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# A New Report of Citrus Fruit Rot Caused by Scopulariopsis Brevicaulis in Egypt

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## Abstract

In 2023, a fungus was isolated from rotting navel orange (*Citrus sinensis*), which presented typical fruit rot disease symptoms for the first time in Ismailia governorate East of Egypt. The pathogen was isolated and identified as *Scopulariopsis brevicaulis* (telemorph: *Microascus brevicaulis*). A pathogenicity test was carried out to fulfil Koch's postulates. The pathogen enters the orange fruit and cause fruit rot through wounds. The fungus has pathogenic effects on other crops, such as Mandarin (*Citrus reticulata*), Lemon (*Citrus limon*), Mango (*Mangiferaindica*), Banana (*Musa acuminata),* Peach (*Prunus persica*), Plum (*Prunus simonii*), Pear (*Pyrus communis*), Apple (*Malus sp.*), Tomata (*Solanum lycopersicum*), Pepper (*Capsicum annuum*), Eggplant (*Solanum melongena*) and Guava (*Psidium guajava*). Symptoms vary in severity depending on the crop species. According to the available literature, this is the first record of orange fruit rot on orange in Egypt.

***Keywords:*** *Citrus*; *Scopulariopsis brevicaulis*; *fruitrot*

## Introduction

Fungal pathogens lead to deterioration and a reduction in the yield of many fruits produced in Egypt. Several fungal taxa, including *Alternaria citri*, *Penicillium digitatum*, *P. italicum*, *Geotrichum candidum*, and *Phytophthora* spp., are known as causal agents of citrus fruit rot worldwide (Whiteside et al.,1988).

*Scopulariopsis brevicaulis* is a type of species of the saprophytic genus Scopulariopsis that belongs to the family *Microascaceae*. It was first described in 1882 as *Penicillium* brevicaule by Saccardo and renamed *S. brevicaulis* by Bainier in 1907. It is a widespread fungus that is mostly known as a soil saprophyte. [Sandoval-Deniset al 2016]. *S. brevicaulis* was obtained and proven through pathogenicity tests on date palm fruit to be the responsible pathogen Anjiliet al. (2016). *Scopulariopsis brevicaulisone* is a fungal pathogen associated with spoilt watermelon fruits in Nigeria Abubakar et al. (2019). Most members of the genus *Scopulariopsis* are soil fungi, which are frequently isolated from food, paper and other materials Sarah Kid et al. (2016). *S. brevicaulis* was isolated from stored in polyethylene Basella alba (Malabar spinach) by Ogunbusola et al. (2018). *S. brevicaulis* can be found worldwide in soil, decaying wood, various other plant and animal products, and moist indoor environments [Park, et al, 2020, Lukassen, et al 2015]. It can be isolated from barley, wheat, rice grains, rice flour, soybeans, mung beans, black pepper, peanuts, groundnuts, and apples. It can also be found in nonfat dried milk, butter, cheese, salami, bacon, and luncheon meat by Pitt and Hocking (2009) and by Flannigan and Miller (2011). Pitt et al. (1998) isolated species of *S. brevicaulis* from soybeans and black pepper from the Philippines and peanuts and mung beans from Indonesia.

## Materials and Methods

Fruit samples that exhibited typical fruit rot symptoms were collected from different markets in Egypt's Ismailia governorate, Egypt (30.58°N, 32.27°E).

Orange fruits with rot symptoms and signs of infection were collected and processed for pathogen isolation. Fruit rot symptoms included soft lesions that later expanded to cause fruit rot. Navel orange (*Citrus sinensis*)-infected fruit peels were cut from the margin of the lesions, surface sterilised with 2% NaClO, and rinsed several times in sterile distilled water. Infected tissues were cut into small segments and plated onto Petri plates containing PDA for 3 days at 25 ± 2°C. The growing fungal isolates were purified via the hyphal-tipped technique and incubated at 25±2°C for 7 days. Colonies of the purified isolates first appeared as light brown powdery colonies with a light tan periphery on PDA after 5 days of incubation, resulting in masses with whitish edges. Pure cultures were identified and confirmed at the Assiut University Mycological Center (AUMC) according to Domschet al. 2007.

### *Pathogenicity test*

Navel orange fruits were surface disinfected with 2% NaClO, wounded, and inoculated, depositing 5 mm fungal discs from the margins of 7-days old culture on 5 mm wounds made by a sterilised cork borer on the surface of healthy fruits. Disinfested, wounded, and inoculated with sterilised PDA were used as controls. After 7 days of incubation at 25 ± 2°C with 85% relative humidity, the inoculated fruit presented symptoms and signs consistent with the original infection from which the isolate was obtained.

A pathogenicity test was performed by injecting healthy orange fruits with a 5 mm disc into the rind of the orange fruits at the early ripening stage. The inoculated fruits were incubated at 25±2°C.

### *Study the effects of temperature on fungal growth*

Five replicas of PDA were inoculated with *S. brevicaulis*. Each plate was inoculated at the center with a 5 mm disc from the edge of a 7-day-old culture. The inoculated plants were incubated at different temperatures, i.e., 10°C, 15°C, 25°C, 30°C, 35°C and 40°C, for 7 days. After the incubation period, the diameter of fungal growth was measured, and the average level of growth was calculated.

### *Host Range Study*

Thirteen different fruit species, viz. Navel orange (*Citrus sinensis*), Mandarin (*Citrus reticulata*), Mango (*Mangiferaindica*), Banana (*Musa acuminata*), Peach (*Prunus persica*), Plum (*Prunus simonii*), Pear (*Pyrus communis*), Apple (*Malus spp.*) Guavas (*Psidium guajava*), tomato (*Solanum lycopersicum*), lemon (*Citrus limon*), pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*), which belong to different fruit families, were tested for the host range of the pathogen. The tests were carried out on fresh fruits. The tests were conducted via the modified technique of DeBoer et al. (1978). The plant parts to be tested were surface sterilised by immersion in 0.3% sodium hypochlorite and then washed twice with sterile distilled water, followed by air drying under a laminar flow hood.

Five replications were performed for each case. The inoculated fruit parts were kept in sterile plastic bags to maintain high humidity. The bags were incubated at 25 ± 2°C, and the observations were recorded on the day to express the first symptom and soft rot intensity after 7 days. Disease intensity was measured and recorded.

## Results

The fungus isolated from the rotten *C. sinensis* fruit had a light brown powdery colony with a light tan periphery on PDA after 5 days of incubation. Colonies are fast growing, varying in color from white, cream, grey, and buff to brown and black but are predominantly light brown. The microscopic morphology shows chains of single-celled conidia produced in basipetal succession from a specialised conidiogenous cell called an annellide. Septate hyphae giving rise to short conidiophores bearing annelides mostly arranged in bush-like groups of pathogens were identified as *Scopulariopsis brevicaulisat* (AUMC) according to Domsch et al., 2007. Colonies of the obtained pathogen *S. brevicaulis* formed light brown powdery colonies with a light tan periphery on potato dextrose agar after 5 days of incubation. The septate hyphae give rise to short conidiophores bearing annelides mostly arranged in bush-like groups. The conidia are 5-8 μm in size, round, and light brown in color. The spores are uniquely shaped, appearing truncated at the base and forming chains resembling lightbulbs in a chain.

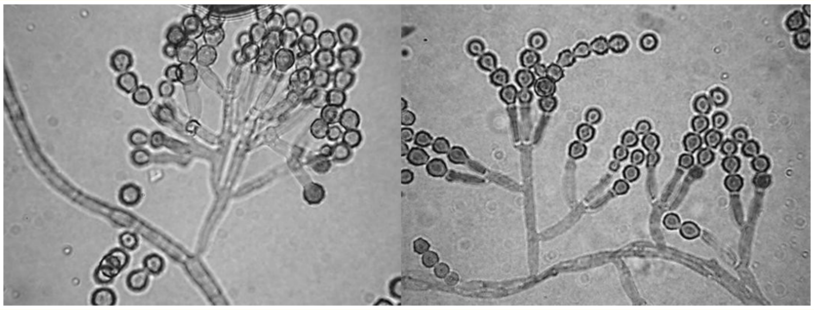

***Figure 1:*** Light microscopy image showing short conidiophores and round, light brown colorconidia and short conidiophores bearing annelides mostly arranged in bush-like groups. Images of *S. brevicaulis* (X1000).

The results presented in Figure 2 indicate that *S. brevicaulis* was capable of growing at temperatures ranging from 15-40°C. The best growth was recorded between 25-30°C, whereas lower growth was observed at 40°C. These results are in agreement with those reported by Pitt JI and Hocking AD (2009), who reported that *S. brevicaulis* prefers moderate temperatures (mesophilic) at 30°C, whereas it can grow poorly, between 15 and 40°C.

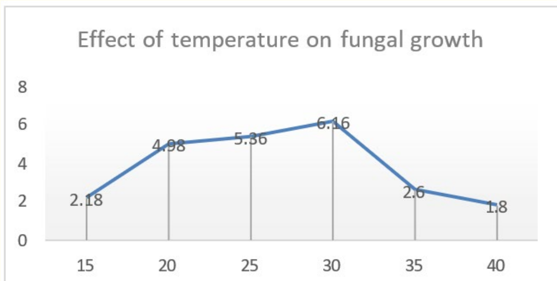


***Figure 2:*** Effects of temperature on fungal growth.

## *Pathogenicity test*

The orange fruits inoculated with the obtained fungus presented fruit rot symptoms consistent with those of the original infection. No symptoms were observed on the control fruits. The reisolation from artificially infected fruits indicated that the same infested pathogen was reisolated.

Host range studies (Fig. 3) of *S. brevicaulis* revealed that the fungus could macerate all the representative species of major tested fruits, viz., orange, mandarin, lemon, apple, pear, eggplant, pepper, tomato, plum, banana, peach, guava and mango.

However, the degree of fruit rot varied among them after inoculation. The greatest degree of fruit rot severity was detected in mango, followed by plum, tomato eggplant orange and apple. The host range of *S. brevicaulis* has been reported to include soybeans and black peppers from the Philippines and peanuts and mung beans from Indonesia (Pitt et al., 1998). Abubakar et al. (2019) also isolated *S. brevicaulis* from spoilt watermelon fruits in Nigeria. Anjili et al. (2016) reported that *S. brevicaulis* was obtained and proven through pathogenicity tests on date palm fruit to be the responsible pathogen.

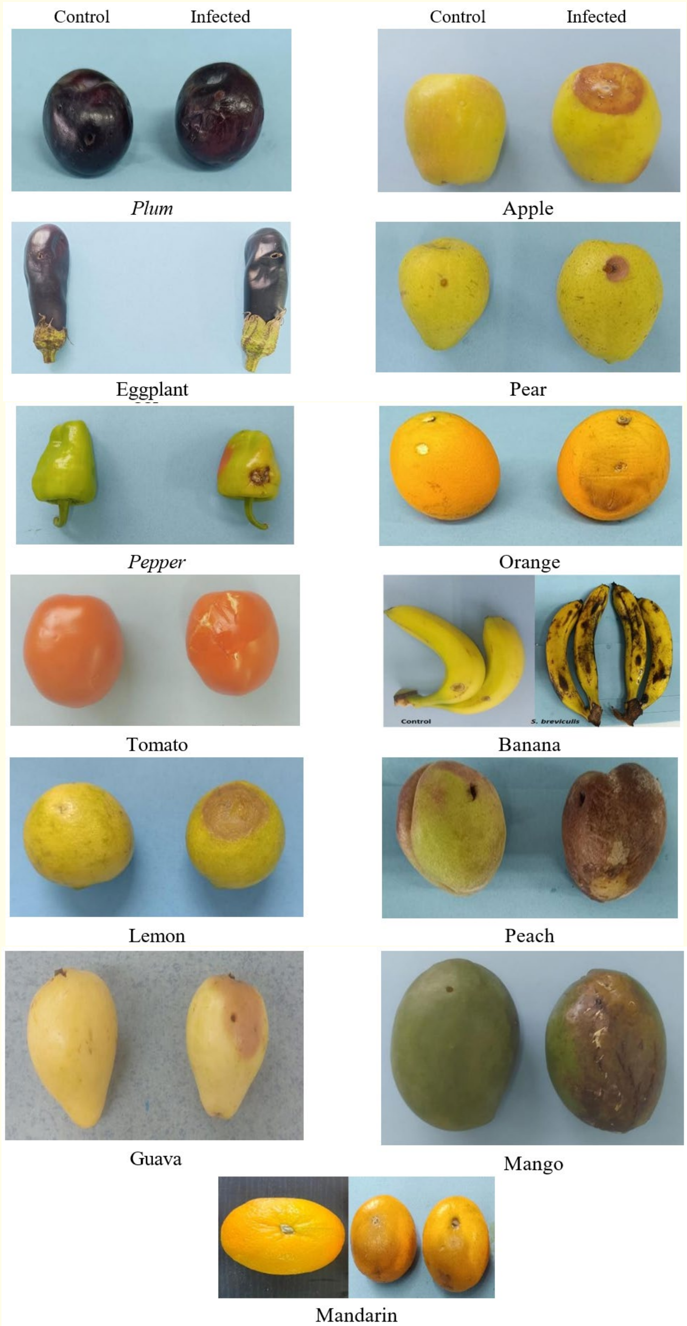


***Figure 3:*** Host rang of *S. brevicaulis*.

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