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# In Vitro Culture of Date Palm (*Phoeruix dactylifera* L) Roots

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## Abstract

The accessional harvested from the 10-year-old Wad Loggi, date palm (*Phoeruix dactylifera* L) class trees planted in open field conditions. Several treatments were performed with the aim of this work to obtain plant roots free of fungal or bacterial contaminants. The root is repeatedly washed with running tap water followed by soaking for different exposure times at different concentrations of "Clorox". The first experiment results showed that for 60 min soaking with a concentration of 30% "Clorox" gave a relatively higher number of contaminant-free culture tubes.

In the second experiment, explants were immersed in an antioxidant solution as a pre-treatment for the "Clorox" for different exposure times. Presoaking in this solution for 60 min led to significant increases in the percentage of sterile implants compared to the "Clorox" treatment alone. A comparative study was conducted using utilizing antiseptic iodine as potential chemical sterility for date palm root explants and as an alternative to "Clorox" In the third experiment, the roots were soaked for different treatment duration in an iodine solution. The results of iodine treatment gave consistently higher rates of sterile root explants compared to "Clorox" treated roots yet were associated with paired iodine treatment at all times of exposure. Thus, the most advisable sequence of disinfestations for date palm roots obtained from mature trees appear to be as follows: frequent washing repeatedly with running tap water before immersing in an antioxidant solution for 60min. The surface of the soak-treated roots in "Clorox" solution for 60 min, rinsed three times before implanted in a nutrient medium.

**Keywords:** Wad Loggi. Date palm. Clorox, Soaking. Exposure time. Roots free contamination. Antioxidant. Presoaking. Sterile. Lodine. Disinfestations.

## Introduction

Traditionally the date palm (*Phoenix dactylifera* L) of the Arecaceae family is propagated by offshoots, the limited number of offshoots a date palm tree produces during its life [1,2]. The low percent it takes to make this method slow ineffective, and the time takes 3 to 4 years to produce a transplantable offshoot. Tissue culture techniques for plant propagation have been looked upon as an alternative. The application of this technique to date palm propagation has been hampered by the absence of the muscular cambium and the absence of a natural branching habit in date palm trees, which limits the number of structural plants available and potential. Obtaining the shoot tips and lateral bud, the excerpt lants of choice plants selected for the clonal desired is difficult, tedious, and laborious [3-5]. Extracted plants obtained from seed or seedlings from maternal tissues [6,7] were used to develop techniques and procedures and then to apply successful methods obtained from selected mature date palm trees. Highly disparate date palm tissue sources have also been used desperately to initiate date palm culture [4-5,8,9].

The morphogenetic responses of these explant types were differentially sporadic cand, irregular, and dependent on the source of the explant's plants and their ontological physiological ages. Large numbers of adventitious roots form naturally at the base of palm tree trunks and are easy to induce and excise without sacrificing the mother tree on its offshoot. The use of root-explants plants to initiate culture in date palm is very limited and the few reported experiments have been discouraging [10,11]. The successful achievement of date palm root culture in the laboratory was reported by [12] in which date palm plantlets were produced through root culture of root apiece has stimulated a surge of interest and enthusiasm for exploiting the potentials of using date palm roots as sources of explants for culture initiation and consequently propagation of date palms.

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The inductions of the adventitious shoot on in vitro cultured citrus roots and for date palm [12]. One of the serious problems associated with the tissue propagation of date palm is the high level of contamination. The trees are grown under field conditions for a long period of time that appears to contain internal and external pathogens that cannot be controlled by surface sterilization. Therefore, several chemical sterilities, have been used to disinfection plant materials prior to in vitro culture [13]. This chemical sterility has been found to be effective enough to kill microbes without any damage to plant tissues. But the balance between the concentration of these chemicals and the duration of treatment is of vitally important [14].

Date palm trees are grown in open fields, a condition that makes initiating culture under in vitro conditions without contamination a difficult task. The pioneers of date palm tissue cultivation experienced high rates of contamination [10,15] and by use of various chemical sterility and physical manipulation to control this problem. Therefore, the aim of this study was to develop an effective procedure for surface disinfestations of date palm rootstocks of palm trees extracted from mature trees in the field.

## Materials and Methods

### Plant Materials

Roots intended for culture were taken from adult "Wad Laggai" trees grown in Khartoum state. A mixture of clay and sandy soils were usually heaped around the trees bases to speed the initiation of new roots to increase productivity of trees, and to support the tree. Roots of 5 -10 cm in length were excised from the selected trees and were cleaned by splashy water and were then placed in an ice container containing an antioxidant solution made of (150 mg ascorbic acid + 100mg citric acid) per liter of distilled water.

### Preparation of Roots

After having been brought to the laboratory, the roots were washed and cleaned carefully with powder soap for 5-7 minutes with continuous shaking to remove dirt from them. The roots were then washed with splashy water for two hours to remove the soap. After that the roots were washed with distilled water and were stored in an antioxidant containing (150 mg ascorbic acid + citric acid) per liter of distilled water. Cleaned roots were then sorted, and were dipped into a beaker containing 1 liter of distilled water plus two grams of activated charcoal

in addition to the anti-oxidant solution were stored in the incubation room at room temperature till culture.

### Basal Nutrient Media used

The basal nutrient medium develops for root culture by [16-20] was used throughout this study. It consisted of full concentration of (MS) inorganic salt mixture [21,22] and the following in (mg\liter): 170 Na $H_2PO_4$.$2H_2O$.60000, sucrose.40, Inositol. ½ the concentration of modified whites' organics; 240 Adenine sulfates, 0.1, 2-4-D, 40000 activated charcoal and 38.02, $Na_2S_2O_5$. The pH was adjusted to 7+ 1 with 0.1N NaOH or 0.1N HCl drops before adding 7.0 grams of agar (Bacto Agar). The medium was heated to dissolve the agar. 20 ml of the medium was then distributed into 25 × 150 mm culture tubes at 25 ml aliquots. The tubes were then covered with plastic Bellco kaputs. The nutrient medium was sterilized in an autoclave at (121°c) for 15 minutes under a pressure of (15 psi), and was left to cool as slant in the culture room until use.

### First Experiment

In the first experiment "Clorox" (commercial bleach, 5.25 g Cl/liter active ingredient) concentration in the range of 5% to 70% was used. The root segments were taken from 10 replicates of each group, a total of 8 groups. Each group was placed in a separate beaker containing "Clorox , with concentrations of 10% , 15% , 25% , 30% , 40%, 50% , 60% or 70% , were freshly prepared. A few drops of the detergent between – 20 were added to each concentration. Then the root segments were immersed into the appropriate test solution for an exposure time of 5, 10, 20, 30, 40, 50, and 60 minutes. The treated roots were then raised three times in autoclaved distilled water for one minute each transferred aseptically to a sterile Petri dish containing a moistened filter paper. One cm long root explants were excised and placed in the nutrient medium, one per test tube.

### Second Experiment

In the second experiment, the effect of an antioxidant solution was tested. This antioxidant solution was made by dissolving 150 mg ascorbic acid, 100 mg citric acid, and one gram of activated charcoal in a liter of distilled water after that stored in a cool system until use. Root explants were divided into groups each containing 10 root explants. The first group was soaked in the antioxidant solution for 15 min, the second group for 30 minutes, the third for 45 minutes, and the last groups were soaked for 60 min before the final "Clorox" treatment.

### Third Experiment

The iodine solution was prepared by dissolving 5 grams of iodine powder in 95 % and making to one liter volume with distilled water. Roots were immersed into an iodine solution, for 10, 15, 30, 60 minutes, followed by rinsing 3 times with sterilized distilled water. One treated root explant was placed in a test tube containing a nutrient. They were then rinsed with sterile distilled water and dipped into a liter of distilled water containing gram activated charcoal with the antioxidant (150 mg/L ascorbic acid+ 100mg/L citric acid). They were shaken vigorously for 1-hour. Treated roots were soaked in 30% chlorex and were shaken for 1-hour. The roots were rinsed with sterile distilled sterilized water and were then cultured on the nutrient medium. All cultures were incubated at 25+2°C with a 16-hours photoperiod provided by cool white fluorescent lamps. The parameter measured was the percent of contaminants cultures tubes. All experiments were conducted using the completely randomized design and data was recorded as percentages after 6 weeks period times from culturing.

## Results and Discussion

The results of the first experiment where a wide range of "Clorox" concentrations was tested with different treatment periods showed that lower centurions (<30%) and shorter treatment periods (<30 min) with all tested "Clorox" concentrations were ineffective in reducing contamination rates (data not presented). However, higher concentrations of "Clorox") < 30%), gave a higher acceptable percentage of contaminates –free culture tubes but the roots were damaged to varying degrees due to treatments. A gradual increase in contamination-free culture tubes was given with increased treatment duration times in "Clorox" concentration < 30% Table 1 and Figure 1. This finding indicates that the treatment of "Clorox" with this concentration is the most effective and least harmful factor for plant tissue [23,24]. It is effective when used at the metrically determined concentration and treatment duration time for the specific explants type is readily available at a low cost. 30% compound therapy of "Clorox" for 60 minutes treatment duration gave a relatively higher number of contaminant-free culture tubes % with the least visible damage to treated root explants. This compound therapy is the primary treatment for cleansing palm roots.

The effects of socking root explants in the antioxidant solution prior to the basic disinfestation's treatment shown in Table 2 and Figure 2. Contaminant-free increased with increasing duration periods of antioxidant and 85% contamination-free cultures were obtained with the highest treatment duration (60 min). Treated root explants looked healthy and kept their pure white color even after completing the experiment. The date palm is consistent with the findings of Idris (1994) who noted an improvement in surface sterilization and the establishment of guava culture. Date palm explants are routinely preserved in an antioxidant solution made by dissolving 150 mg of ascorbic acid and 100 mg citric acid in a liter of water before chemical disinfestations. Activated charcoal is usually added to date palm tissue culture medium at a rate of 0.3% [10]. Both treatments were found to be effective in increasing the survival and cultivation of cultured date palm plants. However, their deeper actions and functions are, largely unknown and there is no exact explanation for their beneficial effects.

We would expect here that antioxidant, in some away, partially inhibited or prevented the cellular leakage of oxidative from the roots of the excised plants to the nutrient and activated charcoal that absorbed and /or adsorbed the resulting toxic by-products creating a healthy environment for the presence of strong vigorous root explants capable of resisting infestation with indicative microbial contaminants. A similar result was reported by [22] how observers were 100% contaminated when dormant buds of peaches use only 3.3 % of contamination was reported with similarly surface-sterilized forced lateral buds. Another worker [23] obtained minimum contamination when the eradicated plant was excised from young field-grown papaya trees compared to their older counterparts.

The treatment duration in 0.05% iodine solution are shown in Table 3 and plotted as a function of treatment duration times in Figure 2. The exposure times tested were dissolved root sterilizers at consistently higher rates than the contamination-free"Clorox" treatments" increased with increasing exposure time and up to 80% of contaminations free culture tubes were obtained with an exposure time of 20 minutes up to 100% with 60 minutes of exposure time. However, exposure of less than time < 20 minutes negatively affected the root explants. Iodine seems to penetrate the root tissue at the cut ends giving the internal time of the root a pinkish-red color. These results are in part consistent with the results of which found that iodine is more effective than chlorinating solutions to obtain sterile palm seeds.

**Table 1**: Clorox concentration and treatment duration on decontamination of date palm root explants. The data was taken after 6 week of incubation period times.

| Treatment duration (minutes) | Percentage of culture tubes free af contaminates % | | | | |
|---|---|---|---|---|---|
| | Clorox concentration (%) | | | | |
| | 30 | 40 | 50 | 60 | 70 |
| 20 | 5 | 10 | 10 | 15 | 20 |
| 30 | 10 | 15 | 20 | 25 | 40 |
| 40 | 15 | 25 | 30 | 40 | 50 |
| 50 | 30 | 40 | 50 | 60 | 70 |
| 60 | 45 | 60 | 70 | 80 | 85 |

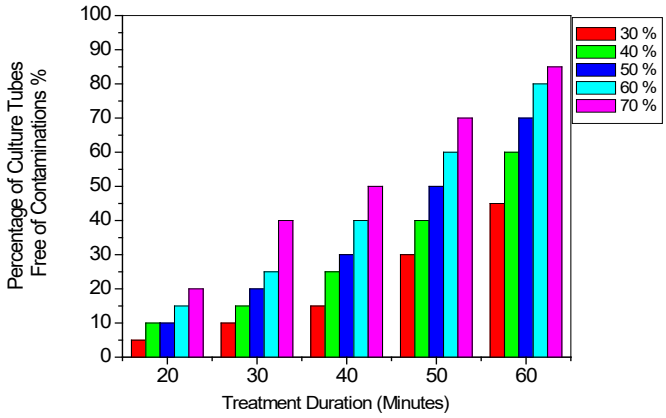


**Figure 1.** Percentage of culture tubes free from contaminants% as a function of Treatment Duration Times by using different Clorox concentration %.

**Table 2**: Treatment duration of pre-soaking in the antioxidant solution on surface disinfestations of date palm root explants by the "Clorox" treatment and the percentage of culture tubes free of contaminations. The data was taken after 6 weeks of incubation period times.

| Treatment duration (minutes) | Percentage of culture tubes free of contaminations % |
|---|---|
| 15 | 40 |
| 30 | 50 |
| 45 | 65 |
| 60 | 95 |

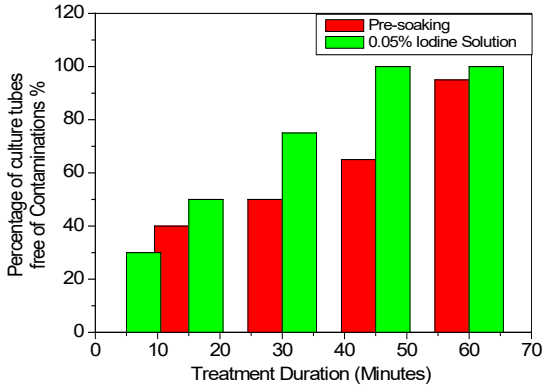


**Figure 2.** Percentage of culture tubes free from contaminants % as a function of Treatment Duration Times.

**Table 3**: Duration of treatment in 0.05% iodine solution on surface disinfestations of date palm root and the percentage of culture tubes free of contaminations. The data was taken after 6 weeks of incubation period times.

| Treatment duration (minutes ) | Percentage of culture tubes free from Contaminations % |
|---|---|
| 05 | 30 |
| 15 | 50 |
| 30 | 75 |
| 45 | 100 |
| 60 | 100 |

The use of chlorinated compounds disinfestations in plant times is unsafe to humans [24] especially in confined space in the presence of the ultra-viaduct indication. They are unstable and hence difficult to store without losing their activity. It has corrosive effects on metallic statements and should be discarded immediately after use. In light of these difficulties, sterilant on alternative chemicals has been the subject of several research. Iodine was chosen in this study because, it known for its bactericidal efficacy relatively low toxicity, and low phytotoxicity.

## Conclusion

This study was carried out to test the effects of various treatments to eliminate surface contamination of date palm roots. The treatments included different concentrations of chlorides, with a varied duration times of treatments. The roots were soaked in activated charcoal and an antioxidant solution prior to final disinfection with 30% chlorex. Extracted and some more chemical solutions were also tested to eliminate surface contamination of the roots. The steps that were developed to disinfestations the date palm were the roots detached from the mother plant and immersed in an antioxidant containing (150 mg/L ascorbic acid +100 mg/L citric acids) placed in a bucket of cold water. Vigorous shaking is very important in all the above treatments to increase the disinfection efficiency to reach all roots or parts of the surfaces, then washing the roots with splashy water for two hours to remove dirt and visible soil dust and washing the roots with soap and detail, After that soaking roots in activated charcoal containing an anti-oxidant solution for 72 hours, Finally immersing the treated roots in a solution of 30% chlorox for 60 minutes duration times.

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