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# First report of dieback of citrus trees caused by Nattrassia Mangiferae in south of Iran

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## Abstract

Wilting, dieback, decline and death of citrus trees have spread out extensively in tropical areas of South of Iran. The aim of this study was identifying the causative agent, dispersion and prevalence of this disease in different regions. About 170 samples suspected of having this disease from different .areas were transferred to the laboratory and finally 18 fungal isolates were extracted. Fungal isolates grew very well in 30-35°C and PDA culture Pycnidia were almost spherical in the medium and they varied from dark brown to black. Arthroconidium stage of pathogenic fungi was identified and arthroconidia were mostly cylindrical under microscope and flat at both ends. Their length was twice their width and without walls, however, they sometimes had walls as well. According to morphologic features and some physiologic aspects along with pathogenicity on detached branches of citrus trees as well as citrus trees in garden conditions and matching them with other sources including Sutton and Dyko article in 1989, the mentioned pathogen was identified to be Nattrassia mangiferae. Results showed that the highest symptoms were observed in Poshtkooh, Marin and Basht areas, in such a way that in Posthkooh region about 75% of lime trees showed the symptoms of this disease. This is the first report of Nattrassia mangiferae as a causal agent of dieback of citrus trees in South of Iran.

Key words : decline, fungi, Wilting Citrus

## INTRODUCTION

Citrus are of Rutaceae family and Aurantioidae sub-family. This subfamily includes over 33 different species among which only three species of Poncirus, Fortchunella and Citrus have economic value and are of special importance in citrus producing countries. Botanically, they are shrubs or tree with dense foliage with 4-8 thick white, red or purple petals, 4-5 sepals and 16-32 stamens [1].

Citrus are important crops that could be cultivated in tropical areas of Iran. In recent years, with regard to advances in drip irrigation systems and government's support, it is being cultivated extensively and this process is still developing [2].

Consuming citrus as fresh fruit or its juice is of special importance in human diet, since they contain minerals and replete with vitamins A, C and B with pharmaceutical and nutritional aspects. Citric acid of these crops is used in food, metal and fabric industries, pharmaceutical and producing synthetic resins [3].

According to information of FAO, Iran stands in the 8th rank in terms of area under citrus cultivation in the world. In addition, among 10 countries in the world, 10 countries accounted for about 75% of production among which China stands in the first rank with 31700,000 tons. Therefore, world trade of citrus has been 16.88million tons in recent years and about 70% of this value has been exported to Europe [2]. Area under citrus cultivation in 2013 has been over 275000 hectares and Mazandaran has been in the first rank with about 41% of area under citrus cultivation. Citrus export of the country in best conditions is 60,000 tons and Iran stands in 25th rank in the world in terms of export value [2].

Citrus in Iran includes all citrus species, the most important of which are oranges (Citrus sinensis), tangerine (C. reticulata), lime (C. limon), Lemon (C. aurantifolia), bitter orange (C. aurantium) and grapefruit (C. rarodisi) [1]. Citrus trees are exposed to various fungal, bacterial and viral diseases. Different parts of the tree including leaves, branches, trunks, roots and fruits may suffer from these pathogenic factors. Wilting and dieback of twigs, decline and death of citrus trees is extensively common in tropical and subtropical areas of Khuzestan province. For instance, in one agro-industry unit of Safi-Abad region, over 150 hectares of Lisbon Lemon trees have been destroyed within 3-5 years after the onset of symptoms and currently more trees are dying and becoming unexploitable [4]. Dieback and decline of deciduous trees was first observed by Nattras in 1933 and he extracted a fungi from these trees and called it *Hendersonula Toruloidea Nat*; however, he hesitated about its being pathogenic [5]. Wilson et al. reported this event from Iranian walnut, as *Juglands regia* in California. They investigated the pathogenesis and the ways of controlling it and they attributed tracheid elements closing to this disease [6-7]. The fungus was reported as endemic on *Eucalyptus camaldulensis* and on citrus trees in Arizona [8]. In China, the fungus was reported to cause butt rot *of Zanthoxylum bungeanum* [9]. *N. mangiferae* was responsible for the mortality of almond, peach, plum and *Eucalyptus* spp., *Populus* spp. and *Pinus* spp. in Iraq [10]. Gummosis of Lemon trees has been widespread and caused by *N. mangiferae.* The fungus also caused losses in bananas [11].In another study, it was revealed that the causative agent enters through a wound and results in dieback and canker in citrus trees in California; however, the causative agent was recognized to be a secondary factor [12]. In the Middle East severe epidemics of dieback causative agent of Lemon tree was reported by Ekila and Stirg [13]. Recently, Micheal reported a disease under the name of dieback in eucalyptus trees in North California [5]. In Iran, the cause of dieback and wilting were reported by Ershad from Khuzestan and Aminayi and Ershad from Kerman [14-15]. In recent years, extensive studies have been conducted on this disease in Khuzestan. Results of these studies suggest that this disease is present in most regions of Khuzestan Province on 18 plant families [2].

Results of studies conducted in Khuzestan revealed that the

**Figure 1 :** The main areas studied of south of Iran.

activity of the pathogen of this lesion starts in early June and develops to its maximum in August and September. Then its activity decreases gradually [4]. Results of studies conducted in Khuzestan revealed that branches cut to 1-20cm are reliable and effective in verifying pathogenesis, severity of pathogenesis of isolates and investigating relative susceptibility [14]. Alizadeh et al., (2000) [12], Reported several fungal species such as Cytospora sp., *Nattrassia mangiferae*, Fusarium sp., were able to produce disease. taheri et al., (2005) [16], reported that propiconazole could reduce mycelia growth of N. mangiferae under in-vivo conditions. (Paxton et al., 1964) [17], mentioned that N. mangiferae thrives better at high temperature (30-35 °C). The fungus produced heavy masses of loose spores under th bark of infected trees that can readily spread the infection. Al Zarari et al, reported N. mangiferae as the causal organism of branch wilt in almond, peach, plum and Eucalyptus spp [18].This disease is currently observed on citrus trees of tropical regions south of Iran included: Kazeroon, Nurabad Mamasany, Rostam, Basht, Gachsaran, Dehdasht, Choram and Behbahan Cities.The aim of this study was identifying infected regions, determining the percentage of infected orchards based on apparent signs of disease, diagnosis of causative agent and pathogenesis verification of the fungi that result in dieback and decline of citrus trees.

## MATERIALS AND METHODS

### Identifying Infected Regions

This disease was investigated within 2010-2011 in tropical areas of Zagros Mountain of Iran including Dehdasht, Lendeh, Choram, , Gachsaran (Gachsaran-Basht, Maleh Barfi- Poshtkooh Basht, Imam Zadeh Ja'far, Bidzard, Sarbisheh, Talkhab Shirin, Mahoor Basht, Marin, Rostam, Nurabad Mamasany, Kazerun, Behbahan) and infected areas were identified (fig1).

### Determining Percentage of Orchards' Infection

The amount of prevalence of this disease in orchards of tropical areas was determined using crossover sampling and factors such as age of the tree, the number of all trees calculated, type of rootstocks and scions in each orchard and percentage of wilting and dieback of trees were used to provide the ID for each orchard.

### Sampling From Infected Orchards:

All infected citrus trees with special signs of wilting and dieback (burning) of branches, canker and gummosis in branches and main trunks were investigated.

Therefore, in all tropical areas of South of Iran, infected orchard were selected. Then, we moved along two diameters of orchards and trees with signs of diseases were selected randomly relative to the area under cultivation and different parts of trees including branches, trucks and middle trunks were sampled, in such a way that of 5 infected trees only one of them was sampled and totally 170 samples were selected. After selecting the samples, the name of area under study, place of isolation, host and number of samples were written and were transferred to laboratory for later studies.

### Isolation and Diagnosis of the Causative Agent

First, the surface of samples transferred to the laboratory were burnt using alcohol and flame and then small pieces of wood were separated from the margin of wounds (between the healthy and ill part) to be transferred to PDA nutrition environment and placed in 30°C incubator in the dark. After 48 hours, small disks (diameter of 5mm) were taken from the margin of colonies and they were again transferred to PDA culture. After 30 hours, they were purified using Hyphal tip method and then purified isolates were recognized using Sutton and Dyko method based on features including shape and color of mycelium in nutrition environment, shape and size of pycnidia, pycnidiospores and conidia of purified isolates [6].

### Verification of Pathogenesis

#### a) Inoculation on detached branches of citrus

For each fungal isolate, 4 detached pieces with diameter of 1-1.5cm and length of 25cm were selected from citrus branches of relatively the same age, in such a way that pathogenesis of each isolate was verified on detached branches of available species in that region. The collected of citrus branches were surface disinfected by sequential washing in 70% ethanol for 20 sec, a bleach solution (5% sodium hypochlorite) for 1 min followed by rinsing with sterile water. Small pieces, between the healthy and infected tissues were excised and plated onto potato dextrose agar (PDA) amended with streptomycin sulfate (0.1 g/l). Then, part of the skin was extracted from the middle of each branch in such a way that cambium was completely exposed. To conduct inoculation, tablets of PDA (with diameter of 5mm) stored in 30°C

incubator for 30 hours were extracted from the margin of growing colonies and they were inoculated in the created wound. To prevent displacement and drying of tablets, the inoculation area was covered using parafilm and adhesive tape and then placed in desiccator containing sterile distilled water with saturated moisture for 7 days in 30°C. Control treatments were inoculated using sterile PDA tablets as well. [19- 20- 21]. Turning the inoculated area into brown was considered a sign of disease compared to control and then the amount of disease development was measured in $mm^2$ and classified as Resistant, moderately resistant, tolerant, sensitive and very sensitive [14].

### b) Inoculation of selected isolates in natural conditions

In this test, Na-9 isolate of *Fungus* separated from lime with higher pathogenesis compared to other isolates, was inoculated in natural conditions on lime Khargi Lemon, orange, tangerine and bitter orange in August similar to the first method and the results were written down 14 days later.

## RESULTS

### Determining distribution areas and infection percentage of citrus orchards

Results from visiting citrus orchards are recorded in Table 1, including the quality of disease distribution, percentage of orchards infection, types of cultivated species, area under cultivation of orchards, elevation of region, age of orchard and type of rootstocks and scions.

The highest symptoms of disease were observed in Poshtkooh, Marin and Basht areas (Figure 2) in such a way that in Poshtkooh region, about 75% of lime trees showed the symptoms of this disease.

These results as well as repeated visits from other citrus growing areas of adjacent provinces like Fars Province indicate that in newly-established orchards, lower infection is observed compared to old orchards. In other words, this lesion is observed

**Figure 2 :** General schematic of an infected orchard in Marin region. Symptoms of disease start from terminal branches of canopy as decline and wilting (Figure 3)

**Figure 3 :** Green-drying, dieback and decline of citrus trees in Choram region. This disease is transmitted from infected terminal branches to other branches and results in their dieback; finally it is transmitted to the trunk and turns it into brown. (Figure5)

**Figure 3 :** Primary signs of disease (dieback of terminal branches) This state exacerbates as temperature rises in such a way that first, the leaves are green-dried and then they turn into brown because of sunlight and they are not often separated from branches. (Figure 4)

**Figure 4 :** Skin turning into brown because of fungi *Nattrassia mangiferae.* The infected trunk turns into dark brown to black and infected parts appear as dark brown in the cross-sectional and longitudinal profiles and in other sections they appear to be normal. (Figure 6)

**Figure 6 :** Cross-section of trunk infected by *Nattrassia mangiferae*. Sometimes, cracks are observed in the skin of infected branch that results in cracking of the trunk skin. (Figure 7).

**Figure 7 :** Cracks created in the skin because of *Nattrassia mangiferae.* Finally, a sooty layer of arthroconidia is formed between the skin and periderm )Figure 8( which sometimes accompanies resin secretion, which is the evident sign of disease. The highest rate of infection was observed on Lemon and lime trees respectively in Poshtkooh Basht and Marin regions.

**Figure 8 :** Sooty layers of arthroconidia between skin and periderm resulted from Nattrassia magnifera.

in young trees less than old ones.

### Isolation and Identification of Causative Agent

Fungi isolates grew very well in 30-35°C in PDA culture. Fungal mycelium is branched or recessed in any medium with colorless to dark brown color spectrum. On the surface of nutrition environment, Pycnidia are relatively spherical and varied in color from dark brown to black. Arthroconidia stage of pathogen fungi was determined and they are usually cylindrical under microscope, flat to both ends, their length is twice their width and they are without wall or sometimes with one wall.

In addition, pathogenesis of separated isolates was verified on detached branches of citrus trees. Therefore, the mentioned pathogen was recognized to be *Nattrassia mangiferae* [23].

### Optimal Conditions for Pycnidial Formation

To from pycnidia, this fungi, in laboratory conditions, is the best state of putting spur of apples in containers of medium and putting the medium containing fungi in the middle of petri in 30°C. In this state, many pycnidia are produced within 3 to 6 months on the surface of spurs inside the petri.

### Response of Detached Citrus Branches to Fungal Isolates

Results from inoculation of citrus trees with fungal isolates showed that all branches are susceptible to this disease but with a different degree of susceptibility. Furthermore, all isolates were pathogenic. The most severe signs of disease were related to lime branch with isolate 9 and the least severe one was related to bitter orange branches (Table 3). However, despite the pathogenesis of isolates, disease signs, including the turning of skin and cambium into brown and black, were observed on all detached branches inoculated using fungi after 7 days.

This method is an accurate and reliable for artificial induction of disease as well as a proper method to evaluate the disease development qualitatively and the results from fungi inoculation in orchard condition is the same as what happened on detached branches in laboratory. In addition, among species studied, Lemon showed the highest susceptibility (1790 $mm^2$) and bitter orange showed the lowest susceptibility (310 $mm^2$).

### Response of Citrus Trees in Natural Conditions

Response of citrus trees in orchard relative to Na-9 isolate of *Nattrassia mangiferae* revealed that inoculated branches wilted and started to dieback from terminal sections. Among these trees, limes were more susceptible and bitter oranges were the most resistant trees. Similar results were obtained comparing detached branches of citrus trees (Table 4).

## DISCUSSION

Results showed that disease is present in (Gachsaran-Basht, Maleh Barfi- Poshtkooh Basht, Imam Zadeh Ja'far, Charbisheh, Bidzard, Talkhab Shirin, Mahoor Basht, Marin, Rostam, Nurabad Mamasany, Kazerun, Behbahan) and it has relatively been observed in all citrus growing areas.

Our results showed the pathogen enters through a wound and infection starts. Since, in old trees there are more wounds available, they become more infected. On the other hand, severe pruning decreases the number of branches extensively that results in sunburn in old trees and provides the path for fungi's penetration and this is verified by other authors as well [4-12-22].

On the other hand, during the visits, it was specified that in

**Table 1 :** Data from visiting citrus planting zones

| Number | City | Region | Elevation | Age of the tree | Number of trees counted | Dieback | | Rootstock | Scion |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Number | Percentage | | |
| 1 | Gachsaran | Emamzade Jafar | 710 | 12 | 25 | 10 | 40 | Bitter orange | Orange |
| 2 | Gachsaran | Emamzade Jafar | 710 | 12 | 20 | 8 | 32 | Bitter orange | Tangerine |
| 3 | Gachsaran | Research Station | 710 | 10 | 25 | 10 | 40 | Bitter orange | Orange |
| 4 | Gachsaran | Emamzade Jafar | 710 | 12 | 25 | 7 | 28 | Bitter orange | Orange |
| 5 | Gachsaran | Emamzade Jafar | 710 | 12 | 40 | 9 | 36 | Bitter orange | Orange |
| 6 | Nurabad mamasani | Bardangun | 880 | 12 | 40 | 7 | 28 | Lemon | Lemon |
| 7 | Nurabad mamasani | Bardangun | 890 | 12 | 50 | 12 | 24 | Bitter orange | Orange |
| 8 | Gachsaran | Emamzade Jafar | 710 | 20 | 100 | 50 | 50 | Lemon | Lemon |
| 9 | Gachsaran | Talkhab Shirin | 700 | 20 | 100 | 50 | 50 | Lemon | Lemon |
| 10 | Rostam | | 875 | 10 | 25 | 10 | 40 | Lemon | Sweet Lemon |
| 11 | Gachsaran | Basht | 820 | 20 | 25 | 60 | 15 | Lemon | Lemon |
| 12 | Gachsaran | Basht | 820 | 35 | 100 | 50 | 50 | Bitter orange | Orange |
| 13 | Gachsaran | Basht | 820 | 35 | 100 | 40 | 40 | Indian bael | Indian bael |
| 14 | Behbahan | | 635 | 35 | 50 | 25 | 50 | Bitter orange | Orange |
| 15 | Kazerun | Chenar Shahijoon | 925 | 35 | 20 | 4 | 16 | Lemon | Lemon |
| 16 | Kazerun | Chenar Shahijoon | 820 | 35 | 30 | 10 | 40 | Lemon | Lemon |
| 17 | Gachsaran | Basht | 820 | 35 | 30 | 10 | 40 | Sweet Lemon | Sweet Lemon |
| 18 | Dehdasht | Cheshme Belgheys | 759 | 25 | 50 | 22 | 44 | Bitter orange | Tangerine |
| 19 | Dehdasht | Choram | 760 | 25 | 100 | 45 | 45 | Bitter orange | Orange |
| 20 | Gachsaran | Marin | 1140 | 30 | 100 | 60 | 60 | Sweet Lemon | Sweet Lemon |
| 21 | Gachsaran | Marin | 1140 | 30 | 100 | 60 | 60 | Sweet Lemon | Sweet Lemon |
| 22 | Gachsaran | Marin | 1140 | 30 | 100 | 60 | 60 | Khargi Lemon | Khargi Lemon |
| 23 | Gachsaran | Marin | 1140 | 30 | 100 | 55 | 55 | Lemon | Lemon |
| 24 | Gachsaran | Bid Zard | 730 | 70 | 100 | 25 | 25 | Bitter orange | Orange |

**Table 2 :** Features of *Nattrassia mangiferae* isolates on citrus trees from tropical areas.

| Number of isolate | Host | Rootstock | Location | Isolation spot |
|---|---|---|---|---|
| Na-1 | Orange | Bitter orange | Gachsaran-Agricultural Research Station | Depth of branch wood |
| Na-2 | Lemon | Lemon | Kazerun-Che | Depth of branch wood |
| Na-6 | Tangerine | Bitter orange | Gachsaran-Agricultural Research Station | Depth of branch wood |
| Na-8 | Lemon | Lemon | Gachsaran-Emamzade Jafar | Depth of branch wood |
| Na-9 | Lemon | Lemon | Gachsaran-Talkhab Shirin | Depth of branch wood |
| Na-10 | Orange | Bitter orange | Gachsaran-Agricultural Research Station | Depth of branch wood |
| Na-11 | Orange | Bitter Orange | Nurabad Mamasani | Depth of stalk |
| Na-12 | Orange | Bitter orange | Basht | Depth of stalk |
| Na-13 | Indian bael | Indian bael | Basht | Depth of stalk |
| Na-16 | Tangerine | Bitter orange | Basht | Depth of stalk |
| Na-18 | Tangerine | Bitter orange | Choram | Depth of stalk |
| Na-19 | Orange | Bitter orange | Choram | Depth of stalk |
| Na-20 | Sweet Lemon | Sweet Lemon | Gachsaran-Marin | Depth of stalk |
| Na-25 | Sweet Lemon | Sweet Lemon | Posht kooh Basht | Depth of stalk |
| Na-28 | Sweet Lemon | Sweet Lemon | Gachsaran | Depth of branch wood |
| Na-30 | Sweet Lemon | Sweet Lemon | Mele barfi Basht | surface of branch wood |
| Na-32 | Sweet Lemon | Sweet Lemon | Gachsaran Chaharbishe | surface of branch wood |

orchards established in higher elevations and lower shadowing, more wilting and burning of branches was observed that could be a reason for sunburn and wound creation and penetration of fungi through this path. In addition, repeated visits from citrus orchards within 2010-2012 revealed that the pathogen starts its activity in the middle of June and reaches to its peak activity in July and August, and then it diminishes and is reduced to minimum in November. It is inactive in the full and spring and similar finding was noted by Akbarpour and Banihashemi also Ershad in Khuzestan Province [4-14].

In addition, our findings supported with Paxton and Wilson that day temperature required by this fungi for rapid wilting of walnut branches is 40 to 43°C along with night temperature of over 24°C continuously [17].

In addition, pathogenesis of separated isolates was verified on detached branches of citrus trees. Therefore, according to mentioned features and their supported with other literature including Sutton and Dyko article in 1989 (23), the mentioned pathogen was recognized to be *Nattrassia mangiferae* [23] and This is the first report of *Nattrassia mangiferae* as a causal agent of dieback of citrus trees in South of Iran.

Based on the our laboratory test, state of putting spur of apples in containers of medium and putting the medium containing fungi in the middle of petri in 30°C is the best method for Pycnidial Formation, these results supported with Alizadeh and Heidarian studies [12].

Sutton and Dyko obtained the best results by inoculating apple sticks using fungal mycelium and exposing them to UV light for 3 to 6 months. In addition, exposing isolates of this fungus against UV light in PDA culture resulted in formation of

**Table 3 :** Response of detached branches of citrus to *Nattrassia mangiferae*isolates.

| Number of isolate | Type of citrus | Response of detached branch | Mean progression of the disease in millimeters | Qualitative responses |
|---|---|---|---|---|
| Na-1 | Lemon | + | 1450 | Susceptible |
| Na-1 | Orange | - | 1100 | Tolerance |
| Control | Lemon | + | | --- |
| Na-2 | Lemon | + | 1500 | Susceptible |
| Na-2 | Orange | + | 820 | Moderately resistance |
| Na-2 | Tangerine | + | 1180 | Tolerance |
| Na-2 | Sweet Lemon | + | 845 | Moderately resistance |
| Control | Lemon | - | | |
| Na-6 | Lemon | + | 1480 | Susceptible |
| Na-6 | Bitter orange | + | 480 | Resistance |
| Na-6 | Khargi Lemon | + | 1770 | Very Susceptible |
| Na-6 | Tangerine | + | 1320 | Tolerance |
| Na-6 | Indian bael | + | 1340 | Tolerance |
| Na-6 | Orange | + | 1620 | Susceptible |
| Na-6 | Sweet Lemon | + | 1580 | Susceptible |
| Control | Lemon | - | | |
| Na-8 | Lemon | ı | 1550 | Susceptible |
| Na-8 | Orange | + | 1300 | Tolerance |
| Na-8 | Tangerine | + | 1280 | Tolerance |
| Na-8 | Sweet Lemon | + | 1605 | Susceptible |
| Control | Lemon | - | | |
| Na-9 | Orange | + | 1050 | Tolerance |
| Na-9 | Khargi Lemon | + | 1550 | Susceptible |
| Na-9 | Lemon | + | 1710 | Very Susceptible |
| Na-9 | Bitter orange | ı | 380 | Resistance |
| Na-9 | Tangerine | + | 1050 | Tolerance |
| Na-9 | Sweet Lemon | + | 1510 | Susceptible |
| شاهد | Lemon | | | |
| Na-10 | Lemon | + | 1520 | Susceptible |
| Na-10 | Sweet Lemon | + | 1300 | Tolerance |
| Na-10 | Khargi Lemon | + | 1610 | Susceptible |
| Control | Lemon | - | | |
| Na-11 | Lemon | + | 900 | Moderately resistance |
| Na-11 | Sweet Lemon | + | 930 | Moderately resistance |
| Control | Lemon | - | | |
| Na-12 | Lemon | + | 1280 | Tolerance |
| Na-12 | Bitter orange | + | 310 | Resistance |
| Na-12 | Khargi Lemon | + | 1100 | Tolerance |
| Control | Lemon | - | | |
| Na-13 | Lemon | + | 720 | Moderately resistance |
| Na-13 | Orange | + | 780 | Moderately resistance |
| Na-13 | Bitter orange | + | 370 | Resistance |
| Na-13 | Tangerine | + | 770 | Moderately resistance |
| Control | Lemon | - | | |
| Na-16 | Tangerine | + | 1310 | Tolerance |
| Na-16 | Lemon | + | 1560 | Susceptible |
| Na-16 | Orange | + | 1340 | Tolerance |
| Control | Lemon | - | | |
| Na-18 | Orange | + | 1570 | Susceptible |
| Na-18 | Tangerine | + | 1470 | Susceptible |
| Na-18 | Lemon | + | 1520 | Susceptible |
| Control | Lemon | - | | |
| Na-19 | Lemon | + | 1540 | Susceptible |
| Na-19 | Orange | + | 1320 | Tolerance |
| Na-19 | Tangerine | + | 1280 | Tolerance |
| Control | Lemon | - | | |

| | | | | |
|---|---|---|---|---|
| Na-20 | Lemon | + | 1500 | Susceptible |
| Na-20 | Sweet Lemon | + | 1480 | Susceptible |
| Na-20 | Indian bael | + | 1100 | Tolerance |
| Na-20 | Bitter orange | + | 760 | Moderately resistance |
| Control | Lemon | - | | |
| Na-23 | Orange | + | 1550 | Susceptible |
| Na-23 | Indian bael | + | 1150 | Tolerance |
| Na-23 | Sweet Lemon | + | 1570 | Susceptible |
| Na-23 | Lemon | + | 1790 | Very Susceptible |
| Control | Lemon | - | | |
| Na-25 | Orange | + | 750 | Moderately resistance |
| Na-25 | Lemon | + | 1540 | Susceptible |
| Control | Lemon | - | | |
| Na-28 | Lemon | + | 1570 | Susceptible |
| Na-28 | Orange | + | 1120 | Tolerance |
| Control | Lemon | - | | |
| Na-30 | Lemon | + | 1570 | Susceptible |
| Na-30 | Khargi Lemon | + | 1530 | Susceptible |
| Na-30 | Orange | + | 1150 | Tolerance |
| Control | Lemon | - | | |
| Na-32 | Lemon | + | 1600 | Susceptible |
| Na-32 | Orange | + | 1170 | Tolerance |
| Control | Lemon | - | | |

+ :+ Detached branches that showed symptoms of skin and sub-skinturning into brown or black are considered to be ill and shown with plus sign.

**Table 4 :** The effect of Na-9 isolate of *Nattrassia mangiferae* on detached branches of citrus trees after 7 days and citrus trees after 14 days

| Species | Rootstock | Response | | Progression of the disease in millimeters | | Qualitative response |
|---|---|---|---|---|---|---|
| | | Detached branches | Treated branched | Qualitative response | Treated branched | |
| Lemon | Bitter orange | + | + | 1750 | 1800 | |
| Khargi Lemon | Bitter orange | + | + | 1500 | 1530 | |
| Sweet Lemon | Indian bael | + | + | 1480 | 1540 | |
| Orange | Bitter orange | + | + | 1150 | 1210 | |
| Tangerine | Bitter orange | + | + | 1120 | 1160 | |
| Bitter orange | Bitter orange | + | + | 710 | 790 | |
| Control | - | - | | | | |

+: trees that show the lowest symptoms of disease such as wilting and dieback are sick and shown with plus sign.
-: trees with natural conditions that do not show dieback and wilting are shown with minus sign.

individual and complex pycnidia within 6 weeks to two months [23].

Our Results showed detached branches technique is the most reliable method to verify pathogenesis of *Nattrassia mangiferae*. That supported by Alizadeh et al studies [12].

In general, results from inoculation of fungi on detached branches of citrus and trees in orchard conditions revealed that all species, including those on bitter orange rootstocks or seed ones are all susceptible to these fungi. However, their degree of susceptibility is different and these results support with the results obtained by other authors [12-14].

## CONCLUSION

Results of this study showed that the causative agent of wilting, dieback, decline and death of trees has been *Nattrassia mangiferae* that has infected citrus trees extensively in tropical areas of Kohgiluyeh-va- Boyer-Ahmad, Fars and Khuzestan Provinces. Therefore, this causative agent has infected over 75% of lime trees in Poshtkooh Basht region. This is the first report of *Nattrassia mangiferae* as a causal agent of dieback of citrus trees in South of Iran. Among citrus species studied, Lemon and lime showed the highest susceptibility and bitter orange showed the least susceptibility. This lesion infects the trees through wounds

and was observed in young trees less than old trees.

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