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# Occurrence of Pre-Harvest Grey Mold Disease of Persian lemon (Citruslatifolia) under field conditions in Egypt

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***Abstract- Citrus species are among the most important fruit trees in the world and are considered as a major export product of Egypt. The growing citrus trees in Egypt include Orange, Mandarin, Lime and lemon. Persian lime (Citrus latifolia) or Shiraz Limoo also known as Tahiti lime or Bearss lime (named after John T. Bearss), is a citrus fruit. During the winter season of 2014, grey mold disease symptoms were observed on seedless Persian lime fruits Citrus latifolia grown in Researches and Production Cultivation Station, National Research Centre located at Nuobariadistrict, Beheira governorate. Disease symptoms appear as a brown leathery decay of the peel, with gray brown to olive spore masses forming around the affected areas of the fruit surface. The Infected fruit turn into sponge's appearance as disease development. The causal fungus was isolated and identified as Botrytis cinerea. According to the available literature, this is thought to be the first record of Persian lemon grey mold disease in Egypt.In the field study, all Persian lime trees were sprayed twice with 15 days interval with the fungicide Kocide 101 (Copper Hydroxide) at the rate of 2.5 g/L. As a result no increase in grey mold incidence as well as disease development was observed even after the first spray application.***

***Index Terms-*** **Persian lemon, Citrus latifolia, grey mold, Botrytis cinerea, pre-harvest fruit diseases.**

## I. INTRODUCTION

Citrus fruits are recognized as an important component of the human diet, providing a variety of constituents important to human nutrition, including vitamin C (ascorbic acid), folic acid, potassium, flavonoids, coumarins, pectin and dietary fibers [1]. Flavonoids in citrus have a broad spectrum ofbiological activities including antibacterial, antioxidant, antidiabetic, anticancer, analgesic, anti-inflammatory and antianxiety [2]. Worldwide production of citrus reached more than 129 million tons from cultivated trees in 140 countries around the world [3]. The statistics Division of the Food and Agriculture Organization of the\United Nations [3] making citrus the leading cultivated tree crop. Citrus production in the Mediterranean Basin (22,441 thousand tons) is third only to China and Brazil [3]and accounts for about 20% of the world citrus production and about 60% of the world fresh citrus trade [4]. Spain is the leading producing country, whereas Italy and Egypt rank second and third, respectively. Egypt represents about 15% of the total citrus production in the Mediterranean Basin [4] and is considered the ninth largest citrus producer in the world with a global market share of 3.1% of the world citrus production [3]. The production is mainly composed of oranges, mandarins and limes, which represent more than 98.8% of the total citrus area [5].

Citrus is considered to be one of the major fruit crops produced in Egypt. The term citrus includes four different types of fruits, namely, oranges, mandarin/tangerine, lemon and grapefruit. The U.S.A. ranks first in citrus production followed by Brazil, Spain, and some Mediterranean countries. Citrus fruits vary in their relative susceptibility to chilling injury: grapefruits, lemons are much more susceptible to chilling in jury than are oranges and mandarins [6,7,8,9].

Postharvest diseases play a major role in limiting the postharvest life of citrus fruits. Blue mold and Green mold are the most important postharvest diseases of citrus fruits in all production areas. In some areas stem–end rots and anthracnose are important. Similarly, sour rot and phytophthora can be a problem during wet seasons [6,7,9,10].

Citrus fruit mold are the most economically important postharvest disease of citrus fruits in all production areas that, like Spain and other Mediterranean countries, are characterized by low summer rainfall [11,12,13]. Actual losses due to green mold are quite variable and, beyond postharvest factors, depend upon the area of production, citrus cultivar, weather and orchard conditions, and especially the extent of physical or mechanical injury to the fruit during harvest and subsequent handling. The mold fungi strict wound pathogens that can infect the fruit in the grove, the packinghouse, and during distribution and marketing. Losses due to postharvest disease may occur at any time during postharvest handling, from harvest to consumption. When estimating postharvest disease losses, it is important to consider reductions in fruit quantity and quality, as some diseases may not render produce un-saleable yet still reduce product value. For example, blemished fruit may not be sold as fresh fruit but may still be suitable for processing, in which case, it brings a lower price. It is also important to take into account costs such as harvesting, packaging and transport when determining the value of produce lost as a result of postharvest wastage [14,15].Gray mold, caused by the fungus *Botrytis cinerea*, is a common postharvest disease of lemons during cool temperature storage under high humidity. *Botrytis cinerea* Pers. Fr., a ubiquitous fungal pathogen, causes gray mold rot on a large number of economically important agricultural and horticultural crops [16]. It is the most common postharvest pathogen of citrus fruits in most regions of the world, leading to severe losses after harvest [18,18].

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The main objective of this study was to isolate and identify the causal organism of grey mold disease of Persian lime (*C. latifolia*), determine its pathogenic ability to infect different lime varieties.

## II. MATERIALS AND METHODS

### *Disease Survey and Detection*

This survey was carried out at winter season (December-January, 2014). The field study located at Researches and Production Station, National Research Centre located at Nuobaria district, Beheira governorate. In this field different citrus species / varieties are growing as follows: Navel orange (Citrus sinensis), Balady orange (*C. sinensis*), Valencia orange (*C. sinensis*), grapefruit (*C. paradisi*), Mandarin (*C. reticulata*), Persian lime (*C. aurantifolia*), Eureka / Lisbon lemons (*Citrus Limon* (L.) Burm.f.syn.). Grey mold disease incidence was observed only on the pre-harvest Persian lime fruits (*Citrus latifolia*). Disease symptoms appear as a brown leathery decay of the peel, with gray brown to olive spore masses forming around the affected areas of the fruit surface. Moreover, brown twigs contacted with the diseased fruits were also observed. The disease spreads rapidly to cover the whole infected fruits and twigs. The Infectedfruitturn intosponge'sappearance and became dry as disease development, then the fruits and twigs died (Figure 1). All the infected fruits were recorded. The percentage of diseased fruits in relative to the whole examined fruits was calculated.

### *Isolation and identification of the causal fungus*

Persian lime fruits and their twigs showing grey mold disease symptoms were collected and transferred to the laboratory for the purpose of isolation of the causal organism. The diseased fruits and twigs were surface disinfected by dipping in 70% ethyl alcohol [19] and left to dry, then placed into Petri dishes contain sterilized moist filter paper and incubated for 5-7 days at 20°C then examined. Then, fungal growth appeared on the surface of decayed fruits and twigs transferred to Petri dishes containing PDA medium. Identification of fungi isolated was carried out according to [20,21]. The purified fungal cultures were maintained on PDA slant for further studies.

### *Pathogenicity test*

The isolated fungus *Botrytis cinerea* was grown on potato dextrose broth (PDB) medium and incubated at 20°C for 14 days. The culture was filtered through sterile glass wool and the collected spores were adjusted to $10^6$ spore/ml using haemaocytometer slide. Fruits of Persianlime (*C. aurantifolia*), Eureka lemon (*C. Limon* (L.)Burm.f. syn.) and Baladylemon (*C. Limon*) cultivar varieties apparently healthy were surface disinfected with 70% ethyl alcohol , allowed to dry and then 4 wounds/fruit each (0.5-1.0 cm deep) were made up atthe fruit neck using sterile scalpel. The wounded fruits were inoculated by spraying them withthe prepared sporesuspension ofthe tested fungus. For development of disease symptoms the lemon fruits were placed in sterilized moist desiccators to maintain a high humidity, held for 7-10 days at 20°C and examined for typical grey mold symptoms. A set of wounded fruits were sprayed with sterilized water and kept as control. Five fruits of each lemon varieties were used as replicates. The percentage of infected fruits was calculated.


**Fig. 1. Grey mold incidence (A), its development on Persian lime (*C. aurantifolia*) fruits (B) and finally the whole infected twigs and fruits are died.**

### *Statistical analysis*

Tukey test for multiple comparisons among means was utilized [22].

## III. RESULTS AND DISCUSSION

Citrus is the main fruit crop in Egypt, its cultivation expanding in the newly reclaimed land. Citrus tree are cultivated across Egypt and occupied nearly 430.000 feddan [23]. Lemon has been recognized as an important

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fruit throughout the world. Lemon as citrus variety,is attacked with several fungal pathogen causing pre-, and postharvest diseases. One of these is the grey mold disease. The first occurrence of this disease was observed and registered at Noubaria District, Beheira governorate, Egypt during the winter season, 2014.

***Disease Survey Detection and the causal organism and its pathogenic ability***

All Persian lime (*C. aurantifolia*), Eureka lemons (*Citrus Limon* (L.)Burm.f.syn.) trees growing at Researches and Production Station, National Research Centre were surveyed for Grey mold infection. It is interesting to note that only Persian lime fruits showed Grey mold disease incidence. No disease symptom on Eureka lemons was observed. Also, it was observed that the grey mold disease was occurred on all growing Persian tress (fruits as well as twigs), although different numbers of diseased fruits/tree was recorded. The pre-harvest naturally occurrence of diseased fruits in relative to the total fruits number was recorded. The percentage of grey mold on fruits was calculated. The obtained results are shown in Table (1).

**Table 1. Survey of grey mold infected fruits on different Lime and lemon trees under field conditions**

| Lime trees varieties | Surveyed trees | Diseased fruits % |
|---|---|---|
| Persian lime | 400 | 17.3 a |
| Eureka lemon | 400 | 0.0 b |

Figures with the same letter are not significantly different (P≤ 0.05).

Results showed that a number of 400 trees of each variety were subjected to disease observation. Presented data indicate that, grey mold appeared only on Persian lime variety. Grey mold infection on Persian lime fruits recorded as 17.3 %, while no disease symptoms was observed on Eureka lemon varieties. Isolation from decayed lime fruits showing symptoms of grey mold revealed the presence of the fungus *Botrytis cinerea*(Fig. 2). Identification was carried out according to morphologically and microscopically characters described earlier [20,21].

*Botrytis cinera* was tested for its pathogenicity on Persian lime and Eureka lemon fruits varieties. Disease incidence was observed only on Persian fruits and no symptoms were observed on Eureka fruit as well as un-inoculated fruits. Disease development was observed as the following symptoms: Initial lesions appeared on inoculated fruits, light to dark brown, slightly raised spots. Following exposure to high humidity, the lesions extended and occupied larger area, then covered with gray color due to sporulation of the pathogenic fungus [24].

Results presented in Table (2) stated that all tested Persian lime fruits proved to be susceptible to attack by the pathogenic fungus and *Botrytis cinerea* causing 100% infection. The re-isolated fungus was found to be identical to these isolated from naturally infected fruits.

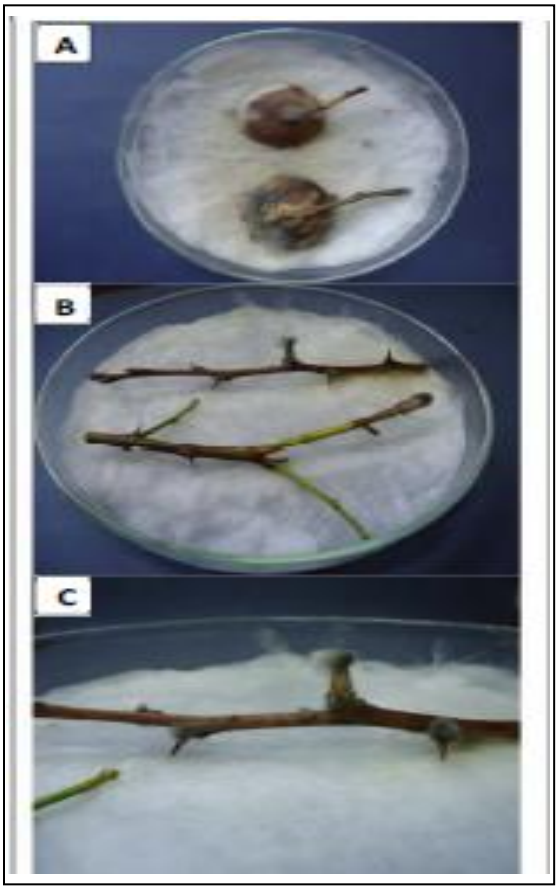


**Fig. 2. Appearance of *Botrytis cinerea* mycelium on diseased fruit (A) and twigs (B&C) of Persian lime under *in vitro* conditions**

**Table 2. Percentage of grey mold infected fruits on different Lime and lemon varieties under *in vitro* conditions**

| Lime and lemon varieties | Diseased fruits % |
|---|---|
| Persian lime | 100 a |
| Eureka lemon | 0.0 b |
| Balady lemon | 0.0 b |

Figures with the same letter are not significantly different (P≤ 0.05).

In this regard, it was reported that the fungus *Botrytis cinerea* sometimes attacks the flowers (blossom blight) and the young fruit of lemons and is characterized by its typical grey to greenish-grey spores. The fungus may develop in blossoms or on small twigs. Botrytis reduces fruit set and injures young fruit resulting in the formation of raised areas or ridges on the rind. The fungus lives on decaying organic matter and its spores are carried by wind, water and insects. Infected petals are the main source of infection of fruit and twigs. The fungus is flavored by prolonged damp, wet and cool conditions and a temperature of 18 $^{0}$C is optimal [25,26,27]. Also, the optimal temperatures for growth of the mold fungi are between 18°C to 23°C. However, some growth will also

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occur at the recommended cool storage temperature [28]. This report agreed with the time of disease survey which carried out, in the present study, at winter season (December-January, 2014) where the air temperatures were between 16-20 $^{o}$C and relative humidity between 80-90 RH.

## IV. CONCLUSION

Fungi causing postharvest diseases may possess a long inactive (quiescent) stage after infection before causing disease symptoms, or symptoms may develop shortly after infection. Such fungi causing pre-harvest grey mold infection and their spores are usually airborne. Good cultural practices that produce trees with minimal deadwood, or removal of deadwood by pruning can aid in reduction of grey mold incidence since dead substrate is required for spore production. Observable reductions in grey mold have been achieved by keeping the orchard floor free of fallen fruit to reduce sporulation on infected fruit and occurrence of air-borne spores that contaminate surfaces of fruit in the tree canopy. Grey mold can be reduced by improving ventilation under the tree canopy to shorten durations of high relative humidity and wetness required for inoculm production. Proper irrigation, soil drainage management and planting of trees on well-drained soils will also reduce conditions conducive to inoculum formation [15,29]. Proper field sanitation, pre-harvest fungicide sprays, and prevention of wounds on the fruit could help reduce the incidence of grey mold [30,31]. In addition, control of grey mold can only be achieved with pre-harvest applications of copper products because no postharvest treatments are available. These products are usually applied between October – November to blocks of trees with a history of grey mold or at the first sign of disease symptoms or the occurrence of disease outbreaks.

In the field study, in the present work, following these previous recommendations were followed. All Persian lime trees were sprayed twice with 15 days interval with the fungicide Kocide 101 (Copper Hydroxide) at the rate of 2.5 g/L. As a result no increase in grey mold incidence as well as disease development was observed even after the first spray application.

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