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STUDY THE EFFECT OF BIOLOGICAL CONTROL FUNGUS INCORPORATED WITHIN EXTRACTS OF SOME PLANTS FOR TREATING OF DRECHSLERA SPP DISEASE IN DATE PALM TREES (IN VITRO)

Z. M. Sarhan*
Department of Plant Protection- College of Agricultural Engineering Sciences- University of Baghdad

***Correspondence to:** Zina Mohammed Sarhan, Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
Email: zzina6677@gmail.com

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Abstract

This research work has been carried out at the University of Baghdad, College of Agricultural Engineering Sciences, Plant Protection Department. The goal of the study was to determine how effectively biological fungus and a few plant extracts could be used to combat the pathogenic fungus that causes date palm blotch disease. Due to the fact that the antagonism was measured in a lab setting, the study demonstrated that the biological fungus Trichoderma Harzianum had a high antagonistic power against the pathogenic fungus Drechslera spp. The outcomes elucidated that the plant extracts of garlic and coriander, as well as biological control fungi have significantly suppressed the growth of pathogenic fungus. Also, the results manifested that the ability of an alcoholic extract of garlic to suppress the pathogenic fungus was superior to an alcoholic extract of coriander at various doses. The biological fungus is able to combat the diseases by surrounding their mycelium with its own, entering their walls, and devouring their nutrient-rich interiors. Additionally, the results of the experiment evinced that the percentage of inhibition for alcoholic extract varied significantly between the plant extracts with garlic extract recording the highest percentage of inhibition 98% and the coriander extract recording the lowest percentage of inhibition 89%. Phenols and saponins were also detected in the compositions under study. And, the zones' diameter of the inhibition for the ethanolic and aqueous extracts, respectively, varied from 4.1 to 14.3 mm and 2.4 to 10.4 mm, respectively.

Keywords: Fungus, Date palm, Drechslera, Trichoderma Harzianum.

دراسة تأثير فطريات المقاومة الحيوية المتضمنة في مستخلصات بعض النباتات لعلاج مرض Drechslera spp في أشجار النخيل (في المختبر)

زينة محمد سرحان*

قسم وقاية النبات – كلية علوم الهندسة الزراعية – جامعة بغداد

***المراسلة الى:** زينة محمد سرحان، قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق.

البريد الالكتروني: zzina6677@gmail.com

الخلاصة

تمت الدراسة في جامعة بغداد، كلية علوم الهندسة الزراعية، قسم وقاية النبات. كان الهدف من الدراسة هو تحديد مدى فعالية الفطريات البيولوجية وبعض المستخلصات النباتية في مكافحة الفطريات المسببة للأمراض التي تسبب مرض تبقع اوراق النخيل. نظرًا لحقيقة أنه تم قياس التضاد في بيئة معملية، فقد أوضحت الدراسة أن الفطريات البيولوجية *Trichoderma Harzianum* لها قوة معادية عالية ضد الفطريات المسببة للأمراض *Drechslera* spp. أظهرت النتائج أن المستخلصات النباتية من الثوم والكزبرة وكذلك الفطريات البيولوجية لها قدرة معنوية على تثبيط نمو الفطريات المسببة للأمراض. أظهرت النتائج أن قدرة المستخلص الكحولي للثوم على قمع الفطريات المسببة للأمراض تفوقت على المستخلص الكحولي للكزبرة بجرعات مختلفة. الفطريات البيولوجية قادرة على مكافحة الأمراض من خلال إحاطة الفطريات المسببة للمرض، ودخول جدرانها، والتهام مساحاتها الداخلية الغنية بالغذاء. أظهرت نتائج التجربة أن نسبة التثبيط للمستخلص الكحولي تفاوتت معنويا بين المستخلصات النباتية، حيث سجل مستخلص الثوم أعلى نسبة تثبيط 98٪ ومستخلص الكزبرة مسجلا أقل نسبة تثبيط 89٪. كذلك تم الكشف عن الفينولات والسابونيات في التراكيب قيد الدراسة. تراوح قطر مناطق التثبيط للمستخلصات الإيثانولية والمائية على التوالي من 4.1 إلى 14.3 ملم ومن 2.4 إلى 10.4 ملم على التوالي.

كلمات مفتاحية: الفطريات، نخيل التمر، دريشسليرا، ترايكوديرما هارزينيوم.

Introduction

The date palm *Phoenix dactylifera L*. belongs to the palm family Arecaceae. Iraq is the original home of the date palm, due to the availability of suitable conditions for palm cultivation in terms of tropical climate and abundance of moisture (2, 12 and 14). Despite the fact that the Arab countries have ideal soil and climate conditions for palm farming, the region's production of date palms are relatively modest when compared to the other nations in the world. Numerous diseases and pests that affect the date palm reduce its ability to produce dates in the Arab world (3 and 13). One of the most prevalent illnesses in most palm-growing regions in the world is the date palm leaf spot disease, which is brought on by a variety of fungi and spread widely in abandoned orchards. As a palm tree ages, so does the likelihood and severity of the

infection. Numerous fungi have been implicated in the development of spot disease, including *Alternaria alternata, Phomopsis sp., Phoma sp., Drechslera sp., Bipolaris sp.,* and *Helminthosporium sp.* (21, 22 and 24). The fungus *Trichoderma* spp. contributes to the improved soil properties by fixing the atmospheric nitrogen, increasing the availability and the uptake of nutrients, like phosphorous, potassium, and microelements, and then enhancing the plant growth and production by enhancing the nutritional status of plants via the soil-based organisms or by the addition of fungal inoculants (9). The fungus *Trichoderma harzianum* plays a crucial role in improving the physical, chemical, and biological characteristics of the soil, especially in increasing the availability of some elements, like nitrogen, phosphorus, and potassium through the release of some enzymes and its high ability to tolerate already-existing or newly-added organic matter in the soil. The biological control fungus affects the pathogenic fungus by working through different mechanisms, such as parasitism or competition for food and space, or the production of anti-substances, or its inhibition of the enzymes of the pathogenic fungus as it acts as a storehouse for many nutrients required for plants, in order to give the plant host high resistance against some pathogens (6).

The strains of *Trichoderma viride* and *Trichoderma harzianum* performed a dual cultural assay to determine which was more effective against *Alternaria alternata*, with inhibition percentages of 75.04% and 67.83%, respectively. *Drechslera halodes* showed the lowest activity, with the growth inhibition percentages of 51.54% and 43.92%, correspondingly (25).

The pathogen's mycelial proliferation was significantly inhibited by all species of Trichoderma. According to the double culture interaction test findings, TvDPs 66.3% were the greatest antagonist at preventing the pathogen growth, pursued via the TDPs 57.4% and the T1s 56.43%. *T. viride* (TvDPs) made volatile metabolites that had the largest growth inhibition 40.91%, pursued via the T1s 25.97% and the TDPs 7.57%, in that order. And, the highest reduction in the radial expansion of the pathogen was visible in the culture filtrate of all antagonists cultured at 25°C. The radial growth of *Fusarium oxysporum* was suppressed to variable degrees by the cell free culture filtrate of *Trichoderma viride* when it was incubated at different temperatures. The culture filtrates of *Trichoderma harzianum* isolates that incubated at 40°C, however, were incapable to restrain the proliferation of pathogen (20).

From the soil samples, six isolates of three different *Trichoderma* species were found. All six Qatari isolates had the ability for growing properly at pH 8 and withstand (70°C/24 hour) after being subjected to the heat shock conditions of 70°C and 90°C for 24 hour. In the tests conducted in vitro and in vivo, *T. harzianium* demonstrated a perfect antagonistic effect versus the 3 incidental agents of date palm leaf spot disease. It's a strong contender to be turned into a bio-pesticide to combat the three infections (7).

Chemical pesticides have assisted in eradicating diseases and protecting agricultural productivity from pests, and they continue to do so. The widespread use of these pesticides and the reliance on them as the primary or only means of pest management, however, resulted in a serious imbalance in the biological balance of

organisms and the creation of fungal strains that are resistant to some fungicides (5). As a result of contaminated food items, soil, and water sources, some fungicides also have hazardous effects on the people and terrestrial and aquatic organisms, in addition to having phytotoxicity on the selected crops (16). Therefore, the quest for less harmful pesticides for plants, the environment, and people began. The most significant of these are the plant extracts that include volatile oils, which have illustrated their efficacy against insects and fungi (8). Due to their safety for both humans and environment, the medicinal and aromatic plants are a significant source of various chemical compounds and essential oils that can be used as insecticides (4).

The aim of present investigation is to evaluate the influence of *Trichoderma Harzianum* upon the *Drechslera* spp fungus that infects the date palm. In addition, it studies the effect of the extraction of the oil plant that incorporated with *Trichoderma Harzianum* on the *Drechslera* spp fungus.

Materials and Methods

Isolation of Fungus: Pieces of the infected date palm, including leaves and wicker, were obtained with a diameter measuring 0.7 cm. These samples were subjected to both sterile distillation and superficial sterilization using a 15% sodium hypochlorite solution from a commercially available preparation. The sterilization process lasted for 3 minutes, after which the samples were thoroughly rinsed with water. After that, the samples were distributed in Petri dishes containing the medium (PDA), chloramphenicol, and antibiotic at a temperature of 26ºC for 4 days, and then they were analyzed and purified at 3ºC ± 250 mg/L.
Figure 1 depicts a leave that infected by *Drechslera* spp. fungus with a brown spots.

Figure 1 *Drechslera* spp fungus (brown spots).

Extraction Method: Garlic was peeled, diced, and crushed in a blender for 60 sec before being steeped in 100 ml of 95% ethanol. It was naturally extracted at the room temperature, the mixture was separated in test tubes by centrifugation, at a speed of 2500 rpm, and the filtrate was dried in a furnace at a 36ºC temperature for 24 hour. And, the completed product was kept at 3ºC.

Coriander seeds were brought to the lab from Baghdad, where they were air dried, and kept in paper bags. 20 gm of dried seeds were removed, crushed, and 200 ml of an organic solvent (ethanol) were poured into a 250 ml beaker, covered with cellophane paper, and left in a dark environment for 15 day to create the plant extract. The extraction was according to (10).
Different concentrations 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mg/l of alcoholic extracts were used.

Testing the disease of *Drechslera* spp: Numerous 10 cm long pieces of date palm or Al-Sayer leaf were taken from the specific location. Before being superficially sterilized for 5 min with a 10% sodium hypochlorite solution of the brand-name product (Komorex), the components were completely cleansed with running water to remove the solution. After drilling a hole in each leaflet piece with an oral drill, a disc was removed from the fungus and cultured on a sterile *Drechslera* spp growth medium in a PDA of 0.6 cm in diameter. The pieces were placed in suitable-sized glass bottles together with 30 ml of sterile distilled water. The nozzles of the glass bottles were then sealed with cotton and aluminum foil for a period of 31 day. The glass bottles were incubated in the incubator for 31 day at a temperature of 26°C. The pathological spot's progression and the fungus's growth on the leaves were noted every four days. In addition, the signs and symptoms were recorded, and the average radius of the injured tissue surrounding the infection site was assessed.

Examining the conflict between biological and pathogenic fungus: On the PDA culture media in petri dishes, the double culture method was built. The petri dish was split in half equally. A sterile cork borer was used to insert a disk of pathogenic fungus that is 8-days-old and growing on the PDA culture media into the first half of the dish's center. The center of the second half of the dish was inoculated with a similar tablet of biological control fungi at the age of 8 days, at a rate of three replicates. After 8 days of double culture in the incubated dishes at 26°C, the radial growth of the pathogen and the bio-resistant fungus was measured. The inhibition percentage was calculated by the following formula (11):

$$Inhibition\ (\%) = \frac{Growth\ before\ treatment - Growth\ after\ treatment}{Growth\ before\ treatment} \times 100$$

Identifying effective chemical compounds in the studied plants:
Detection of saponins: The aqueous extract of garlic and coriander plants was found to contain saponins; the discovery was confirmed by the emergence of dense foam that lingered on the extract's surface for a considerable amount of time.
Detection of phenols: Garlic and coriander plant extracts were combined with 1% of an aqueous ferric chloride carrier, and the development of a bluish-green precipitate indicated the presence of phenols.

Results and Discussion

The ability of biological control fungi to repel the pathogenic fungi: The antagonistic ability test in the dishes revealed that the biological control fungi *Trichoderma*

harzianum possessed an elevated level of antagonistic capacity versus the pathogenic fungus *Drechslera* spp growth, as the degree of antagonism was 1.8.

Through a variety of processes, including parasitism, competition for resources (food and space), the creation of anti-substances, or the inhibition of pathogenic enzymes, the biological control fungus *Trichoderma Harzianum* influences the pathogenic fungus (26). Additionally, the biological control fungus has the capacity to fight the diseases by encircling their mycelium with its own before penetrating their walls and consuming their nutritional contents. Table 1 shows the biological control fungus treatment against the pathogenic fungus.

Table 1 The treatment of biological control fungus against the pathogenic fungus.

Treatment	Growth rate of pathogenic fungi
Control	9 cm
Trichoderma Harzianum	2.94 cm
Inhibition ratio (%)	67

Effect of plant extracts on inhibiting the pathogenic fungus *Drechslera* spp growth in vitro: Table 2 depicts that, to varied degrees and in accordance with the concentration of each extract, the test treatments all resulted in the inhibition of harmful fungus growth on the culture medium. The experiment's findings revealed that there were substantial differences in the percentage of inhibition across the plant extracts for alcoholic extract, with Garlic extract recording the highest percentage of inhibition 98% and Coriander extract recording the lowest percentage of inhibition 89%.

Garlic and Coriander extracts' ability to prevent the growth of harmful fungi may be attributable to the chemical components these plants possess that, when added to the culture medium, changed the medium's natural characteristics and rendered it less appropriate for the growth of fungi. These substances include phenols, saponins, and tannins, which have antifungal activity and inhibit the growth of harmful fungus. The investigated plants also contained those chemical compounds that might be responsible for the inhibitory activity because these compounds have the ability to penetrate the cell membrane and obstruct the active sites of specific enzymes that may be required for the growth and reproduction of the organism inside the fungal cell, thereby preventing the growth of pathogenic fungi (23).

This study's objective was to assess the antifungal effectiveness of indigenous Sisaket, Thailand, types of garlic, chili, and shallot extracts versus the pathogenic fungus, *Phomopsis* spp, which were isolated from the para rubber's infected leaves (Hevea brasiliensis Muell. Arg.). The all isolates' development was significantly slowed down by the garlic extract, which completely stopped it at 80% concentration. The garlic extract had the largest percentage inhibition of mycelial growth, pursued via shallot and chili extracts, in that order. And, the study also portrayed that several polyphenols, including tannic acid, kaempferol, quercetin, catechin, gallic acid, and apigenin, which being well-known chemicals with antifungal action, were present in these plant extracts (15).

It is evident from the data that the ethanol extract of garlic was more active than the aqueous extract against *the Fusarium* spp and *Rhizopus* spp. The inhibition zones for the ethanolic extract ranged in diameter from 4.1 mm to 14.3 mm, whereas those for the aqueous extract ranged from 2.4 mm to 10.4 mm.

Table 2 Influence of different concentrations of extractions loaded with *T. Harzianum* against pathogenic fungi.

Concentration of Extraction (mg/l)	Inhibition ratio of Garlic extraction + *T. Harzianum* VS *Drechslera* spp (%)	Inhibition ratio of Coriander extraction + *T. Harzianum* VS *Drechslera* spp (%)
50	73	69
100	76	71
150	79	72
200	81	74
250	84	76
300	86	79
350	89	82
400	91	85
450	94	87
500	98	89

Chemical detection of the presence of some active substances in plant leaves: The active components of the saponins and phenols in garlic and coriander leaves were successfully detected chemically, and alcohol has the power to extract these compounds, Table 3. (19) noted that these substances, phenols and tannins, have anti-fungal activity and are distinguished by their capacity to bind with cell protein and sediment it in nature. He also noticed that they function as good solvents for fatty substances, carrying the membranes of living cells, as a result of which the fungal and bacterial cells die. This importance against fungi is increased by the phenols and saponins positive chemical detection. (17) showed that the other ingredients include saponins, phenolic compounds, organosulfur compounds, and polysaccharides. And, their highly important pharmacological and biological influences are ascribed to their organosulfur compounds, which being isolated as well as extracted for the therapeutic applications. The garlic-derived compounds have been displayed to possess antibacterial, anti-inflammatory, and antioxidant properties, with favorable influences versus the cardiovascular illnesses, cancer, and immune regime disorders. The garlic's saponins, organosulfur compounds, phenolic compounds, and flavonoids, are highly in charge for its antioxidant action. And, such prospective is bigger into water-soluble organosulfur compounds with antioxidant capability into aqueous extracts than the new garlic or else arrangements. Additionally, these chemicals have been found to work in concert with flavonoids, saponins, and certain micro- and macronutrients found in aqueous garlic extracts to produce an antioxidant effect (18).

Table 3 Detection of saponins and phenols in garlic and coriander.

Effective compounds	Garlic	Coriander
Saponins	+	+
Phenols	+	+

Conclusion: From the results, it can be concluded that the biological fungus, after performing the improvement process by adding some effective plant extracts, has an important role in eliminating the pathogenic fungus. There is an evidence to suggest that both garlic and coriander have antifungal properties that can be effective against the fungal infections in date palm trees. Coriander has been manifested to have antifungal properties against the *Drechslera* spp, which is another common pathogen of date palm trees. Garlic has been evinced to have effective and stronger antifungal properties against the various fungal pathogens, including *Drechslera* spp, which is a common pathogen of date palm trees. Both garlic and coriander contain bioactive compounds, such as saponins and phenols that have been elucidated to have antifungal properties against the fungal pathogens in the date palm trees. And, the present investigation portrayed that the garlic and coriander effectiveness to kill the fungi cell can be attributed to phenols which are organic compounds that contain a hydroxyl group attached to an aromatic ring. They can interact with the fungal cell membranes, causing changes in their permeability and fluidity. This disruption can ultimately lead to the death of the fungal cell. Saponins, on the other hand, are glycosides that contain a steroid or triterpenoid core structure with attached sugar molecules. They have been demonstrated to disrupt the fungal cell membranes by binding to their sterols, which are the important components of the fungal cell membrane. This binding can cause changes in the membrane's permeability and lead to the leakage of cellular contents, ultimately resulting in the fungal cell death.

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