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# Expanding the Shelf Life of Tomato Fruits (*Solanum Lycopersicum Mill*) Using N-Hexan Extract of Date Seeds as Antifungal Agent

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## Abstract

Tomato fruits are a global commercialised vegetable which are consider as one of the faster perishable fruits. Microbial spoilage cause an important loss from the yield at pre and post harvesting periods.. To reduce fruits harm, a several postharvest application techniques are use, and the environmentally friendly aspects of these methods attract attention. This study was conducted to test the antifungal activity of n-hexan extract of date seeds against fungi which associated with tomatoes and cause spoilage.

Samples of tomatoes (local cultiver) were collected from markets in Erbil city during December 2018. Fruites were kept seperately for seven days in plastic cups with lids at lab environment. Seven fungal genera were isolated and identified- *Absidia corymbifera, Alternaria alternata, Aspergillus niger, A. fumigatus, A. flavus, Fusarium oxysporum, Penicillium sp, Rhizopus stolonifer, Saccharomyces cerevisiae*. The occurrence % of *A. alternata* and *A. niger* was the highest (60% and 58% respectively). The n-hexan extract of date seeds(DSE) were prepared for two extraction periods(15 and 20 hours), they were tested externally to reduced the rate of rotten fruit, to test their anifungal activity against *A. alternata (invitro)*, and to copare with mint and thyme oils. Results showed that DSE reduced the rate of rotted fruits to 30%. It also showed antifungal activity against *A. alternata* in both *invitro* and *invivo* tests. The comparison test showed that DSE less activity than oil of mint had highest thn oil of thyme .

**Keywords:** Tomato; Date Seeds Extract; N-Hexane; Shelf Life; Antifungal; Alternaria

## Introduction

The plant/Tomato *Solanum lycopersicum Mill* (Solanaceae) is one of the most popular and widely grown crops in the world. Its yearly global production reaches 160 million tons [1]. Because of their high-water content and other physiological characteristics [2], tomatoes are one of the most perishable vegetable crops. Tomatoes spoilage resulte from pre-harvest as well as post-harvest factors such as field temperature and humidity followed by poor handeling during harvesting, transporting, and storage. Such effects are markedly increase the instances of microbial rot [3].

### Treatment of Tomato Crop by Plant Oils

There are several applications to control and to minimize harms in fresh and soft fruits, including tomatoes. These techniques are necessary and accompany the crops from the field to the consumers. Using essential oil to reduce fruit decay and their vapours attract more attention than chemicals. They are safe for humans to use and affect the environment less than chemechals [4,5]. Several noteworthy plant-based oils come from cloves, oregano, rosemary, thyme, sage and

vanillin are mentioned as effective agents against bacteria which cause tomatoes spoilage, the antibacterial effect of these oils related to phenolic components, he also stated that the vapour from *Eucalyptus* and *Cinnamon* oils represented a notable lowering of strawberry and tomato fruits decay [6].

*Thyme* oil showed activity against *Botrytis cinerea*, and *Alternaria arborescens* developed experimentally on tomato fruits. *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Saccharomyces cerevisiae, Alternaria alternata, Penicillium digitatum* and *Geotrichum candidum* are the most common causes of tomatoes harm [7]. Other effective fungi were listed by include *Aspergillus phoenicis, Absidia spp*, *Trichoderma spp*, *Alternaria alternata*, *Fusarium oxysporum, F. moniliformis, Aspergillus niger, Mucor spp*, *Rhizopus stolonifer, Penicillium spp, Geotrichum spp* and *Phytophthora spp* [8,9].

### Date Palm Seed Oil

There are many different uses of date palm seeds, and one of the most important is the therapeutic applications [10]. The extractions of seeds via water or organic solvents showed antibacterial and antifungal activity [11]. The water, methanol, and acetone extracts of pits revealed significant activity against numerous species of *Fusarium, Alternaria*, and *Trichoderma* [12].

In the current study the antifungal activity of n-hexan extract of date palm seeds (*Phoenix dactylifera*) was (*invivo &invitro*) tested against *Alternaria alternata* as the most comon fungal isolet associated with tomato samples.

## Materials and Methods

During December 2018 a thirty sisty fruits of local cultivar of tomato were collected from several markets in Erbil city, they were approximately with the same size. Fruits were kept sigelly in clean, plastic cups, with caps for five days in a lab environment. The developing fungi were identified according to morphological characteristics based on [13,14]. A Pure culture of *Alternaria alternata* was prepared and was kept in the refrigerator for next tests. Occurrence% of fungal genera was estimated [15].

### Preparation of Date Seeds Powder

The mature date palms (CV Berhee) were purchased from Basrah province market (south of Iraq). Seeds were cored manually from the fleshy fruits; they were cleaned by tap water then were air dried in lab environment for seven days. The dried seeds were carefully grinded by electrical grinding machine (Electrical Power Grinder/DE-1000g). The grinded material has been sifted by manual sieve (pores=1mm), a closed glass container was used to kept the powder at 4°C.

### Preparation of Date Seed Extract (DSE)

Twenty five gram of seed powder and 125 ml of n-hexane were used in soxhlet apparatus to get the seed extract. Seed powder was loaded in a thimble of extractor while the solvent was poured into the round flask (500 ml). Hexan was heated to 68°C, and two periods of extractions were conducted, 20 hours (T1) and 15 hours (T2). At the end of extraction period, the resedual hexan in the round flasc was evaporated by heated waterbath. The final extractions looked as thick brown liquids, and they were kept in a universal screw cap at 4°C for further examination.

### The Antifungal Activity Tests

They involved conducting four consecutive tests:

- Primary evaluation of (T1 and T2) DSE effectiveness against fungi associated externally with tomatoes fruits .
- Use the the disc diffusion method as *in vitro* test to compare between the antifungal activity of (T1 and T2) of DSE against the predominat isolet *Alternaria alternata* .
- Use the expermintally contaminated tometoes to explain the actevity of DSE (T1and T2) aginst *Alternaria alternata*.
- Comparison the antifugal activity of DSE with oils of mint (*Mentha sp*) and thyme (*Thymus sp*).

The tests were conducted as following:

- A three groups of (20 fruits) were used for the primary evaluation the antifungal activity of (T1 and T2) DSE. All selected fruits approximately had the same size, colour, and without blemishes nor injuries, they related to the same yield patch. Two drops of DSE were spread on the surface of the fruit, while the untreated group was regarded as a control test. The samples were kept separately in clean plastic covered cups and were checked after 7 days to record the rate of fungal development.
- The *in vitro* antifungal activity of (T1 and T2) extracts were carried out against *A. alternata* isolate. A disc (5mm in diameter) of 7 days old of *A. alternata* pure culture was placed on the centre of growth medium surface. Sterilized discs (6mm) of filter paper (Whatman no.1) were soaked for two minutes in the DSE, and after removing the excess extraction, the discs were placed around the fungal disc (2 cm apart). The plates were done in duplicate and were incubated at 25°C. The result was adopted when *Alternaria* growth reached the control disc. Antagonism of T1-DSE has compared again with n-hexan by disc diffusion method using separated culture plates.
- *In vivo* test for antifungal activity of (T1-DSE). The test was carried out-after modification-according to [16,17]. Healthy tomato fruits were externally sterilized by 2% of sodium hypochlorite (laundry bleach), thereafter

they were intensively washed by sterilized distilled water then were air-dried inside the laminar airflow. The fruits were experimentally infected by *A. alternata* spores suspension, a sterile lancets had been used to wound fruits superficially (two crossed lines with1cm length), the wounds were contaminated by a drop of spores suspension, after 30 minutes two drops of DSE was droped on the wonds by sterilized droppers. Fruits without contaminated wounds were conducted as a control test. Mycelial growth on the wound indicates to fungal colonization. The distingwishable conidia of *A alternata* was recognized again from the contaminated injuries a. Six fruits were used for each test.

- The antifungal activity of DSE was compared with mint and thyme oils which were purchased from privet plants pharmacy in Erbil city. Disc diffusion method, as well as *in vivo* test (using experimentally infected eruits ) were followed as mentioned above in [4].

## Results and Discussion

Extending the shelf life of tomatoes was a goal of several previous works. Different environmentally friendly applications were used to control physiological and mechanical changes [18-20]. In contrast, less attention is given to use these phytochemicals as an antifungal for extending tomatoes shelf life. The presumption is that current study is a sign to use DSE in this subject. A local tomato cultivar was chosen in the current study due to its short shelf life, and is prone to damage, compared to imported cultivars. The local tomatoes fruit are lobed, juicy and soft with thin cuticle coat that makes it easier to get injuries (Figure 1).


**Figure 1:** Tomatoes cultivars in Erbil markets (L=local, I=imported).

Nine isolates related to seven fungal genera were identified from tested tomaoes, *Absidia corymbifera, Alternaria alternata, Aspergillus niger, A.fumigatus, A. flavus, Fusarium oxysporum, Penicillium sp, Rhizopus stolonifer*, *Saccharomyces cerevisiae*. They were comonly causes of tomatom spoilag [21]. *Alternaria alternata* and *Aspergillus niger* had the highest occurrence % (Table 1). *Alternaria* is one of the most common fungi that cause tomatoes postharvest disease in Iraq Firas, et al. [22], so it was selected as a target for study.

| | Fungi | O% |
|---|---|---|
| 1 | Alternaria alternata | 60% |
| 2 | Aspergillus niger | 58% |
| 3 | Penicillium sp. | 33% |
| 4 | Fusarium oxysporum | 23% |
| 5 | Rhizopus stolonifer | 16% |
| 6 | A.fumigatus | 6% |
| 7 | Saccharomyces serevisiae | 4% |
| 8 | Absidia stolonifer | 2% |
| 9 | A. flavuss | 2% |

**Table1:** The occurrence% (O%) of isolated fungi.

All untreated fruits exhibit fungal growth(damage =100%). Date seeds extract reduced the rate of damaged fruits to (30%) as well as minimize fungal growth. T1-DSE reduced fungal growth more than T2-DSE (Figure 2).

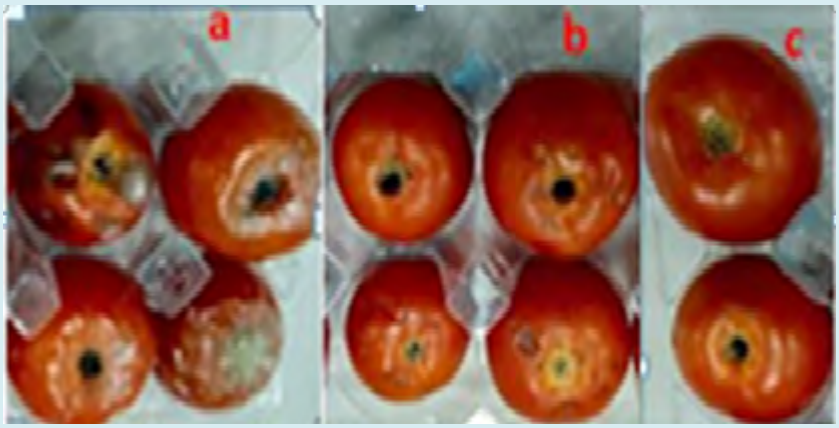


**Figure 2: A:** untreated fruits, **B:** fruits treated by T2- DSE, **C:** fruits treated by T1-DSE.

Date seeds extract had several phytochemicals such as flavonoids, anthocyanidins, and glycosides, which act as fungal growth inhibitors [23,24]. The different effectiveness between T1 and T2 related to the difference in their compenets ratios. Several studies reported that the extraction time effect on the ingredients retios besides the type of solvent, and particles size [25]. *In vitro* comparison between the activity of T1-DSE and T2-DSE against *Alternaria alternata* was recorded when the fungal growth reached the edge of the control disc(n-hexane). The test showed that the inhibition zone of T1-DSW was 4mm while it was 2mm for T2-DSE. (Figure 3-A). The activity of T1-DSE was confirmed and was compare by separating culture plates (Figure 3-B & C), the range of clear zone was 8 -20 mm for T1-DSE (Figure 3-B), while there is no effect for n-hexan as control (Figure 3-C).

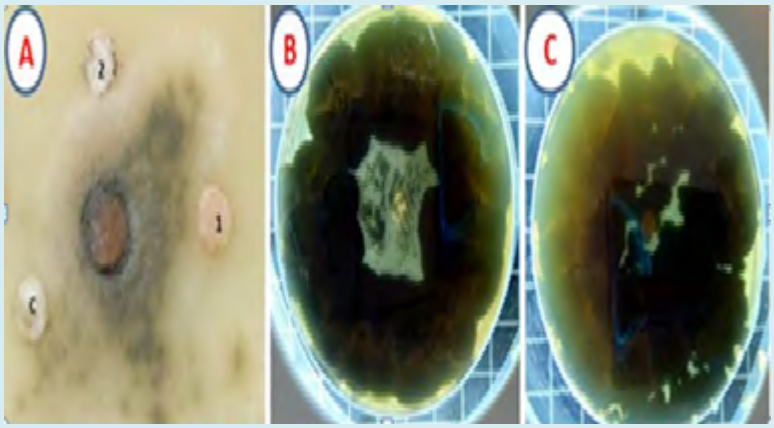


**Figure 3: A:** Discs diffusion method: 1=T1, 2=T2, 3=n-hexane, **B:** T1 disc in the centre, **C:** n-Hexane disc in the centre.

The effectiveness of T1 DSE on experimentally infected fruits showed that *A. alternata* failed to colonize the treated fruits (inhibition=100%), while fungal growth was observed on 91.6% of untreated tometoes of which 81.8% was *Alternaria*. This means that activity of DSE was not affeceted by plant tissue environments. The antifungal activity of DSE was compared with that of mint and thyme oils which have a distinguished effecacy against several fungi [26-28]. Oil of mint showed the largrst zone of inhibition followed by DSE and thyme oil (6mm, 4mm, 1-2mm respectively) (Figure 4).


**Figure 4:** Disc diffusion test of (mint oil=M), (DSE=D), (thyme oil=T) and (n-hexane=C) against *A.alternata*.

The comparison test by expermantaly infected tomatoes(*invitro*) showed the same results.Mint oil caused an absolute disappearance of *Alternaria* growth on infected fruits (inhibition= 100%). (Figure 5-M). Thyme oil (Figure 5-T) showed low antifungal activity rate (33% inhibition) in compare with DSE (66%) (Figure 5-D).

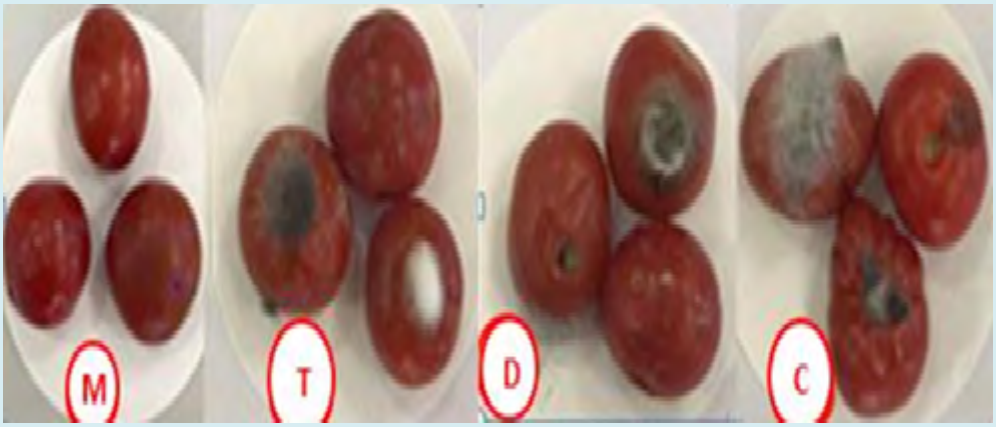


**Figure 5:** Antifungal activity of (mint oil=M), (thyme oil=T), (DSE=D), and (n-hexane=C) against *A.alternata* in artificially infected fruits.

## Conclusion

According to the results of current study, the n-hexan extract of date seeds has ihibitory action against fungal contamination of tomatoes. It may use to prevent tomato spoilage successfully and extending their shelflife. Further studies should be conducted for several safety applications of date seeds extract as a natural antifungal agent.

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