Journal of Agricultural Technology 2015 Vol. 11(6):1371-1380
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

# First Record of Core Rot Disease on Apple Fruit cv. Anna 106 Local Cultivar in Egypt.

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El-Mohamedy R. S. Riad and El-Sayed H . Ziedan (2015) First Record of Core Rot Disease on Apple Fruit cv. Anna 106 Local Cultivar in Egypt. Journal of Agricultural Technology 11(6):1371-1380.

Fruits of apple plants (*Malus domestica* L.) cv. Anna 106 collected from different orchards and markets of Egypt were exhibited typical core rot disease symptoms at the first time in Egypt during 2012 and 2013seasons.The incidence of infection is much more prevalent in a wet spring. There are often no visible symptoms of disease until after harvest. In some cases, infected fruit may color and fall prematurely in the orchard. Disease symptoms include mold growth in the core region of the fruit. The rot does not spread into the flesh, and can be seen only when the fruit is cut open. Isolation trials yielded several fungi, i.e., *Alternaria* spp , *Penicillium* spp and *Fuarium* spp . *Alterania* spp. was the common fungal associated with diseased tissue. Pathogenicity test indicated the ability of the all *Alterania* spp. isolates to cause core rot disease with typical symptoms on apple fruits cv. Anna 106.The pathogen was isolated and identified as *Alternaria alternata* (Fr.:Fr.) Kiessl. According to the available literature, this is the first record of core rot on apple cv. Anna 106 in Egypt.

**Key words**: Core rot disease – Apple- *Alternaria alternate* .

## Introduction

Alternaria core rot is a major postharvest disease of apple fruit in world wide of apple cultivations. Core rot is a post-harvest disease, with three symptoms, namely mouldy core (MC), dry core rot (DCR) and wet core rot (WCR).These

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symptoms are caused by various pathogenic fungi, including Alternaria and Penicillium (Serdani *et al.,*1998).Core rot occurs worldwide in susceptible apple cultivars such as 'Starking' and 'Red Delicious'. These cultivars have a wider, open calyx tube which results in an open core area.. The disease isn't noticed until the fruit are cut open (Serdani *et al*.,1998 ). Ntasiou *et al*., (2015) indicate that Alternaria core rot represents a major threat of apple fruit production not only due to quantitative yield losses but also for qualitative deterioration of apple by-products. Although MC is not economically important, DCR and WCR are, as they affect the flesh of the fruit .The moldy core fungi colonize the flower parts as soon as the blossoms open. The fungi then enter the developing fruit through an opening in the calyx. Moldy core is primarily a problem during years with light fruit set or when dry weather in early summer is followed by heavy rains in late summer. In addition, wet weather during bloom may cause conditions favorable for the fungi to produce spores. Moldy core is characterized by the growth of fungus mycelium within the locules (the seed cavity), without penetration into the flesh of the fruit. External symptoms are rare, except infected fruit may color and fall prematurely.

*Alternaria* spp. have been associated with several economically important plant diseases (Rotem, 1994), and have also been cited as the main cause of dry core rot of apples (Combrink and Ginsburg, 1973; Ellis and Barrat, 1983; Combrink *et al*., 1985a, 1985b; De Kock *et.al.,* 1991). Gao *et al.,* (2013) found that *Alternaria alternata, A. tenuissima, A. arborescens, Cladosporium cladosporioides,* and *C. tenuissimum* were the main pathogens causing core browning and moldy core. They also noted that fungi associated with core rot of 'Starking' apple fruit in South Africa included *Alternaria alternata,*

*Pleospora herbarum, Coniothyrium* sp., *Penicillium funiculosum, P. expansum, P. ramulosum***.** Fugler,(1990) noted that Core rot of red apple cultivars is presently regarded as one of the most serious diseases of harvested apples in South Africa, resulting in huge annual losses of income. A number of fungi cause fruit infection by first colonizing or infecting the blossoms .Varieties with an open sinus extending from the calyx to the core are more susceptible to moldy core.

The present investigation was aimed to isolate and identifies the causal pathogens causing core rot disease on apple fruits cv. Anna 106 collected from various apple orchards after harvesting as well as from different marketing of Egypt .

## Material and Methods

### *Isolation and identification of causal agents*

Samples of apple fruits were collected during harvesting in 2012 and 2013 from three commercial orchards (OR1 , OR2 and OR3 ) of apple c.v. Anna 106 a local variety of Egypt during the harvesting season; the trees were approximately 8 to 12 years old and these orchards with history of fruit fail before maturity stage Harvested fruit were stored at 4°C before the isolations were conducted.

Apple fruit were rinsed under running tap water and then allowed to air dry. Whole fruit were disinfested with 70% ethanol. The fruit were then dissected along the longitudinal axis to permit examination of the core region. Tissue showing signs of core rot or mouldy core was dissected, and plated onto 90 mm Petri dishes containing PDA. Dishes were incubated on the laboratory bench at 25 C, and emerging mycelia hyphal-tipped and transferred to PDA slants incubated at 25 C. For identification, fungal isolates were transferred to Petri

dishes containing potato carrot agar (PCA) (Simmons and Roberts, 1993), and were incubated for 7-10 days at 22 C under a 10/14 h cool-white fluorescent light/dark cycle. Isolates of *Alternaria* spp. were categorized into different groups according to their sporulation patterns at 50 x magnification, and conidium morphology (Simmons and Roberts, 1993). Isolates of *Penicillium* spp. were transferred to Czapek agar (Samson and Van Reenen-Hoekstra, 1988), and incubated on the laboratory bench. Colony margins were transferred onto (PDA) which as a part of the culture purification process by single spore isolation technique and fungal isolates were identified according to (Ellis, 1971).

***Pathogenicity test***

For inoculations, freshly harvested apple fruit c.v. Anna 106 were sterilized with 70% ethanol and cut into halves along the longitudinal axis with a flame-sterilized knife. Fruit halves were inoculated without additional wounding, using sterile swabs to gently daub the spore suspension into the core region, away from the area injured by the original cut. Spores were harvested from cultures by adding a small amount of sterile distilled water to each dish and gently rubbing the sporulation mycelia mats with a bent glass rod (18). The spore concentration was determined and adjusted with the aid of a hemacytometer to obtain a series of five suspensions, containing $1 \times 10^4$ to 1 × 108 spores/ml. Each apple was inoculated with several isolates from every species and each isolate inoculation was repeated three times in each of the 2 years of study. The control fruit were treated similarly to the inoculated fruit but with sterile water instead of spore suspensions. After inoculation, fruit were placed on trays, covered with plastic wrap to maintain high humidity, and held at 25°C in the dark. Fruit were examined daily and types of symptom expression were recorded.

## Results and Discussion

### Isolation, identification and pathogenicity test of the isolated fungi:

Samples of apple fruits cv. Anna 106 collected from different commercial orchards of apple with history of fruit fall prematurely in these orchards, as well as from two markets in Cairo , Egypt were used in isolation trials after cutting the fruits and observed core rot symptoms Fig(1) . Several fungi isolates of *Alternaria spp., Penicillium spp* . and *Fusarium spp* were isolated from diseased tissue of infected apple fruits showing core rot disease Table (1). Results show that 42 fungal isolates represented three genera *i.e*., *Alternaria* (21isolates) , *Penicillium* (19 isolates) , *Fusarium* (19 isolates) and unknown isolates (5 isolates) were isolated from the flesh of infected apple fruits in all samples .*Alternaria* spp. were the most dominate representing 50 % followed by *Penicillium* pp. and *Fusarium* spp. (19.04%) . The highest records of *Alternaria* spp . were noticed in Orchard 3 (O3) and market 2(M2) .On the basis of sporulation patterns and spore morphology, all isolates of *Alternaria* spp. were divided into two differ species *Alternaria alternatia* (15 isolates) and *Alternaria* spp .(6 isolates) .This findings are in agreement with Combrink *et al.,* 1985, they noted that several facultative parasitic fungi have been isolated from diseased apples, of which *Alternaria alternata* (FT.: Fr.) Kiessl. has proven to be the most dominant. Facultative parasites growing aerobically in the seed cavity, they eventually penetrate surrounding mesocarp tissue, are the main cause of core rot and in view of the prominent role, *Alternaria* spp. play in core rot (Combrink *et al*., 1985; Fugler,1990; De Kock *et al.,* 1991 ; Gao *et al.,* 2013) .

Table (1) Incidence of fungi recovered from apple fruit cv. Anna collected from different orchards and markets of Egypt during 2012/2013 seasons.

| Orchard/market | Total numbers and frequency (%)of isolated fungi | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Alternaria spp* | | *Penicillium spp* | | *Fusarium spp* | | others | | Total | |
| | No | % | No | % | No | % | No | % | No | % |
| Orchard A | 3 | 60.00 | 0 | 0.0 | 1 | 20.00 | 1 | 20.00 | 5 | 11.90 |
| Orchard B | 4 | 44.44 | 1 | 11.11 | 2 | 22.22 | 2 | 22.22 | 9 | 21.42 |
| Orchard C | 8 | 61.53 | 2 | 15.38 | 3 | 23.07 | 0 | 0.0 | 13 | 30.95 |
| Market 1 | 1 | 25.00 | 1 | 25.00 | 0 | 0.00 | 2 | 50.00 | 4 | 9.52 |
| Market 2 | 5 | 45.45 | 4 | 36.36 | 2 | 18.18 | 0 | 0.0 | 11 | 26.19 |
| Total | 21 | 50.00 | 8 | 19.04 | 8 | 19.04 | 5 | 14.28 | 42 | 100 |

Fig.1. Internal symptoms of apple core rot disease caused by *Alternaria alternata* (Fr.:Fr.)Kiessl

According to the numbers of isolated fungi from infected apples fruits , *Alternaria alternate* responsible of core rot disease of all sample ,as the highest number of this pathogens were isolated from infected fruits especially orchard three(O3) and market two (M2).

Pathogenic ability of five isolates of *Alternaria alternate* on apple fruits (cv,Anna ) was investigated Several isolates of *A. alternata* fungi was isolated

from diseased tissue of infected apple fruits showing core rot disease. All isolates were able to causing core rot of wounded. Fungal isolates varied of their pathological potential on apple fruits Table (2) .All isolates of *Alternaria alternate* induce typical core rot disease symptoms on apples fruits c.v. Anna 106 artificially inoculated with spore suspensions of each isolate with different degree of infection. *Alternaria alternate* isolate No.O3 was the aggressive isolate, caused high record of fruit infection. Our observation are clearly indicated that *Alternaria alternata* (Fr.) Keissler causing core rot disease of apple Fig (1). In this respect, *A. alternata* is common pathogen of apple core rot it is recorded a post- harvest disease of apple by many investigators (Combrink *et al*., 1985 ; Spotts, 1988 ; De Kock *et al*., 1991; Serdani,*et al*., 1998; Gao *et al*., 2013; Ntasiou *et al.,* 2015 ) .

Table (2) Pathogenicity of fungi isolated from core rotted apple fruits on apple fruits c.v. Anna local variety of Egypt.

| Fungi | % Core rot incidence after treatment | | | |
|---|---|---|---|---|
| | 7 day | | 14 day | |
| | No. of infected fruit | % infection | No. of infected fruit | % infection |
| *Alternaria alternata* O1 | 9b | 36 | 14b | 56 |
| *Alternaria alternata* O2 | 10c | 40 | 20c | 80 |
| *Alternaria alternata* O3 | 12c | 48 | 23c | 92 |
| *Alternaria alternate* M 1 | 7b | 28 | 15b | 60 |
| *Alternaria alternata* M 1 | 10c | 40 | 21c | 84 |
| Control | 00 a | 00 | 00a | 00 |

Figures with the same letters in each column are not significantly differed ($P \leq 0.05$)

Most apple cultivars susceptible to core rot have an open calyx tube,the pathogen enters the seed cavity via this route(Spotts, 1988). However,

Combrink *et al.* (1985) found that at harvest more than 50% of all fruit and seed cavities of apples with either an open (Starking) or a closed calyx tube (Golden Delicious and Granny Smith) were colonised by fungi, suggesting that other factors were also involved with disease development. Combrink (1983) speculated that the low fumaric and malic acid content of Top Red apples could predispose them to infection. Ellis and Barrat (1983) stated that disease development could possibly be accelerated by frost, while Miller (1959) found that a rupturing of the calyx tissue due to abnormally fast growth following heavy rains after a dry period also played a role. This observation was further substantiated by Combrink & al. (1985) who found growth cracks appearing in the cavities of Red Delicious types 2 wk. after full bloom, and a sporulating *Alternaria* sp. in the calyx tube 16 wk. after full bloom. Previous studies have suggested that infection may occur 3-6 wk. before harvesting , or during and shortly after full bloom (Ellis and Barrat, 1983; Combrink *et al*., 1985).Conidia of this fungus may accumulate on plant surfaces during dry weather, where they are able to survive ultraviolet radiation due to their pigmented, multi-celled conidia, thus accounting for elevated disease levels after dry periods. As soon as free moisture appears, these conidia may germinate, thus causing infection (Rotem and Aust, 1991; Rotem, 1994). According to, this is the first record of core rot on apple cv. Anna in Egypt . As far as the writers are aware and the available literature ,this is first record of *A. alternata* as a new pathogens causing core rot disease of apple fruit cv. Anna106 in Egypt.

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(received 30 July 2015; accepted 1 September 2015)