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Effect of Water Salinity on *Thielaviopsis paradoxa* and Growth of Date Palm Seedlings

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Abstract. *Thielaviopsis paradoxa* was isolated from rotted roots and leaves rachis tissues obtained from date palm trees showing characteristic black scorch symptoms. Results of artificial inoculation of date palm seedlings from ten different palm cultivars revealed that Nabtat Aly and Om-Khashab were more susceptible than the other tested cultivars. Increased water salinity (12.9 ds/m) reduced growth of date palm seedlings as expressed by length or fresh weight of the seedlings. Growth and sporulation of *T. paradoxa* decreased as the conductivity levels in the solid agar media increased. Fungal growth; however, was not dramatically inhibited even at the highest conductivity level tested (30.9 ds/m). Increasing the salinity levels (1.4 (tap water), 6.9, 12.9, 18.4, 26.5 ds/m) of the water used for irrigation of six-months-old date palm seedlings tended to increase the infection rate of inoculated seedlings as compared with the control.

Introduction

Black scorch caused by *Thielaviopsis paradoxa* (De Seynes) v. Hohn has been reported from most date palm producing countries including Saudi Arabia [1, p.70]. The most striking symptom of this disease is dark brown to black, rough, irregular, necrotic lesions on the leafstalk. Such lesions may coalse to cover the whole leafstalk which then looks as if it has been exposed to fire. The fungus may also invade the inflorescence and fruit stalk causing their blackening and decay. The fungus has also been associated with fruit rot [2]. Finally in a few cases the fungus, may invade the terminal bud leading to tree death [3, p. 21].

Although this disease has been reported in Saudi Arabia since 1954 [4], it has attracted relatively little attention. The sporadic distribution of the affected trees may be the reason for this negligence. Varietal resistance and environmental factors may play a role in the sporadic distribution of this disease. The accumulation of salts due to saline irrigation in many parts of Al-Gassim region [5] may represent an important environmental stress leading to increased plant susceptibility. No studies have been conducted regarding the effect of increased salinity on diseases commonly occurring on date palm.

The objectives of this research were to test the susceptibility of several date palm cultivars to infection with *T. paradoxa*, to determine the effects of various conductivity levels on growth and sporulation of the fungus, and the potenial effect of salinity on predisposition of date palm seedlings to infection with *T. paradoxa* under greenhouse conditions.

Materials and Methods

A) Isolation of the causal agent

Isolations were done from symptomatic leaves rachis tissues as well as rotted roots. Plant material was washed thoroughly under a fine spray of tap water. Tissue pieces (± 6 mm) were surface disinfested with 0.5% NaOCl for 2 min, rinsed in sterile distilled water (SDW) for the same period and left to dry on sterile filter paper in a flow bench. Plant sections were placed on acidified potato dextrose agar (APDA) medium. The pH of the medium was aseptically adjusted to 4.5 after autoclaving by addition of 0.1N H Cl Plates were incubated at 27°C ± 1 for one week. After incubation, the developed fungal growth was purified and identified according to morphological and cultural characteristics [3, p.19; 6, p.88; 7, p.54].

B) Pathogenicity tests

Fungal cultures were grown for 10 days at 27°C ± 1 on APDA medium. The inoclum was prepared by flooding the agar surface of each petri dish with 10 ml of SDW and scraping it with a spatula. The resulting spore suspension was filtered through four layers of cheese cloth, and the spore concentration was adjusted to 1 x 10^6 spores /ml using a hemacytometer.

The date palm cultivars listed in Table 1 were used in this study. Seeds of these cultivars were surface sterilized for 10 min. with 0.5% NaOCl, soaked in SDW for 24 hrs., then planted in 12 x 15 x 24 cm pots filled with steamed sandy loam soil. Five seeds of each cultivar were planted in each pot and five pots were used for every cultivar. Pots were kept in the greenhouse and plants were inoculated six months after seedling emergence. Two milliliters of *T. paradoxa* spore suspension were injected into the base of the cotyledon of each seedling using a sterile 5 ml plastic syringe. Another

set of seedlings from each cultivar were injected with SDW to serve as a check. After inoculation, pots were watered and covered separately with plastic bags for 48 hrs to maintain high humidity. Plastic bags were removed and pots were randomly arranged under greenhouse conditions. The percentage of infected plants in each cultivar was calculated three months after inoculation.

C) Effects of irrigation water salinity on date palm seedlings growth

Two sets of date palm seedlings derived from seeds of the previous cultivars were prepared. Six months after seedling emergence, one set was irrigated daily with saline water where each pot was irrigated with 100 ml of Na Cl solution with Ec value of 12.9 ds/m. In the other set, each pot was irrigated with 100 ml of tap water with Ec value of 1.4 ds/m. Three months after the irrigation treatments, seedlings in each set were harvested, washed thoroughly and seedlings lengths as well as fresh weights were determined.

D) Effects of salinity on mycelial growth and sporulation of *T. paradoxa*

A 6-mm-diameter disk was cut from a 7-days-old APDA culture of *T. paradoxa* and placed in a 8.5 cm - diameter petri dish containing 15 ml of potato dextrose agar medium to which Na Cl was added at different concentrations to give Ec values of: 11.4, 17.3, 24.7, 30.9 ds/m. No NaCl was added to the control APDA medium which had an Ec value of 4.9 ds/m. Plates were incubated at 27°C ± 1 and the radial growth of the fungus on the various media was measured after 3 days while the numbers of spores per ml were determined by 7 days after incubation using a hemacytometer.

E) Salinity level effects on the number of infected plants in the greenhouse

Date palm seedlings derived from seeds of Shagra and Rothan varieties were used in this study. Irrigation treatments were applied after seedling emergence, while inoculation tests were conducted six months after seedlings emergence. Plants in every pot were irrigated daily with 100 ml of NaCl solution with Ec values of: 1.4 (tap water), 6.9, 12.9, 18.4, 26.5 ds/m. There were 10 pots (replicates) of each salinity treatment for each cultivar each pot contained five seedlings. Plants of five replicates from each treatment were inoculated with *T. paradoxa* spore suspension as described before, while seedlings of the other five replicates were injected with SDW to serve as a check. All pots were maintained in the greenhouse with a maximum temperature of 30°C and a night minimum of 25°C. Three months after inoculation, the number of infected plants in each treatment was determined.

A completely randomized design was used for all experiments. All experiments were repeated twice and data were combined. Means were compared using Duncan's multiple range test.

Results

A) Fungal isolation

Thielaviopsis paradoxa was consistantly isolated from rotted roots as well as leaves rachis obtained from date palm trees showing the characteristic black scorch symptoms. Fungal colonies on APDA are first white in color, but after one week they obtain a dark grey to blackish color. The fungus produces two types of conidia : cylindrical, hyaline microconidia, measuring 10 to 15 u in length and 4 to 7 u in width. This type of conidia is produced internally within the conidiophore and thus is called endoconidia. The other type is egg-shaped, dark brown in color and measure 15 to 20 u in length and 9 to 12 u in width. The latter type is referred to as chlamydospores. These findings agreed with the description of *T. paradoxa* given by several authors [3,p. 19; 6, p. 88; 7, p. 54]. There are all gradations in size, shape, and brown color between these two types as stated by Fawcett and Klotz [3, p. 19].

B) Pathogenicity tests

Susceptible date palm seedlings reacted to infection with *T. paradoxa* in the form of tip necrosis. Necrosis progressed downward within 30 to 50 days until the entire cotyledon was severely necrotic. For the purpose of this study, seedlings necrotic with one fourth of its length were considered infected. No symptoms were observed in any of the control seedlings of the ten cultivars used over the three months after inoculation.

Date palm seedlings derived from different cultivars reacted differently to the inoculation with *T. paradoxa* (Table 1). Cultivars Om-Khashab and Nabtat Aly were more susceptible than the other tested cultivars. On the other hand, Helwa was the most highly resistant cultivar in comparison with the other cultivars.

C) Salinity effects on growth of date palm seedlings

Seedlings growth of most tested date palm cultivars was suppressed due to irrigation with saline water (12.9 ds/m) for three months (Table 2). The cultivar Shagra showed a significant ($P= 0.05$) reduction in both mean fresh weight and mean length as compared with check (irrigated with tap water). On the other hand, seedlings of the cultivar Rothan showed better growth under saline water irrigation regime for three months (Table 2). The two previously mentioned cultivars were selected for the following greenhouse experiment to study the effect of water salinity on infection of date palm seedlings with *T. paradoxa*.

D) Influence of salinity on mycelial growth and sporulation of *T. paradoxa*

T. paradoxa is a fast growing fungus, its mycelial growth filled the plate on APDA medium after 3 days. Both mycelial growth and spore production was negatively affected by the increase of salinity level in the culture medium (Figs. 1,2). The reduction in radial growth; however, even at the highest salinity level tested (30.9 ds/m) was not

dramatic (Fig. 1), the fungus continued to grow at a fairly high rate and covered more than two thirds of the plate in three days. Sensitivity to salt concentration varied between the two types of spores produced by the fungus (Fig. 2). The numbers of both endoconidia and chlamydospores were reduced as the salinity level increased. Chlamydospores production, was totally inhibited at Ec value of 30.9 ds/m (Fig. 2).

Table 1. Disease incidence on nine-mon.-old seedlings of various date palm cultivars, artificially inoculated with *Thielaviopsis paradoxa*

Cultivar	Plants infected (%)
Helwa	5
Kuereia	16
Maktoomy	28
Nabtat Aly	35
Om-Khashab	39
Roshody	31
Rothan	16
Sabaka	29
Shagra	18
Sukkary	22

* Data are combined from two experiments and results of each experiment are based on 25 seedlings per cultivar.

Table 2. Growth of date palm seedlings, irrigated with saline water (12.9 ds/m) for three months, as compared with seedlings irrigated with tap water (1.4 ds/m).

Cultivar	Seedlings mean fresh weight (g)		Seedlings mean length (cm)	
	Tap water	Saline water	Tap water	Saline water
Helwa	6.3 a[x]	5.9 a	40.5 a	37.3 b
Kuereia	7.2 a	6.0 b	36.5 a	33.8 a
Maktoomy	9.1 a	8.2 a	40.0 a	37.5 a
Nabtat Aly	5.3 a	4.1 b	37.8 a	35.0 a
Om-Khashab	10.8 a	9.7 a	38.5 a	36.3 a
Roshody	9.3 a	8.5 a	37.0 a	34.5 a
Rothan	6.5 b	7.9 a	36.2 b	39.7 a
Sabaka	6.2 a	5.4 a	38.0 a	34.8 b
Shagra	8.7 a	6.5 b	39.0 a	31.3 b
Sukkary	9.5 a	8.6 a	40.3 a	37.8 a

[x]Means within a row (horizontally for each character) followed by the same letter are not significantly different (P =0.05) according to Duncan's multiple range test.

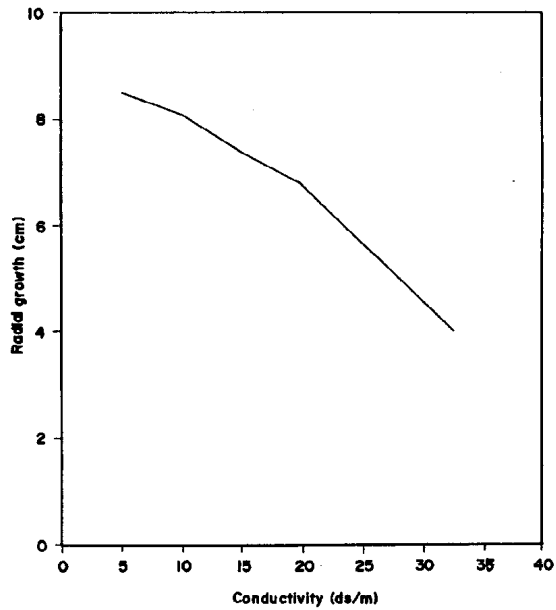

Fig. 1. Effect of various conductivity levels using NaCl in solid agar media on radial growth of *T. paradoxa* after 3 days of incubation.

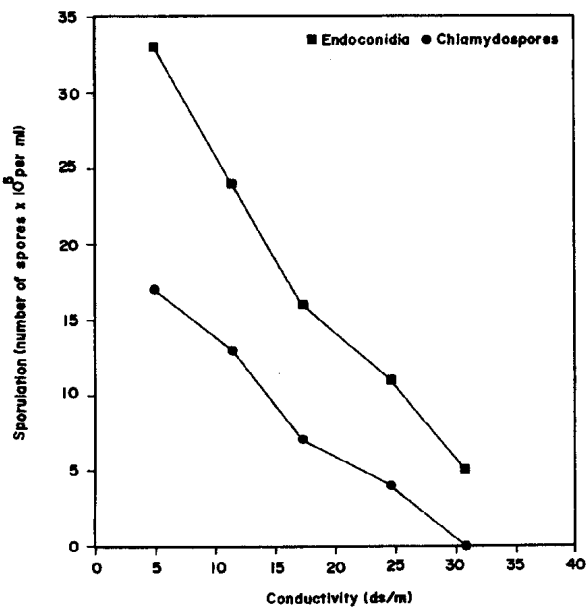

Fig. 2. Effect of various conductivity levels using NaCl in solid agar media on sporulation of *T. paradoxa* after 7 days of incubation.

E) Salinity level effects on the number of infected plants in the greenhouse

In case of Shagra, the number of infected date seedlings increased gradually with the increase in salinity level (Fig. 3). The salt tolerant cultivar (Rothan); however, expressed first a reduction in the number of diseased seedlings as the salinity level increased from 1.4 ds/m (tap water) to 6.9 ds/m or even 12.9 ds/m (Fig. 3). But as the conductivity level increased further the number of infected seedlings increased sharply and at conductivity level 26.5 ds/m, the percentage of infected plants of the salt tolerant cultivar (Rothan) (57%) was more than those of the salt sensitive one (Shagra) (46%) (Fig. 3).

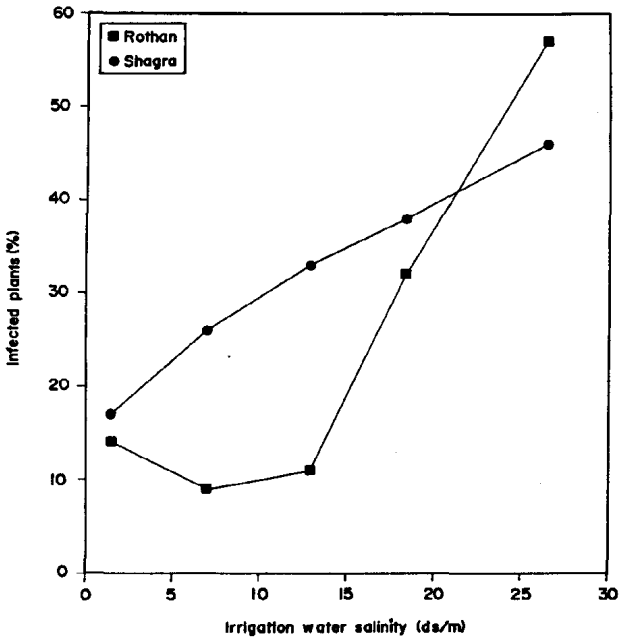

Fig. 3. Effect of irrigation water salinity on number of data palm seedlings (cultivars: Shagra and Rothan) infected with *T. paradoxa* after 3 mon. of incubation.

T. paradoxa was easily recovered from infected seedlings in all inoculated treatments but was never recovered from uninoculated plants.

Discussion

During the course of this study, *T. paradoxa* was consistantly isolated from rotted roots as well as leaves rachis of date palm trees exhibiting black scorch symptoms. These results contradict what has been stated by Djerbi [1, p. 70] that *T. paradoxa* was not isolated from the roots of naturally infected trees. This may be due to the presence of various biotypes of the fungus in different regions.

In the greenhouse, all tested palm cultivars were found to be infected with *T. paradoxa* but with various degrees of susceptibility. Increasing the salinity level of irrigation water resulted in the increase in the number of seedlings infected with *T. paradoxa*. Salinity stress increased Phytophthora root rot of citrus [8], and of tomato [9], Pythium blight of creeping bent grass [10], common root rot of spring wheat and barley caused by *Cochliobolus sativus* [11] and increased seed rot of soybean sown in soil infested with *Rhizoctonia solani* [12]. In the present study, the primary effect of increased water salinity was apparently on the plant, because salinity had little effect on mycelial growth of the fungus. Also endoconidia were produced with relatively high numbers even at the highest water salinity level tested. Increased disease incidence could result from increased tissue susceptibility and/or inhibition of root growth and regeneration as suggested by Blaker and Mac Donald [8].

Salinity levels around various trees in the same area may vary considerably over the course of time depending on irrigation variables such as irrigation frequency, duration, water quality, soil permeability as well as grass cover. Fluctuations in salinity levels along with varietal resistance may contribute to the sporadic occurrence of black scorch of date palm in the field.

References

[1] Djerbi, M. *Diseases of the Date Palm*, Beirut: Al Watan Printing Press Co., 1991.

[2] Al-Hassan, K. and Abbas, G.Y. "Biology of *Thielaviopsis paradoxa* and Its Role in Date Fruit Rot". *Date Palm J.*, 2 (1983), 37-54.

[3] Fawcett, H.S. and Klotz, L.J. Diseases of the Date Palm, *Phoenix dactylifera*." *Univ. of California Bull* Berkeley, 522 (1932).

[4] Nixon, R.W. "Date Culture in Saudi Arabia." *Date Grower's Inst. Ann. Rep.*, 31 (1954), 15-20.

[5] Abou-Taleb. E.M., Tohamy, A.M. and Madkour, M.A. "Pathogenesis of Certain Soil-borne Fungi as Affected by Salinity." *Alex. J. Agric. Res.*, 33 (1988), 151-166.

[6] Barnett, H.L. and Hunter, B.B. *Illustrated Genera of Imperfect Fungi*. 3rd. Minneapolis, Min.: Burgess Publishing Co., 1972.

[7] Nag Raj, T.R. and Kendrick, B. *A Monograph of Chalara and Allied Genera*. Waterloo: Wilfred Lanvier Press, 1975.

[8] Blaker, N.S. and Mac Donald, J.D. "The Role Of Salinity in the Development of Phytophthora Root Rot of Citrus." *Phytopthology*, 76 (1986), 970-975.

[9] Swiecki, T.J. and Mac Donald, J.D. "Soil Salinity Enhances Phytophthora Root Rot of Tomato but Hinders Asexual Reproduction of *Phytophthora parasitica*." *J.Am. Soc. Hortic.* Sci., 116 (1991), 471-477.

[10] Rasmassen, S.L and Stanghellini, M.E. "Effect of Salinity Stress on Development of Pythium Blight in A *grostis palustris*." *Phytopthology*, 78 (1988), 1495-1497.

[11] Duczek, L.J. "The Effect of Soil Salinity on Common Root Rot of Spring Wheat and Barley." *Can. J. Plant Sci.*, 73 (1993), 323-330.

[12] Abdel-Aal, S.I., Sabrah, R.E., Rabie, R.K. and Abdel Magid, H.M. "Evaluation of Ground Water Quality for Irrigation in Central Saudi Arabia". *Arab Gulf J. Scient. Res.*, 15 (1997) (In press).

تأثير ملوحة ماء الرى على الفطر *Thielaviopsis paradoxa* وعلى نمو بادرات نخيل البلح

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ملخص البحث. تم عزل الفطر ثيلافيوبسز بارادوكسا من الجذور المتعفنة وقواعد الأوراق المأخوذة من أشجار تظهر عليها أعراض الإصابة بمرض اللفحة السوداء. أوضحت نتائج العدوى الصناعية التى أجريت على بادرات عشرة أصناف مختلفة من النخيل ، أن هذه الأصناف استجابت بدرجات متفاوتة للإصابة بالمرض. كان الصنفين نبتة على وأم الخشب هما أكثر الأصناف قابلية للإصابة.

أدت زيادة ملوحة ماء الري باستخدام كلوريد الصوديوم (١٢,٩ ديسيسمتر لكل متر) إلى نقص نمو بادرات النخيل بصفة عامة وذلك من حيث الوزن الرطب أو الطول للبادرات. كذلك أظهرت النتائج المعملية أنه كلما ازدادت نسبة الملوحة في بيئة زرع الفطر *T. paradoxa* ، كلما تناقص معدل النمو الفطري وتناقص كذلك عدد الجراثيم التى يكونها. ومع هذا فإن الفطر قد استمر في النمو وإنتاج الجراثيم حتى عند أعلى نسبة ملوحة بالبيئة تم اختبارها (٣٠,٩ ديسيسمتر لكل متر). كما أدت زيادة ملوحة ماء الرى من ١,٤ (ماء الصنبور) الى ٦,٩ ، ١٢,٩ ، ١٨,٤ ، ٢٦,٥ ديسيسمتر لكل متر ، إلى زيادة نسبة البادرات المصابة بالفطر *T. paradoxa* مقارنة بالنباتات المروية بماء الصنبور.