

## Short Communication

# Heart rot caused by *Aspergillus niger* through splitting in leathery skin of pomegranate fruit

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**The pomegranate trees are not affected by any serious disease but the fruit can be damaged by heart rot caused by different fungi and bacteria or after invasion of the insect. Twenty-six (26) samples of splitting pomegranate fruits from different orchards near Cairo, Egypt were examined, and they showed that they contain a spore of *Aspergillus niger* which may reach the heart of the fruits during the period of growth until harvesting the mature fruits. The aim of the study was to prove if the infection happened during blooming or through splitting of leathery skin and other microorganism's subsequently the fungi, such as *Erwinia* sp. and *Saccharomyces* sp.**

**Key words:** Pomegranate, disease, *Aspergillus niger*, split, heart rot.

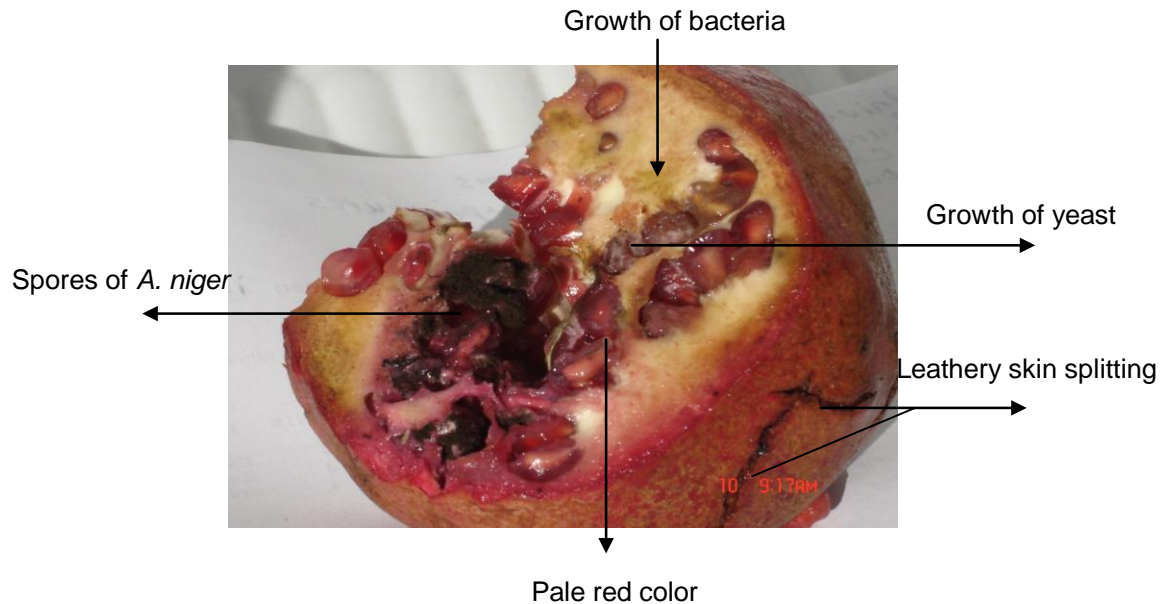
## INTRODUCTION

The pomegranate, *Punica granatum*, a popular fruit and ornamental of Mediterranean peoples for centuries, is a native from Iran to Himalaya that has been grown in Southern Nevada since pioneer days. The name pomegranate comes from a Latin word meaning apple with many seeds. The fruit which is about the size of an apple is depicted in ancient Assyrian and Egyptian Sculpture and is one of the oldest fruits used by man. It has been widely cultivated by man. It has been widely cultivated throughout India and drier parts of Southern Asia, Malaysia, the East India and tropical Africa. The most important growing region is Egypt, China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, India, Burma and Saudi Arabia (Morton, 1987). In Turkey, Pomegranate's economical value has been recognized in recent years as the production have reached to 208,502 tons in 2010 from 80,000 tons in 2005 (TSI, 2011).

There is no fruit crop that has high medicinal value compared to that in pomegranate. It has some cultural significance in Iran (it signifies immortality, fertility, or reproductive energy), which is found in the central courtyard of every home on the Iranian plateau. Pomegranate juices, either fresh or in the form of grenadine, is a common souring agent in western Asia and may used, example, in the Turkish or Arabic salad (tabuleh) made from precooked wheat (bulgar), parsley

and possibly raw vegetables. Lastly, dried pomegranate seeds make an interesting alternative for raisins in cakes and other European sweets, Damania (2005).

Perhaps the most serious problem with pomegranate is the occurrence of a fungal disease which affected both the leaves and the fruit, causing premature leaf loss and also resulting in fruit rot on the plant. While the leaf drop may begins to mature. Dry rot caused by *Phomopsis* sp. or *Zythia versoniana* may destroy as much as 80% the crop, unless these organisms are controlled by appropriate spraying measures. Excessive rain during the ripening season may induce soft rot. A post harvest rot caused by *Alternaria solani* was observed in India on 1970. It is particularly prevalent in cracked fruits. Discoloration of fruits and seeds results from infestation by *Aspergillus castaneus* (Morton, 1987). Seeram et al. (2006) declared that the fungi caused decay of arils of pomegranate fruit ranging from sections to all the arils within the rind without external symptoms except for slightly abnormal skin color or soft spot (The lack of obvious external symptoms makes black heart identification a challenge for sorters in the packinghouse or processing line. They also mentioned that a fast non-destructive technique capable of probing the interior of pomegranate and detecting the infection was needed for fruit used for the fresh market and processing. If the



**Figure 1.** *A. niger* (hypha and black spores) distributed in the pomegranate seed and subsequently the growth of bacteria and yeast and color changes.

alteration in cell structure and tissue constituents in arils could be identified after fungal infection, it would be possible to detect the black heart in pomegranate caused by *Alternaria* spp. and *Aspergillus* spp.

The aim of this study was to prove which way *Aspergillus niger* can invade the fruits of *P. granatum*; though splitting happened in the leathery skin or during blooming season and cause heart rot disease.

## MATERIALS AND METHODS

*A. niger* was isolated from pomegranate on potato dextrose agar (PDA) and stored at 4°C. *Erwinia* sp. was isolated on nutrient agar and *Saccharomyces* sp. was isolated on Sabouraud agar. The pomegranate fruits were collected from different orchards near Cairo, Egypt and observation was on the sample which had a splitting in its leathery skin. By using scalpel the cross section was done in the fruits. Photographic pictures were taken for the section by a digital camera (Sony Cybershot, 5.1 mega pixel). The fungi, its spores, bacteria and yeast were examined by compound microscope.

## RESULTS AND DISCUSSION

Different examination cross sections of the 26 samples of splitting pomegranate fruits is shown in Figure 1, it was found under microscope that fruits contained spores of *A. niger*, also, the crust of the fruit had a longitudinal and transfer splitting in its leathery skin. Following how the fungus could reach to the heart of the fruit, changed the shape of the fruits and the color of the seeds from its bright red to pale red or brown. It could be concluded that *A. niger* fungus has two ways to reach the heart of the

pomegranate fruit and caused the disease to the fruits, first during consisting of the bloom by spreading the spores through storm or the rain droplet, second by invasion through splitting incident of the leathery skin.

It has been estimated that microbial pectinases account for 25% of the global food enzymes sales. Microbial pectinase can be produced from bacteria including actinomycetes, yeast and fungi. *A. niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes (Sharma et al. 2011). The fruit rot of *P. granatum* was also followed by the growth of yeast such as *Saccharomyces* sp. also bacteria as *Erwinia* sp., the two kinds of microorganisms also have pectinolytic activity.

The physiological disease of pomegranate can cause a fruit splitting in a small and big fruits. Many people's thought that this splitting could be due to the water humidity or the imbalance in water activity between earth and weather. They actually split when the storm blow due to the increase of the growth of the crust and resulted in a high pressure from inside the fruit to outside. It was found also that nitrogen fertilizers and its care with regular irrigation lead to decrease of splitting ratio.

Although the pomegranate tree is very drought-tolerant; it needs adequate moisture to produce good crops. Its summer water requirements are considered by some overseas authorities to be almost as great as those of citrus. Adequate soil moisture as fruit approaches maturity is said to reduce the susceptibility of the fruit to splitting. Overseas experience indicates that fully grown trees will benefit from one or more applications of fertilizer which in total provide 0.25 to 0.5 kg nitrogen

annually. Nitrogen application should be split in lighter soils. Timing of application should be strategic to prevent excessive growth, delayed ripening and poor coloring of the fruit (Johnson, 1983)

Sharma et al. (1982) isolated *Aspergillus varicolor* from pomegranate (*P. granatum* L.) and revealed a causative disease characterized by softening of rind and underlying pulp. The affected rind initially turns brown and then blackish brown at advanced stage of infection. They reported that *Aspergillus* namely, *awamori*, *A. fumigatus*, *A. flavus*, *A. niger* and *Drechslera rostrata* have caused fruit rot of *P. granatum*. The pomegranate is not affected by any serious insects or disease if the hulls and debris are removed each year after harvest. The fruit can be damaged by heart rot caused by *Alternaria* fungus. Heart rot infections take place in the bloom by spreading of the spores to the interior of the fruits. The central cavity of the infected fruit can be partially or totally decayed while the rind remains unaffected. There is no chemical control recommendation currently, however, removal of old fruits from plants during pruning may help to eliminate sources of fungus as well as shoot dieback for the following year. Rain during blooming season seems to promote more infection (Crites et al., 2004). Different postharvest disease such as *A. niger*, *Botrytis cinerea*, and *Alternaria* sp. cause the fruit to split before ripening (Tous and Louise, 1996). The practice of picking early in the season to avoid fruit cracking is known to result in poor quality fruit pomegranate (Kumar and Mohan, 1990). Heart rot this may be caused by *Aspergillus* sp. and *Alternaria* sp. Affected fruit showed a slightly abnormal skin color, and internally a mass of blackened arils. The disease develops while the fruit is on the tree. Affected pomegranate can be detected and removed by sorters in the packinghouse.

Pruning is necessary for good fruit production. If a tree-type plant is desired, then prune to a single trunk. Basal shoots will continue to appear and must be removed each year (Crites et al., 2004).

*Alternaria alternata* has been reported to cause a leaf spot disease on pomegranate in India (Madhukar and Reddy, 1976). However, fruit rot is not included in this report. Fruit rot caused by *Alternaria* sp. has previously been reported in USA, Mexico (Farr et al., 2007) and as a postharvest disease in Greece (Julia et al., 1973). Although this disease has been detected before in Greece, it was noticed again only recently probably due to the recent expansion of pomegranate cultivation and changes in weather conditions. This is the first report of *A. alternata* species causing fruit decay on pomegranate in Greece. Pomegranates are marketed as intact fresh fruit, extracted arils, or juice. Fruit diseases such as black heart caused by *Alternaria* spp., *Aspergillus* and *Penicillium* rots, are considered the most important diseases of pomegranate fruit (Michailides et al., 2012).

In Sri Lanka, there are several fungus diseases which are economically important in pomegranate cultivation

and are detailed below as follows: Anthracnose fruit rot caused by *Sphaceloma punica*, Aspergillious fruit rot caused by *Aspergillus* sp. and Penicillium fruit rot caused by *Penicillium* sp. Zhang et al. (2012) explained that, Black heart, which has also known as "heart rot", is a major pomegranate disease impacting production in California. Although black heart is always recognized as a postharvest quality problem, the infection begins in the orchard. Their results agreed with our finding that the main heart rot was caused by *Alternaria* spp. or *Aspergillus* spp. which enters the fruit during bloom and early fruit set, grows and spreads within the fruit as the fruit develops. After penetration into the host tissue, fungal pathogens attack the fruit by producing cuticle and cell wall degrading enzymes, toxins, and detoxifying resistance compounds in the host. They reported also that the host responded to the fungal infection, by producing antimicrobial compounds, such as phytoalexins and active oxygen species, reinforce the physical barrier, or initiate localized cell death to prevent the pathogen from spreading. The pathogen–host interaction may induce a number of alterations in the physiological and biochemical processes or in the host tissue constituents (Barkai-Golan, 2001).

## Conclusion

From the above discussion, it could be concluded that the pectinolytic microorganisms specially fungus as *A. niger* can invade pomegranate fruits through splitting or during blooming season and caused heart rot and followed by the growth of bacteria (*Erwinia* sp.) and yeast (*Saccharomyces* sp.).

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