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Evaluation of the efficacy of isolates of *Trichodermaharzianum* and *Ziziphusspina-christi* extract on the growth of *Penicilliumdigitatum* fungus growing on orange fruits

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Abstract

Orange fruits are susceptible to many fungal diseases that lead to their spoilage, especially during the collection and marketing stages. The most prominent of these diseases is green mold, caused by the fungus *Penicilliumdigitatum*. This study aims to investigate the potential of alcoholic extract of *Ziziphusspina-christi* and the *Trichodermaharzianum* fungus as biological control against *P. digitatum* fungus. Materials and Methods : *Trichodermaharzianum* and *Ziziphusspina-christi* extract (50% concentration) were used to study their effect on the growth of the pathogenic fungus *P. digitatum*. Results: *T. harzianum* inhibited the growth of the pathogenic fungus *P. digitatum* growing on orange fruits in 7 days of incubation up to 77.96%. As for the extract of *Ziziphusspina-christi*, it reached a rate of inhibition of 82.50%. Discussion: The research results showed that the use of biological control methods using microorganisms and plant extracts was effective in combating diseases that affect plant crops and limiting their losses. Recommendations: Completing **the** research by conducting field experiments with isolates of microorganisms and natural plant extracts.

Keywords: *Trichodermaharzianum*, *Ziziphusspinachristi* (L), *Pe nicilliumdigitatum*.

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تقييم كفاءة عزلات فطر *Trichodermaharzianum* ومستخلص نبات السدر *Ziziphusspina-christi* على نمو فطر *Penicilliumdigitatum* النامي على ثمار البرتقال

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المستخلص:
تتعرض ثمار البرتقال للعديد من الأمراض الفطرية التي تؤدي إلى تلفها خاصة خلال مراحل الجمع والتسويق، ومن أبرز هذه الأمراض العفن الأخضر الذي يسببه فطر *Penicilliumdigitatum*. تهدف هذه الدراسة إلى البحث في إمكانية استخدام المستخلص الكحولي لنبات السدر وفطر *Trichodermaharzianum* كمكافحة حيوية ضد فطر *P. digitatum*. أظهرت النتائج أن فطر *T. harzianum* ثبط نمو الفطر الممرض بنسبة 77.96%، بينما وصل تثبيط مستخلص السدر إلى 82.50% بعد 7 أيام من التحضين. توصي الدراسة باستكمال البحث بإجراء تجارب حقلية باستخدام العزلات الميكروبية والمستخلصات النباتية الطبيعية كبدائل آمنة للمبيدات الكيميائية.

الكلمات المفتاحية: فطر التريكوديرما هارزيانوم، نبات السدر، فطر البنسيليوم ديجيتاتوم.

Introduction

Oranges are considered one of the most important agricultural crops that provide a type of food that is very rich in vitamins and volatile oils that are used in many food and pharmaceutical industries and are a good and safe source for the consumer due to their nutritional value (Abulnaga, 2013). Orange fruits are infected with the fungus *Penicilliumdigitatum* by wounding or scratching the fruits, especially during handling operations (collection, transportation, marketing), and not taking care of the fruits, sorting them, and disposing of the infected ones (Al-Jali and Bianco, 2019). The infection with fungus has reached digitalis the orange yield during

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storage and refrigeration for the Clementine variety was 16.5% and for the Abu Sura variety 14.1% (Khafta, 2019).

The fungus belongs to the division Fungi imperfecti. It is called incomplete because the sexual phase (telomorph) disappears in some stages of fungal development (Raven et al., 2004). The morphological characteristics of the fungus are clear, as the mycelium is divided and the conidiophores are bi-verticillate and are known as the stalk, which divides at its apex, forming a broom-like structure called *Pencillus*, which are specialized cells that resemble a flask (Siyala and Al-Alusi, 1994). The spores are in oval or round chains when examined microscopically and form a smooth, dark green surface in the mycelium (Aerosol and Mustafa, 2016). Isolates of *Trichoderma* spp. T22 and T34 were highly effective in controlling the fungus *P. digitatum*, the causal agent of green mold on fruits, with an inhibition rate of 100% (Harman, 2000). *Trichodermaasperellum* reduced the fungal growth on fruits by 79.90% (Vu et al., 2021). Filtrates of *T. harzianum* at concentrations (20%, 50%, 75%) inhibited the growth of the pathogen *Fusariumsolani* by 100% (Enespa and Dwivedi, 2013). Strains of *T. harzianum* Pp002272 and *T. longibrachiatum* Pp002254 were effective in inhibiting green mold on oranges caused by *P. italicum* with inhibition rates of 71% and 77% (AsmaElnagar et al., 2024).

The global trend in recent years has been to reduce the use of chemical pesticides in plant protection by adopting eco-friendly alternatives such as biological control and preventive biological applications. The use of plant extracts helped suppress the growth of several pathogens such as blue mold on citrus fruits (Alihia, 2007). Agricultural applications of *Trichoderma* fungi are among the most important biological agents for controlling many plant diseases, as they produce bioactive compounds such as Butenolide, Harzinolide, and Harziandione (Yuliu Cheng et al., 2020). One of the uses of *T. harzianum* isolates is the reduction of *P. digitatum* growth when applied as a biofilm suspension (SalivinaPanebianeo et al., 2015).

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The Aim of Study

1. Isolation and identification of the pathogenic fungus *Penicilliumdigitatum* growing on orange fruits.
2. Study of the effect of *Trichodermaharzianum* fungus in the fight against the fungus that causes green rot disease on orange fruits.
3. Study of the effect of sidr plant extract (*Ziziphusspina-christi*) 50% concentration on *P. digitatum* growth.

Materials and Methods

Antagonism test: Culture plate assay method was used (Swapan et al., 2013). In a sterile environment, a 7-day-old colony of the pathogenic fungus was taken using a sterile cork punch (5 mm diameter) and placed on PDA nutrient medium. Two discs of the control fungus *T. harzianum* were placed on both sides of the plate at a distance of 2.5 cm from the pathogenic fungus. Plates were incubated at 26°C for 7 days, and growth inhibition was measured.
Preparation of alcoholic extract: Weigh 20 grams of the plant, dissolve in 200 ml of ethyl alcohol for 24 hours. Filter and use a rotary evaporator at 55°C to obtain the active ingredients (Stok). The growth inhibition percentage was calculated according to the formula: PIRG(%) = (R1 - R2) / R1 * 100 Where: PIRG = percentage of growth inhibition; R1 = Average fungal diameter in control; R2 = average diameter in treatment.

Results and discussion.

The results showed that *Ziziphusspina-christi* extract led to a reduction in the growth of *P. digitatum*. The inhibition rate reached 13.43% in 3 days and 82.50% in 7 days (Table 2). This is due to its content of active substances such as phenols and glycosides that reduce the pH to 4 on the wall of pathogenic fungi (Dahri et al., 2007). These results were similar to a study using *Ziziphus vulgaris* which inhibited *Aspergillusochraceus* (Daghman, 2023).

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TABLE 1. Average growth diameter of the fungus *Penicilliumdigitatum* treated with *Ziziphusspina-christi* extract (50%) for 3-7 days.

Transactions	Average diameter of dishes over 3 days (cm)	Average diameter of dishes over 7 days (cm)
Untranslated	1.2667	5.333
Laboratories	1.1167	0.933

The results presented in Table (1) demonstrate clear differences in colony diameter between the two treatments over time. After 3 days of incubation, colony diameters were relatively similar between treatments, with untranslated recording 1.2667 cm and Laboratories 1.1167 cm, indicating comparable early growth rates by day 7, a pronounced difference was observed. The Untranslated treatment showed a substantial increase in colony diameter (5.333 cm), reflecting active and continuous mycelial growth. In contrast, the Laboratories treatment showed limited growth (0.933 cm), suggesting a strong inhibitory effect over time. These results indicate that treatment effects became more evident with prolonged incubation, with significant divergence in fungal growth observed after 7 days.

Figure (1) showed that the extract of volatile oils in the jujube plant works as antimicrobials for plant diseases.(Malik, BS. 1996), the use of crude oil from the clove plant reduced the appearance of disease symptoms on the fruits by injection, and the disease inhibition rate reached 64.1% (Aerosol. JihadMuhammad, et al.2016)

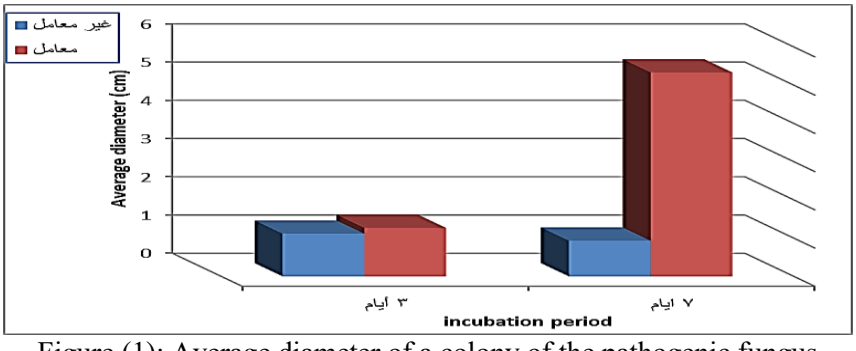

Figure (1): Average diameter of a colony of the pathogenic fungus Penicilliumdigitatum treated with Ziziphusspina-christi extract 3-7 days 50% concentration

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TABLE 2. Effect of *Ziziphusspina-christi* extract on the inhibition rate of *Penicilliumdigitatum* for 3 -7 days.

Incubation period	The inhibition rate
3 days	13.43%
7 days	82.50%

The previous table (2) shows the growth inhibition rate of the pathogenic fungus *Penicilliumdigitatum*. Using the extract of *Ziziphusspina*-Christi, it reached 82.50%, which is probably due to phenolic substances such as glycosides and theanines (Pallavi. I. Mahajan, K.*et al* 2016), as well as due to the effectiveness of the production of the enzyme 3 hydroxyl-3 methyl glutase, which prevents the formation of the Ergosterole compound, which is important in building the fungal cell wall (Po S. Cheu. Y,et al.2016).

Figure (2)Between the use of *Ziziphusspina* extract - christi It contains active ingredients such as phenols, glycosides, and acids such as malic, citric, and acetic acids, which reduce the pH to 4 on the wall of pathogenic fungi(Jat JG and Agalave HR, 2013**).**

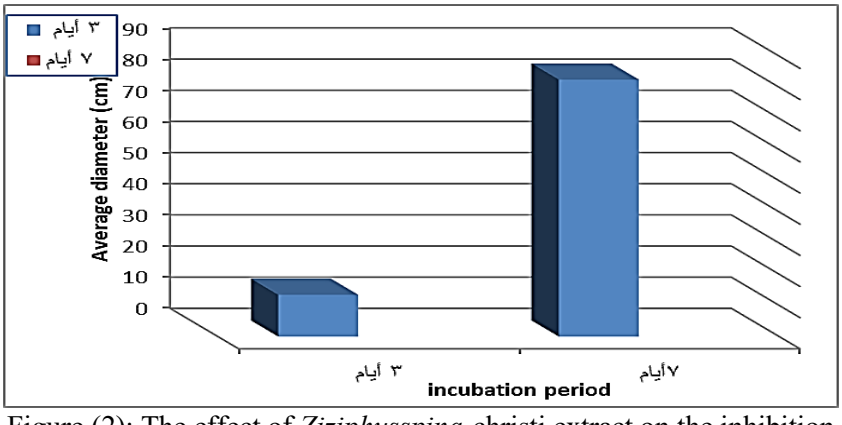

Figure (2): The effect of *Ziziphusspina*-christi extract on the inhibition rate of the pathogenic fungus *Penicilliumdigitatum* in 3-7 days.

TABLE 3. Average growth diameter of *P. digitatum* treated with *Trichodermaharzianum* (3-7 days).

Transactions	Average diameter (3 days) (cm)	Average diameter (7 days) (cm)
Control	1.1500	3.7667
Treatment	1.6667	1.1000

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The diameter of the pathogenic fungus growth decreased. *Penicilliumdigitatum* As shown in Table (3), this is consistent with a study conducted by in (Berry, C. Fernando, W,*et al.*2010)hich isolates of *Trichodermaharzianum* When used as a spore suspension on fruits, isolates of Trichoderma SPP T22-T34 were very effective in controlling the growth of P. digitatum. Growing on orange fruits up to 100% ,(Daghman, et al.2023).

Figure (3) illustrates that treatment with Trichoderma harzianum progressively reduced the colony diameter of Penicillium digitatum compared with the control, with the inhibitory effect becoming clearly evident after 7 days of incubation.

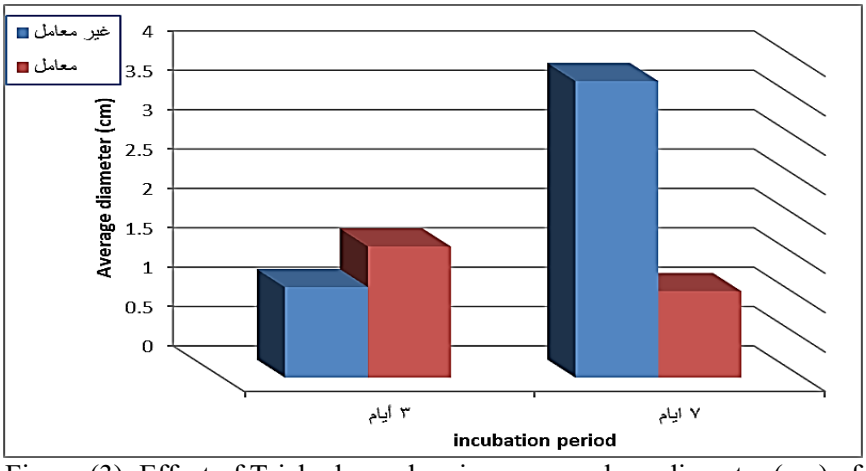

Figure (3): Effect of Trichoderma harzianum on colony diameter (cm) of Penicillium digitatum at 3 and 7 days of incubation under laboratory conditions.

TABLE 4. Effect of *Trichodermaharzianum* on the inhibition rate of *Penicilliumdigitatum*.

Incubation period	Inhibition ratio
3 days	44.93%
7 days	77.96%

Penicilliumdigitatum was In 3 days 44.93% and reached up to 77.96%, the inhibition rate was similar to the study. *T.harzianum* PP002272 and T.longibrachitum PPOO2254 were used and were excellent in inhibiting blue mold on orange fruits caused by

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P.italcum. By 77% and 71% respectively due to the production of active enzymes such as Catalase(AsmaElnagar , Yasser S,*et al.*2024).

Figure (4) Defensive applications of *Trichoderma fungi* In the field of biological control against diseases and pests of agricultural crops, because of its ability to inhibit The growth of pathogenic fungi is due to the secretion of many active compounds such as Butenolde, harzinolide, and harziandion and (Alihia , S.A. 2007)the production of active enzymes such as Catalase (Asma Elnagar,et al.2024).

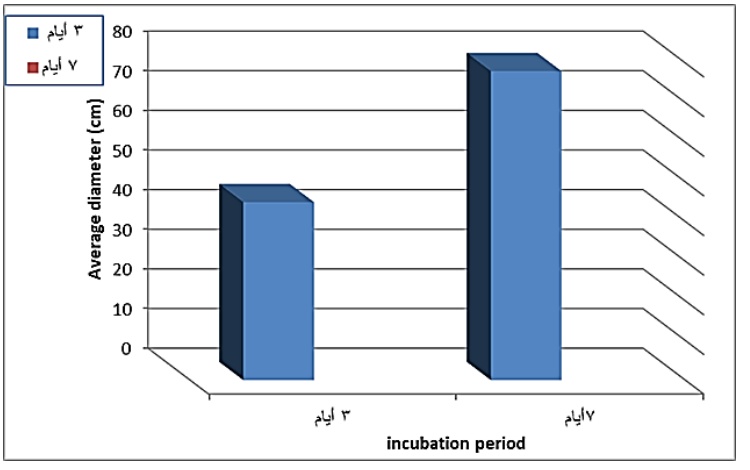

Figure 4: Effect of *Trichodermaharzianum* on the inhibition rate of *Penicilliumdigitatum in* 3-7 days.

Conclusion

The present study showed that both the alcoholic extract of *Ziziphusspina-christi* (50%) and *Trichodermaharzianum* isolates were effective in reducing the growth of *Penicilliumdigitatum* on orange fruits. The jujube extract achieved a high inhibition rate (82.50%) after 7 days of incubation, while *T. harzianum* inhibited fungal growth by 77.96% over the same period, confirming their potential as eco-friendly alternatives to chemical fungicides in postharvest citrus disease management. Field-scale trials using microbial isolates and plant extracts are recommended to validate

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their effectiveness and safety under commercial storage and marketing conditions.

Recommendations

Completing the research by conducting field experiments using isolates of microorganisms and natural plant extracts as safe and environmentally friendly alternatives to chemical pesticides.

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