



## Pathogenicity test of different fungi on date palm causing sudden decline disease of date palm (*Phoenix dactylifera* L.) in Khairpur, Pakistan

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### Abstract

The date palm (*Phoenix dactylifera* L.) is considered as one of the most important crop of the country and holds a very significant position on the agriculture horizon of Sindh. Khairpur is the biodiversity center of date palm. Dates play a major role in the socio-economic stability of the country to increase the national income of Pakistan and is grown in all four provinces. Since last few years the date palm orchards of district Khairpur are suffering from decline disease of unknown etiology. This disease has destroyed hundreds of orchards and puts the negative effects of farmers in those affected areas by creating social and economic problems. The disease has caused not only yield loss but also loss of a major source of income of the farmer of date palm. During the present investigation, the date palm orchards of different areas of Khairpur, Sindh, Pakistan, were found to suffer from a decline disease that is severely damaging the trees and becoming epidemic in some areas. Typical disease symptoms include whitening and dying of the foliage followed by sequential death of the branches and whole tree. The pathogenicity test on date palm seedlings was carried out on most commercial growing date palm varieties like Aseel, Fasli and Karbalian, by three different methods. The frequency of *F. solani* followed by *P. ucladium* and *H. sativum* was significantly highest on all the studied date varieties ranging from 30-57% on Aseel (57%). Var. Aseel appeared as the most susceptible as significantly higher fungi were isolated from it as compared to the vars. Fasli and Karbalian. The inoculations of *F. solani* alone or with *P. ucladium* or *H. sativum* confirm the pathogenic nature of *F. solani* which not only greatly reduced the plant growth but also caused significant plant mortality and disease severity. Whereas, *P. ucladium* and *H. sativum* were found less pathogenic causing disease development and plant mortality. The *Fusarium solani* alone showed typical symptoms of disease in soil inoculation methods followed by stem inoculation method and spraying method. Whereas such symptoms were not observed on plants inoculated with combination in *Fusarium solani* + *Phoma ucladium* followed by *Fusarium solani* + *Helminthosporium sativum* and *Phoma ucladium* & *Helminthosporium sativum* alone.

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## Introduction

Date Palm (*Phoenix dactylifera* L.) is a dioecious perennial monocot plant belongs to Arecaceae family having 200 genera and 1500 species (Dawson, 1982). Date Palm plant is attacked by a number of fungal pathogens causing diseases and disorders worldwide such as: inflorescence rot by *Mauginiella scaettae*, black scorch/bud rot by *Ceratocystis paradoxa*, Graphila leaf spot by *Graphiola phoenicis*, brown leaf spot by *Mycosphaerella tassiana*, rachis or Diplodia by *Diplodia phoenicum*, Pestalotiopsis leaf spot by *Pestalotiopsis palmarum*, Belaat disease by *Phytophthora palmivora* and *Fusarium* wilt by *Fusarium oxysporum* (Mani *et al.*, 2005).

Pakistan is considered one of the top ranked date palm cultivated regions of the world. In Pakistan, date palm cultivation is mainly concentrated in Khairpur (Sindh) and Turbat (Balochistan) districts (Jatoi *et al.*, 2009). Date palm holds a significant position in the socio-economic development at Khairpur where several thousand peoples are related with date palm cultivation, trade and market. The date palm industry is now a days threatening by a sudden decline disease (SDD) problem caused by a soil borne fungus *Fusarium solani* which have been destroyed enormous number of date palm trees in the region (Maitlo *et al.*, 2009; Abul-Soad *et al.*, 2011; Maitlo *et al.*, 2013). The symptoms of this disease are more or less similar to bayoud disease caused by *Fusarium oxysporum* f. sp. *Albedinis* which have been destroyed millions of date palm trees in North African countries (Djerbi, 1983; Al-Akaidy, 1994). Similarly the sudden decline disease or pseudo bayoud is severely damaging the trees and becoming epidemic in other areas of the region (Maitlo *et al.*, 2009; Abul-Soad *et al.*, 2011; Maitlo *et al.*, 2013).

The decline disease of date palm has also been reported by several workers from different date palm growing areas of the world. However, they reported various fungal pathogens responsible for this disease such as *Fusarium oxysporum* f. sp. *albedinis*, *Fusarium moniliforme*, *Fusarium proliferatum* and *Fusarium solani* (Abdalla *et al.*, 2000; Sarhan, 2001;

Rashed and Hafeez, 2001; Masood *et al.*, 2011; Maitlo *et al.*, 2013).

There is no any work have been carried out on this devastating disease of date palm in Sindh, Pakistan before. Maitlo *et al.* (2009) first time conducted research on this disease and isolated & identified the causing pathogens of which the most predominant fungus was the *Fusarium solani*. The current study is a continuous series of the above work and focused to investigate the disease severity as well as pathogenicity test of predominantly isolated fungal species by Maitlo *et al.*, (2009) associated with sudden decline disease *viz.*, *Fusarium solani* alone and in combination with *Phoma ucladium* and *Helminthosporium sativum*.

## Material and methods

### *Susceptibility of different date palm varieties to different isolated pathogens*

The susceptibility of different varieties of date palm *viz.* Aseel, Fasli and Karbalian to sudden decline disease caused by different pathogenic fungi, *viz.* *Fusarium solani*, *Phoma ucladium*, *Helminthosporium sativum*, *Alternaria alternata*, *Aspergillus niger* and *Penicillium chrysogenum* were studied.

### *Pathogenicity test*

The pathogenicity test of most frequently isolated fungi (*Fusarium solani*, *Phoma ucladium* and *Helminthosporium sativum*) causing sudden decline disease was under taken either alone or in combination in the earthen pots. For this purpose, the commonly growing date palm varieties Aseel, Fasli and Karbalian were selected. The three different methods of inoculation *viz.*, soil inoculation, stem inoculation method and spraying methods were used for pathogenicity test. The two-month-old healthy and uniform date palm seedlings of each variety were obtained from nursery of Date Palm Research Institute, Shah Abdul Latif University, Khairpur. The plants were transferred in sterilized earthen pots containing 2 kg autoclave sterilized soil. In soil inoculation method, the spore suspensions of tested

pathogens were incorporated in the potted soil before seedling transfer. The seedlings amended with only distilled sterilized water (without pathogen) served as control. Similarly, in stem inoculation method, the spore suspensions of tested pathogens were injected in the stem of the date palm seedlings (after one month of transferring in the pots) with the help of the syringe. The date palm seedlings served as control given only 5 ml distilled sterilized water. In spraying inoculation method, the prepared spore suspension of each pathogen was inoculated by spraying approximately at 5 ml/plant. The seedlings sprayed with distilled sterilized water were served as control.

The plants were irrigated regularly after inoculation. After two weeks of inoculation, plants were monitored for the development of disease symptoms. After 45 days of inoculation, the seedlings were uprooted and data was recorded on seedling mortality and infection percentage. The re-isolations were also made from the roots of the uprooted plants to confirm the pathogenic nature of the inoculated fungal species.

The experiment was arranged in randomized complete block design (RCBD) with three replications and three methods of inoculation. The details of the treatment are as under statistical analysis.

#### *Layout of the experiment*

Each treatment comprised of three replicates. The randomized complete block design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using L.S.D test at 5% and 1% level of significance (Steel *et al.*, 1997).

- T1: Control (No inoculum)
- T2: *Fusarium solani* (alone)
- T3: *Fusarium solani* + *Phoma ucladium*
- T4: *Fusarium solani* + *Helminthosporium sativum*
- T5: *Phoma ucladium*
- T6: *Helminthosporium sativum*

#### *Disease severity assessment*

The disease severity was calculated with following

formula as described by Baudian (1988) and Abdalla *et al.*, (2000) using scale:

- 0-5 = 0: No infection.
- 1 = 1-10% damaged or dark area around the point of infection
- 2 = 11-25% gradual wilt occurred on plantlets
- 3 = 26-50% gradual wilt occurred on plantlets
- 4 = 51-75% gradual wilt occurred on plantlets
- 5 = 76-100% Died plantlets

#### *Re-isolation of pathogens*

To satisfy Koch's postulates, re-isolation was also done from inoculated and un-inoculated plants as described above to confirm the pathogenic nature of the tested fungi. For this purpose roots, leaves, flowers and strands were washed thoroughly with tap water and cut into small pieces and Surface sterilized with 5% sodium hypochlorite and plated on PDA.

Plates were incubated at 25°C and the subsequent growth of the pathogen was recorded. The recovery of inoculated fungi was recorded and infection percentage was calculated with the help of following formula:

$$\text{Infection\%} = \frac{\text{Number of pieces colonized by the fungus}}{\text{Total number of pieces studied}} \cdot 100$$

## **Results and discussion**

### *Susceptibility of different date palm varieties to different isolated pathogens*

To confirm the etiology of date palm decline disease, the pathogenicity test was carried out on most commonly cultivated date palm varieties of Khairpur region e.g. Aseel, Fasli and Karbalian, by three different methods.

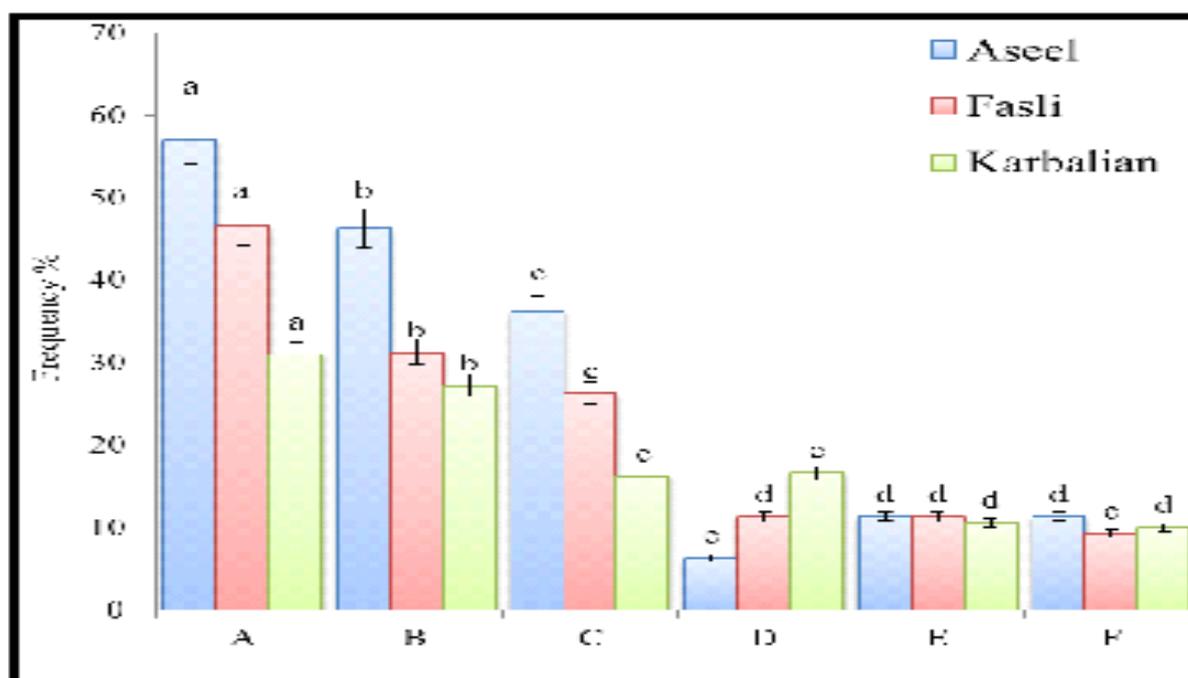
The most predominantly isolated fungi *Fusarium solani* was inoculated alone and in combination with *Phoma ucladium* and *Helminthosporium sativum*. The association of different fungi was greatly varied with different date palm varieties. Among the six fungi, the frequency of *Fusarium solani* followed by *Phoma ucladium* and *Helminthosporium sativum* was significantly high on all the date palm varieties studied (Fig. 1).

**Table 1.** Effect of different pathogens on disease severity of different date palm varieties.

Treatments	Disease severity		
	Aseel	Fasli	Karbalian
<i>Fusarium solani</i>	5.0000	3.6667	2.0000
<i>Phoma ucladium</i>	4.0000	2.6667	2.0000
<i>Helminthosporium sativum</i>	3.0000	1.3333	1.3333
<i>Alternaria alternata</i>	2.0000	1.6667	1.3333
<i>Aspergillus niger</i>	0.6667	0.6667	1.3333
<i>Penicillium chrysogenum</i>	1.0000	0.3333	0.0000
Control	0.0000	0.0000	0.0000
LSD at 0.05	1.34	1.44	1.22

Similarly, El-Deeb *et al.* (2007) conducted experiment on three date palm varieties *viz.* Zaghloul, Sammany and Hayany to study their susceptibility against the pathogenic fungi, *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, *Botryodiplodia theobromae*, *Thielaviopsis paradoxa*

and *Rhizoctonia solani*. Our results are in agreement with the findings of Baraka *et al.* (2011) who tested three varieties and found them susceptible to infection by the isolated pathogenic fungi. Furthermore, var. Hayany was the most susceptible followed by vars. Sammany and Zaghloul.

**Fig. 1.** Susceptibility of different date palm varieties to different isolated pathogens.

Where: A= *Fusarium solani*, B= *Fusarium solani*+*Phoma ucladium* C= *Fusarium solani*+*Helminthosporium sativum* D= *Phoma ucladium*, E= *Helminthosporium sativum*, F= Control

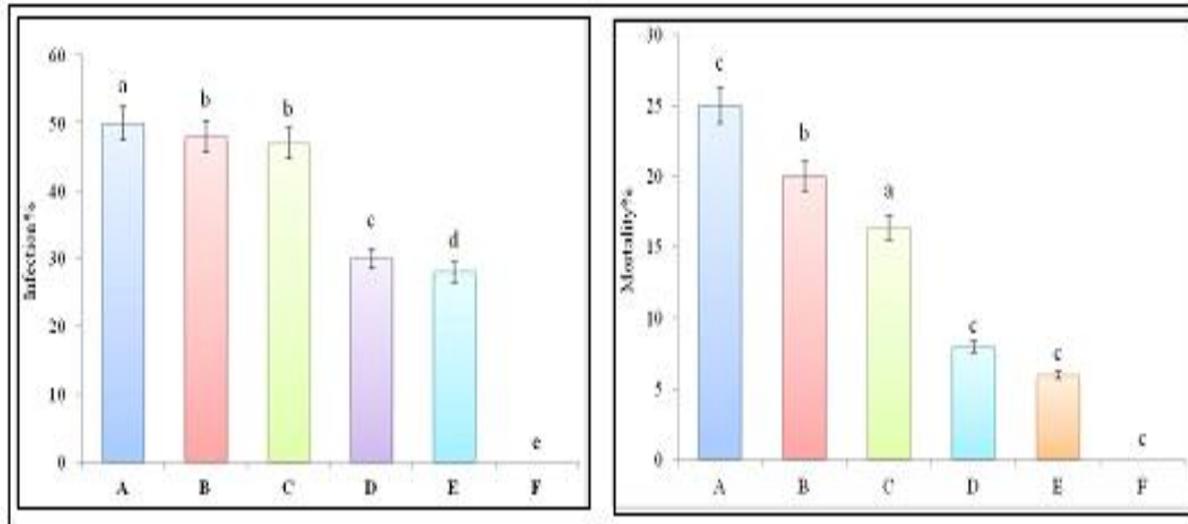
#### Effect of different pathogens on disease severity of different date palm varieties

The significantly highest disease severity was recorded on var. Aseel (10-100%) followed by var. Fasli (10-50%) and var. Karbalian (10-25%) caused by

different pathogens. The *Fusarium solani* caused (100%) disease severity followed by *Phoma ucladium* (75%) and *Helminthosporium sativum* (50%). The lowest disease severity was recorded on *Penicillium chrysogenum* (10%) followed by *Aspergillus niger*

(5%) and *Alternaria alternata* (20%) on different varieties (Table 1). The frequency of *F. solani* in all varieties was ranging from 30-57% with highest on var. Aseel (57%). Whereas, the frequencies of *Phoma ucladium* and *Helminthosporium sativum* was

ranging from 26-45% and 15-35% respectively. The var. Aseel appeared as the most susceptible variety. The maximum frequencies of fungi were isolated from var. Aseel as compared to other two varieties (Table 1).



**Fig. 2.** Effect of different fungi on infection and plant mortality by spraying inoculation method.

Similarly, Baraka *et al.* (2011) tested different varieties of date palm to check the disease severity against different fungal pathogens and found *Fusarium oxysporum* and *Fusarium solani* as most virulent ranging from (23.01-14.35%), while *Thielaviopsis paradoxa* and *Fusarium moniliforme* (12.59-10.46%) were moderately virulent, However, *Botryodiplodia theobromae* and *Rhizoctonia solani* (8.24-9.63%) were proved to be the weak virulent.

#### *Effect of fungi on plant mortality and infection through stem inoculation method*

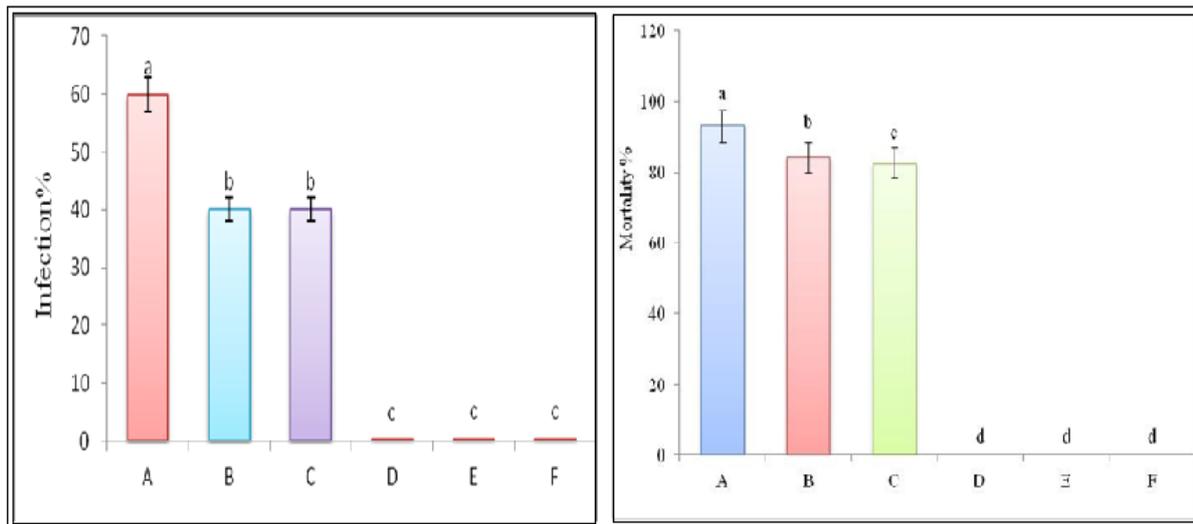
Plant inoculated with *Fusarium solani* alone showed significantly mortality (40%) followed by *Fusarium solani* + *Phoma ucladium* (37%) and *Fusarium solani* + *Helminthosporium sativum* (33%) as compared to control (Fig. 2). No plant mortality was observed in recovered in significantly greater frequencies in plants inoculated either *Fusarium solani* alone or *Fusarium solani* + *Phoma ucladium* or in combination with *Fusarium solani* + *Helminthosporium sativum*. Similarly, the tested pathogen of *Fusarium solani* alone was recovered from the roots of inoculated plants with maximum

frequency (60%) followed by *Fusarium solani* + *Phoma ucladium* (40%) and *Fusarium solani* + *Helminthosporium sativum* (40%). The recovery of *Phoma ucladium* and *Helminthosporium sativum* was negligible from the inoculated plants (Fig. 2).

Feather *et al.*, (1989) confirmed the pathogenic capability through stem and seedling injection. Our results are in close confirmation with the findings of Abdalla *et al.*, (2000) who conducted experiments on Pathogenicity test of toxigenic *Fusarium proliferatum* from date palm in Saudia Arabia by injecting 1 ml of hyphal or spore suspension on six-month-old plants and evaluated at 30, 45, 60 and 90 days after inoculation and recorded disease infection and plant mortality.

#### *Effect of fungi on plant mortality and infection through spraying inoculation method*

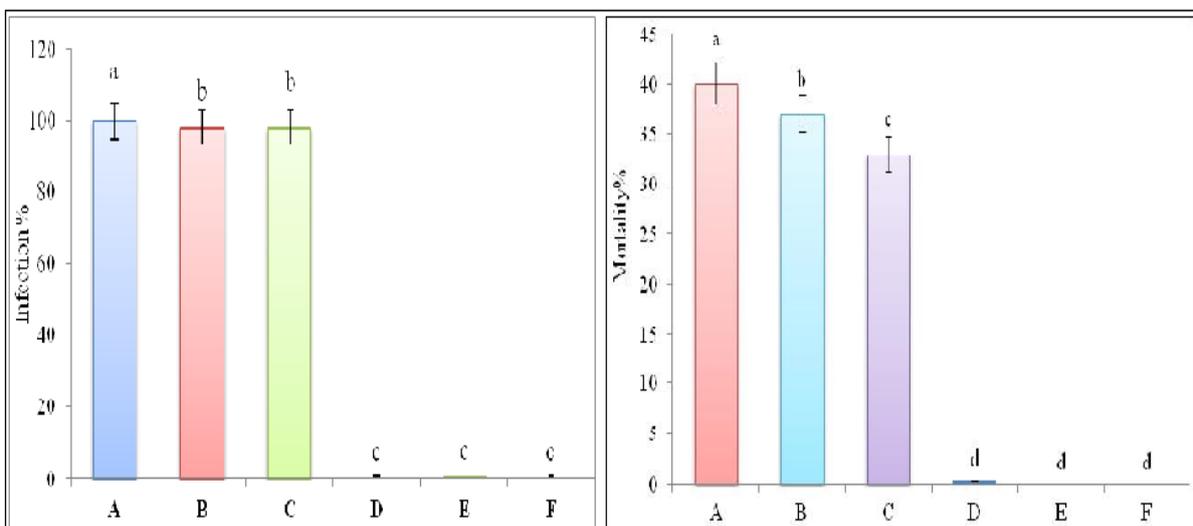
Plant inoculated with *Fusarium solani* alone showed significant mortality (24.66%) followed by *Fusarium solani* + *Phoma ucladium* (20%) and *Fusarium solani* + *Helminthosporium sativum* (16.4%) as compared to control (Fig. 3).



**Fig. 3.** Effect of different fungi on infection and plant mortality by stem inoculation method.

The tested pathogen of *Fusarium solani* alone was recovered from the roots of inoculated plants with maximum frequency (50%) followed by *Fusarium solani* + *Phoma ucladium* (48%) and *Fusarium solani* + *Helminthosporium sativum* (47%) inoculated plants. whereas *Phoma ucladium* (30%) and *Helminthosporium sativum* (28%) alone or in

combination with *Fusarium solani* (Fig. 3). The results of the present studies in close confirmation to those of Rashed and Abdel-Hafeez (2001) who found that *Fusarium moniliforme* and *Fusarium solani* were frequently associated with declined date palm trees in Egypt.



**Fig. 4.** Effect of different fungi on infection and plant mortality by soil inoculation method.

Where: A= *Fusarium solani*, B= *Fusarium solani*+*Phoma ucladium* C= *Fusarium solani*+*Helminthosporium sativum* D= *Phoma ucladium*, E=, *Helminthosporium sativum*, F= Control.

#### *Effect of fungi on plant mortality and infection through soil inoculation method*

Plant inoculated with *Fusarium solani* alone showed significant mortality (93%) followed by *Fusarium solani* + *Phoma ucladium* (84%) and *Fusarium*

*solani* + *Helminthosporium sativum* (82%) as compared to control. Whereas no plant mortality was observed in *Phoma ucladium* and *Helminthosporium sativum* alone (Fig. 4). The tested pathogen of *Fusarium solani* alone was recovered from the roots

of inoculated plants with maximum frequency (100%) followed by *Fusarium solani* + *Phoma ucladium* (98%) and *Fusarium solani* + *Helminthosporium sativum* (98%) inoculated plants, whereas *Phoma ucladium* (0.1%) and *Helminthosporium sativum* (0.5%) alone (Fig. 4). The *Fusarium solani* (either alone or with *Phoma ucladium* and *Helminthosporium sativum*) adversely affects the plant growth. Similarly, El-Zawahry *et al.* (2000) added 100 ml/hyphal or spore suspension ( $4 \times 10^6$  /ml) to soil and conducted pathogenicity test of different fungi and irrigated every 3-4 days to ensure distribution of the tested fungus.



**Fig. 5.** Pathogenicity test by soil inoculation method.

Among three methods of inoculation, soil inoculation method followed by stem inoculation was appeared as more effective in establishing the pathogenicity of the inoculated pathogens and to confirm etiology of the date palm decline disease. The *Fusarium solani* alone showed typical symptoms of disease in soil inoculation methods (Fig. 5 & 6) followed by stem inoculation method and spraying method where as such symptoms were not observed on plants inoculated with combination in *Fusarium solani* + *Phoma ucladium* followed by *Fusarium solani* + *Helminthosporium sativum* and *Phoma ucladium* alone and *Helminthosporium sativum*. No or negligible disease was appeared on plants inoculated without *Fusarium solani* or un-inoculated plants.



**Fig. 6.** Soil inoculation method Un-inoculated (left) Inoculated (right).

The plants inoculated *Phoma ucladium* + *Helminthosporium sativum* alone showed very low level of disease severity and plant mortality. The soil inoculation method was also appeared highly effective in term of recovery of inoculated pathogens, as *Fusarium solani* was recovered in very high frequencies (98-100%) from inoculated plants.

Whereas stem inoculation method and spraying method failed to produce typical disease symptoms or no infection was recorded but it was in very low frequency.

Sarhan *et al.* (2001) and Al-Yaseri *et al.* (2006) reported that *Fusarium oxysporium* and *Fusarium solani* were the most frequent and most abundant fungal species in the roots of date palm trees showing decline disease and wilt disease in Iraq. Similarly, Mansoori and Kord (2006) reported that *Fusarium solani* is associated with yellowing and death of the fronds of date palm.

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