INCIDENCE OF PATHOGENIC MICROORGANISMS IN DATE PALM STOCKS PRODUCED BY TISSUE CULTURE TECHNIQUE

BY

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B. Sc. Agric. Sci.(Plant Pathology), Fac. Agric., Cairo Univ., Egypt, 1998

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APPROVAL SHEET

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ABSTRACT

The survey proved that certain fungal diseases of date palm plants were occurred at varies degrees according to the locality. Isolation procedures yielded *Aspergillus niger*, *Alternaria* sp., *Penicillium* sp., *F*usarium *oxysporum*, *Phomopsis* sp., *Pythium* sp. and *F. moniliforme*.

The selected fungi which more pathogenic to date palm plants and more virulent were *F. oxysporum*, *Phomopsis* sp. and *F. moniliforme*.

All pathogenic fungi were able to produce PME, PG and CX enzymes either *in vitro* and *in vivo.* Phenol contents were higher in infected tissues than in the healthy ones but, sugar contents were higher in healthy tissues than in inoculated.

Microscopic examination of sections, taken from basal parts of date palm infected leaves, indicated that the pathogenic fungi were able to change cells structure.

Soaking the roots of date palm plant or injecting their leaves bases in deferent concentrations of Ascorbic acid or Salicylic acid reduced the development of diseases caused by the tested fungi compared with control.

Saprol completely inhibited growth of all tested fungi by using tested concentrations (40, 50, 100 and 150 ppm). The same was for Topsin M_{70} and Bendazin against *F. moniliforme* and *Phomopsis* sp. but, reduced the growth of *F. oxysporum*. The same fungicides were used to control the tested fungal diseases in infected tissues of date palm plants.

Spraying with fungicides carried out ten days after artificial inoculation with fungi. Decrease in disease severity was recorded ten days after treated with fungicides. Topsin M_{70} was the most effective against all tested fungi.

Key words: Tissue culture, date palm, induced resistance, *Fusarium oxysporum, Fusarium moniliforme*,*Phomopsis* sp.

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CONTENTS

INTRODUCTION

Date palm (*Phoenix dactylifera L.*) is considerd one of the most important fruit crops in Egypt. It is cultivated all over the the country specially in Aswan, Sinai, New Vally, Sharkiya, Giza and Beheira governorates. Generally, date palm is attacked by several fungal diseases in fields and nurseries.

The most important and common diseases are, Black scorch caused by *Thielaviopsis paradoxa (*Klotz and Fawcett, 1932), Diplodia and Botroyodiplodia diseases caused by *Diplodia phoenicum* (Klotz and Fawcett, 1932) and *Botryodiplodia theobromae* (Baraka *et al*., 1985), respectively.

Inflorescence rot (Khamedj) caused by *Mauginilla scaettae*, Graphiola leaf spot caused by *Graphiola phoenicis* and there are many of Fusarium species affecting palm species, *Fusarium moniliforme* and *F. semitectum* were isolated in India from leaves and spears of oil palm (Babu, 1989).

(Ammar, 2003) Isolated the same fungi mentioned above from date palm (*Phoenix dactylifera L.*) in Egypt.

(Prasad *et al*., 1992) isolted the same fungi in India from ovule of sago palm (*Cycas revolute*).

F. proliferatum was isolated in Saudia Arabia and Spain from root, crown area and basal parts of leaves (Armengol *et al.*, 2001)

Nowadays, tissue culture micropropagation of date palm plants aids to produce large numbers of genetically uniform palms without diseases.

However, contaminations appear during any stage of propagation of tissue cultured date palm plants especially during acclimatization stage are considered dangerous and affect the micropropagation of tissue cultured plants of date palm. Sabet *et al.* (2007).

The objectives of the presented studies were to investigate the following aspects:

1. Isolation and identification of the fungi which cause diseases to tissue cultured date palm plants during acclimatization stage and their pathogenic capabilities.
2. Enzymatic activity and biochemical changes due to infection of the most virulent fungi.
3. The effect of abiotic inducers on the development of diseases through infected tissues and the defence of plants.
4. Histopathological features of fungal development within the infected tissues.
5. The effect of different fungicides on fungal growth *in vitro* and on diseases control.

REVIEW OF LITERATURE

Date palm (*Phoenix dactylifera* L.) is usually subjected to several diseases caused by certain pathogens belonging to fungi and / or bacteria as well as phytoplasma. Fungal diseases are mainly responsible for the majority of losses caused in date production or in the number of plantlets produced during tissue culture techniques.

1. Symptoms and causal fungi

Feather et al. (1989) found that *F. oxysporum* in association with *Gliocladium vermoesenii* (*P. vermoesenii*) caused wilt and dieback of *Phoenix canariensis* (canary island palm), disease complex. Symptoms reduced canopy, death of pinnae on one side of the rachises and cause vascular browning, and black-brown dry rot in bud and rachises tissues.

Abbas *et al.* (1990) in Iraq, isolated *Phomopsis phoenicola* (Traw. and Pessa) and *F. equiseti* [(Cord) Sacc.] from date palm trees showing symptoms of decline. *Phomopsis phoenicola* was also isolated from root, trunk, rachises and bud samples of affected palms which were exhibtied symptoms of decline, dying and terminal bud rot.

Turney (1999) revealed that the causal agent of cankers on camellias (*Camellia sasanqua*) in southern California has been identified as *Phomopsis* sp. On the other hand, Veselig and Arsenijevic (1999) reported that *Phomopsis* sp. caused necrosis and die-back on blackberry canes in Yugoslavia.

2. Fungi and involved date palm disease

Klotz (1930) showed the cracking of distal end of the date fruits resulting from the physiological disorder known as “black nose” which offers a good harbor for air borne fungi such as *Alternaria spp., Phomopsis spp., Rhizopus spp*. and *Hormodendron sp*.

Bliss (1939) suggested that *Penicillium vermoesenii* (Biourge) could cause diseases to *Phoenix canariensis*, *Washingtonia filifera* and *Cocos plumose*. Synonymous is *Penicillium roseum* (Link). The disease was described as producing discoloured linear streaks on leaves of *P. canariensis*, the pinnae become yellowish brown and dying. The disease seemed to spread by contact from one leaf base to another, death of the palm occurred when the terminal bud was invaded.

On *W. filifera*, stunted and deformed leaves, retarded terminal growth, and great masses of rose-colored spores between the layers of leaves characterized of the disease. On date palms the disease was usually observed in association with the Diplodia disease.

Turrell *et al.* (1940) found that the two most important fungi cause spoilage were *Aspergillus niger* and *Alternaria spp*., which incitant of calyx-end rot, causing spot decay of date palm fruits. Almost 50% of the loss was due to fungal decay.

Carpenter and Klotz (1966**)** reported that species of *Alternaria, Helminthosporium* and *Macrosporium* may infect date fruits directly, at the beginning of Khalal stage. While, *Aspergillus niger* and *Citromyces ramosum* may cause a calyx end rot at the late of Khalal or early Rutab stage.

Cohan (1972) isolated *Alternaria citri, A.stemphylicides, Aspergillus niger, A. phoenicis, Catenularia fuligines, Fusarium moniliforme, F. semitectum, F. lateritium, Penicillium roseum, Diplodia phoenicum* and *Phomopsis phoenicola* from preharvest and dropped date fruit. All obtained isolates exhiited pathogenic capabilities, when tested on date fruits under laboratory conditions.

Hartley (1977) stated that the causal of lethal bud rot of oil palm is oscure. Insects, fungi and bacteria had been suspected, while unfavorable environmental circumstances such as poor drainage, compacted soils and unbalanced nutrition have been put forward as predisposting factors. Fungi which had been isolated from diseased spears include *Fusarium oxysporum*, *F. moniliforme* and *Botryodiplodia sp.* Also, invasion of bud tissue by bacteria was also presented in diseased spears, and pathogenicity of these organisms had not yet been established.

El-Arosi *et al*. (1982) stated that *A. alternata, Aspergillus fumigatus*, *A. japonicus*, *Botryodiplodia sp., Fusarium moniliforme*, *F. lateritium*, *Nigrospora sp.* and *Penilicillium sp*. were isolated from calyxes of infected date fruits.

Baraka *et al*. (1985) isolated *Alternaria sp., Cladosporium sp., Thielaviopsis paredoxa, Aspergillus niger, Rhizopus nigricus* and *Penicilluim spp.* from naturally infected Samany and Zaghlool dates. The fruits of the two varieties were more susceptible in Rutab stage.

Lamberti *et al*. (1985) isolated *F. oxysporum* from the trunk and primary roots of Socorro coconut palm which exhibited wilt disease in Philippines. Also, Babu (1989) isolated *F. moniliforme* (*Gibberella*

fujikuroi) and *F.semitectum* (*F. pallidoroseum*) from rotted spears and from infected leaves of oil palm in India.

Feather *et al*. (1989) found that *Fusarium oxysporum* in association with *Gliocladium vermoesenii*, causes wilt and die back on *Phoenix canariensis*. They also added that both fungi were pathogenic, by stem injection, singly and in combination to seedlings.

Mishra *et al*. (1989) reported that *Gliocladium roseum* in associated with *Helminthosporium halodes*, *Gloeosporum, Fusarium* spp. and *Macrophomina phaseolina* caused leaf rot disease complex of coconut palm.

Abbas *et al*. (1990) in Iraq, isolated *Phomopsis phoenicola* (Trav. and Pessa.) and *F. equiseti* ((corda) Sacc.) from date palm trees showing symptomos of decline. *Phomopsis phoenicola* was also isolated from root, trunk, rachises and bud samples of affected palms exhibited sympotoms of declining, dying and terminal bud rot.

Matheron and Benbadis(1990) isolated *Fusarium oxysporum* f.sp.*albedines* from date palms and found that Deglet Nour is susceptible to this fungus.

Prasad *et al*. (1992) isolated *F. moniliforme* and *F. moniliforme* f.sp. *intermedium* from Sago palm (Cycas revoluta) exhibited symptoms of ovule rot. It caused a rot of immature ovules on *C. revoluta* in India.

Lopez-Llorca and Orts (1994) recorded that *penicillium vermoesenii* (Biourge) (*Gliocladium vermoesenii* (Biourge) Thomos) was the causal agent of palm disease, commonly termed “Pink but rot”. The fungus had been observed in several species of palms, normally

causing necrosis and sunken lesions on new leaves. If the infection reaches the central but it caused the death of the palm.

Rashed and Abdel-Hafeez, (2001) isolated *F. moniliforme* from rachies of date palm in Egypt, showimg decline symptoms. However, Armengol *et al*. (2001) isolated *F. proliferatum* from root, leave bases and crown area of different palm species in Spain.

Ammar (2003) reported that *Phomopsis spp., Alternaria sp., penicillium sp., Fusarium moniliforme, F. semitectum* and *Gliocladium roseum* from different geographic locations in Egypt from date palm. *F. moniliforme* and *F. semitectum* exhibited heart rot symptoms. Also, Ammar *et al*. (2004) isolated *F. moniliforme* frome leaves bases and crown of tissue cultured date palm (*Phoenix dactylifera L*.) showing canker symptoms after acclimatization stage. Sabet *et al*. (2007) isolated *F. moniliforme*, *F. oxysporum* and *Phomopsis* sp.from leaves, crown and roots of tissue cultured date palm plants during acclimatization stage.

3. Enzymatic activities of the causal pathogens

Several investigators mentioned that causal pathogens were able to produce cellulolytic and pectinolytic enzymes during their metabolism under laboratory conditions or in the infected tissues.

Trique (1971) stated that all strains of *Fusarium oxysporum* f.sp. *elaeidis*, the causal agent of oil palm wilt, showed the same ability for liberating pectinases *in vitro*, mainly endopolygalacturonase and methylesterase; transeliminase activities were very slight being nil.

Abdel Sattar (1978) found that *Ceratocystis paradoxa, Colletotrichum musae, Botrytis cinerea* and *Botryodiplodia*

theobromae were varied in PME, PG and CX production. Enzymes activity reached its maximum after 15 days of incubation. He added that loss in viscosity was greatly increased in inoculated banana fruits compared with non- inoculated one.

Srivastava (1986) reported that adding of apple pectin and carboxy methyle cellulose in a synthetic media enhanced the production of pectinolytic and cellulolytic enzymes, respectively. The optimum incubation period was 9 days for maximum production of both enzymes, produced by *Alternaria alternata, Aspergillus flavus, Cladosporium herbarum,* and *Myrotheciumroridum.*

Rashed (1991) found that *in vitro* PME, PG and CX activities of the isolate of *B. theobromae* from Rasheed (R) were higher in culture filtrates of the fungus than Qanater (Q) isolate but, he found that *in vivo*, the (Q) isolate of *B. theobromae* was more active in producing pectinolytic (PG and PME) and cellulolytic (CX) enzymes than (R) isolate in infected tissues of date palm rachises.

EL-Deeb (1994) observed that the four tested fungal pathogens (*F. oxysporum; Thielaviopsis paradoxa, Diplodia phoenicum* and *B. theobromae*) were able to produce cellulolytic and pectinoytic enzymes during their metabolism under laboratory conditions. Activity of cellulolytic and pectinolytic enzymes were detected in both healthy and diseased date palm tissues, with higher levels in the latter.

Nafea, azza (1995) revealed that the activity of (PME) could not be detected in healthy Zaghlool and Samany varies as well as in infected Samany fruits inoculated with *T.paradoxa; Cladosporium herbarium* and *Alternaria alternata*. Infected Zaghlool fruits with