packaging on postharvest quality and bioactive compounds of pomegranate fruit cv. 'Hicaznar'. Scientia Horticulturae. 2018;**235**:235-243

[94] Singla M, Pareek S, Kumar N, Sagar NA, Fawole OA. Chitosan-cinnamon oil coating maintains quality and extends shelf life of ready-to-use pomegranate arils under low-temperature storage. Journal of Food Quality. 2022;**2022**:1-18

[95] Ranjbar A, Ramezanian A, Shekarforoush S, Niakousari M, Eshghi S. Antifungal activity of thymol against the main fungi causing pomegranate fruit rot by suppressing the activity of cell wall degrading enzymes. LWT - Food Science and Technology. 2022;**161**:113303

[96] Kim YH, Kim GH, Yoon KS, Shankar S, Rhim JW. Comparative antibacterial and antifungal activities of sulfur nanoparticles capped with chitosan. Microbial Pathogenesis. 2020;**144**:104178

[97] Griffith CM, Woodrow JE, Seiber JN. Environmental behavior and analysis of agricultural sulfur: Environmental behavior and analysis of agricultural sulfur. Pest Management Science. 2015;**71**(11):1486-1496

[98] Shankar S, Jaiswal L, Rhim JW. New insight into sulfur nanoparticles: Synthesis and applications. Critical Reviews in Environmental Science and Technology. 2021;**51**(20):2329-2356

[99] Priyadarshi R, Riahi Z, Rhim JW, Kim JT. Comparison of physicochemical and functional properties of elemental sulfur, sulfur nanoparticles, and sulfur quantum dots for sustainable biological applications. Materials Today Sustainability. 2022;**20**:100243