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Evaluating the Compatibility of *Trichoderma harzianum* with Three Commercial Systemic Fungicides Against Some Phytopathogenic Fungi

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دراسة توافق فطر *Trichoderma harzianum* مع ثلاثة مبيدات فطرية جهازية تجارية ضد بعض الفطريات الممرضة للنبات

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ABSTRACT

Background: The compatibility of *Trichoderma harzianum* with some recent commercial fungicides by combining them to promote an efficacious IDM for controlling plant pathogens and prohibiting chemical resistance evolution in pathogens.

Materials and Methods: laboratory experiments were conducted to study the compatibility of *Trichoderma harzianum* with three commercial systemic fungicides: Al-Sary (Carbendazim 50%WP), Top Topsin (Thiophanate-methyl 70%), and Tachichem (Hymexazol 30%WP) at recommended doses (2500 ppm for Al-Sary fungicide, 500 ppm for Top topsin, and 1.5 ppm for Tachichem) by the food poisoning technique against six pathogenic fungi: *Alternaria sp., Aspergillus flavus, Aspergillus niger, Aspergillu wentii, Penicillium sp*., and *Rhizopus sp* .

Results:*T. harzianum* displays various degrees of antagonism against the tested pathogenic fungi in dual culture. *Asp. wentii* was most sensitive to the *T. harzianum* antagonistic activity, with an inhibition percent of 67.91%, while *Rhizopus sp.* displayed complete resistance to the antagonistic activity. The results showed *Asp. niger*, *Asp. wentii*, and *Penicillium sp*. were completely inhibited by Al-Sary fungicide. *Asp. wentii* and *Penicillium sp*. were totally inhibited by Top Topsin fungicide, *Rhizops sp.* was not affected by the two fungicides. *Asp. niger* displays high sensitivity to *Tachichem* fungicide (87.23%), whereas *Penicillium sp.* was resistant to the fungicide (0.0%). Tachichem fungicide showed complete compatibility with *T. harzianum*,but Al Sary and Top-Topsin fungicides were remarkably toxic to *T. harzianum*. The effect of fungicides on *T. harzianum* antagonistic activity was estimated in PDA medium.

Conclusion The compatible fungicide Tachichem can be applied with *T. harzianum* in a comprehensive way to disease control techniques.

Key words: Compatibility, *T. harzianum*, Chemical fungicides, In vitro

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INTRODUCTION

The widespread and continuous application of chemical ingredients called agriculture toxins in modern agriculture has made it too difficult to manage crop diseases [1].To improve agricultural systems' productivity, these chemicals have been used extensively recently [2,3]. A promising alternative to reducing modern agriculture's intensive dependence on costly chemical fungicides, which pollute the environment and promote the emergence of resistant strains, is biological control of plant pathogens [4] *Trichoderma sp.* are the most promising biocontrol agents for soil-borne plant disease management [5]because it possess different mechanisms such as Mycoparasitism, antibiosis, competition for nutrients or space, improved root and plant growth, prompt resistance, sequestration and solubilization of inorganic nutrients, and pathogens' enzyme inactivation, colonization the rhizosphere, fungicides tolerance, adaptation to different ecological conditions, host specificity, high reproductive capacity and short life cycles [6].Thus, the application of eco-friendly materials and bioagents for plant disease management should be the main goal of researchers. The application of bio-agents along with suitable fungicides might boost plant disease control. Therefore, the compatibility of different fungicides with bioagents resulted in identifying safe fungicides to be used in combination with bio-agents like *Trichoderma* species [7]. Combining various disease management technologies for soil pathogens minimizes the intensity of chemical pesticides. Currently, numerous investigations on the compatibility of Bio-agents with commercial pesticides [8] Combining antagonists with chemical fungicides reduce the possibility of resistance development and restricts the use of fungicides. In consideration of these reasons, a laboratory study was achieved to test the compatibility of *T. harzianum* with three recent commercial fungicides by combining them to promote an efficacious Integrated Disease Management (IDM) for controlling plant pathogens and prohibiting chemical resistance evolution in pathogens.

Materials and Methods

The experiments were achieved in the fungal laboratory of the Biotechnology Department, College of Science, AL-Anbar University, Iraq.

- **Isolation and identification of pathogens:**

Alternaria sp. was isolated from infected fruits of *Solanum lycopersicon* , and *Rhizopus* sp. was isolated from the infected fruits of *Cucumber melo* (local market of Ramadi, Anbar) according to [9]. The isolated pathogens were identified based on morphological and microscopic features according to the classification keys of [10] Pure cultures of *Aspergillus flavus, Aspergillus niger , Aspergillus wenti,* and *Penicillium sp*. were obtained from the fungal laboratory in the Biotechnology Department / College of Science / Anbar University/ Iraq.

- **Culture of *Trichoderma harzianum***:

A pure culture of *Trichoderma harzianum* was obtained from the Ministry of Agriculture/ Integrated Management Projects for Crop Production and Protection/ Crop Protection Department and subcultured on Potato Dextrose Agar (PDA) medium for experiments.

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- **Fungicides:**

Three fungicides were used and obtained from the local market in Ramadi. The details of these fungicides are shown in table [1]:

Table 1. Tested chemical fungicides

Commerce designation	Active substance	Formulation	Mechanism
Al-Sary	Carbendazim	50% WP	Systemic fungicide
Top Topsin	Thiophanate-methyl	70% WP	Systemic fungicide
Tachichem	Hymexazol	30% WP	Systemic fungicide

- **Dual culture of T. *harzianum* and pathogenic fungi on PDA medium:**

The antagonism of the tested fungi was assessed by placing 6-mm-diameter discs that were 5-7 day-old and contained active growth of *T. harzianum* and pathogenic fungus at the ends of 8.5-cm-diameter Petri plates that contained 20 milliliters of P.D.A. with a 6-cm gap between them. One disc of each tested pathogen was put individually on a P.D.A plate as a control. All plates were incubated at 28 C○. Radial growth of the pathogens was measured daily until the control plates achieved the highest growth. The percent of inhibition of radiative growth was determined according to the following Equation[11] :

Inhibition percentage = (R1-R2) /R1 × 100

R1 = Radiate Growth (millimeter) of pathogenic fungi alone

R2 = Radiate Growth (millimeter) of pathogenic fungi in dual culture

- **In vitro influence of the chemical fungicides on pathogenic fungi growth :**

A poisoned food technique was used to assess the chemical fungicide's effect on the pathogenic fungi radiate growth, P.D.A medium poisoned with final concentrations of 2500 ppm for Al Sary fungicide, 500 ppm for Top topsin, and 1.5 ppm for Tachichem was used. PDA medium without fungicides served as controls. A 6 mm disc of each pathogen pure culture was put in the middle of PDA plates, and the plates were incubated at 28 C°. The radial growth was determined after the control plates were filled. The percent inhibition of radiate growth was determined by applying the equation mentioned in [12].

$$I = (C-T)/ C \times 100$$

Where

I = Percent of Inhibition, C = growth of pathogenic fungi in control plate, T = growth of pathogenic fungi in treated plate

- **The compatibility of *T. harzianum* with fungicides in P.D.A:**

The study looked at how much three fungicides stopped the growth of *T. harzianum* using the food poison method at the same amounts of fungicides mentioned earlier. The compatibility test was achieved using the International Organization of Biological Control (OILB) scale [13].

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Based on the percent of inhibition compared to the control, the classification groups (<30%: safe; 30–75%: little poison; 75–90%: mildly poison;>90%: poison) exhibit compatibility between *T. harzianum* and chemical fungicides [14]

- **Effect of the fungicides on the antagonistic capacity of *T. harzianum* in vitro**

Dual cultures of *T. harzianum* and phytopathogenic fungi were performed to study the effect of chemical fungicides on *T. harzianum's* antagonistic activity against pathogenic fungi. PDA plates were inoculated at one end by a 6 mm disc taken from 5-7 old days *T. harzianum* culture previously poisoned with concentrations of 2500 ppm for Al Sary fungicide, 500 ppm for Top topsin, and 1.5 ppm for Tachichem. At the opposite end the plate was inoculated with another disc from 5-7 days-old of fungal pathogen cultures. The percent inhibition of radiate growth (PIRG) of the fungal pathogens was calculated according to [12].

Bell et al [15] scale has been applied to evaluate the mycoparasitism capability of *T. harzianum* attack and colonization on the hyphal surface of pathogenic fungi.(Table 2)

Table 2. Bell scale of Mycoparasitism classification [15]

Category	Features
1st	*T. harzianum* has grown entirely above pathogenic fungi and filled the whole mid-surface.
2nd	*T. harzianum* occupied an area of two-thirds of the mid-surface
3rd	*T. harzianum* and pathogenic fungi nearly occupied half of the mid-surface
4th	Pathogenic fungi comprised an area of two-thirds of the mid-surface.
5th	Pathogenic fungi grew entirely above *T. harzianum* and covered all mid-surface

- **Statistical analysis**

Data analysis used a completely randomized design, with **t**hree replicates of each treatment. Gen stat software (version 12) was used for an analysis of variance, and the LSD test was applied to comparison means at the level of 0.05 significance. Growth inhibition percentages were converted by the angular arccosine function ($\sqrt{x} + 1$).

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RESULTS AND DISCUSSION

- **Antagonistic efficacy of *T. harzianum* against pathogenic fungi**

Results in Table 3 stated *T. harzianum* reduced the mycelial growth of tested pathogenic fungi : *Alernaria sp.* (by 51.53%), *Aspergillus flavus* (by 60.41%), *Asp. niger* (by 52.41%), *Asp. wentii* (by 67.91%) and *Penicillium sp.* (by 54.9%), whereas *Rhizopus sp.* has not been influenced by the antagonistic activity (0.0%).

T. harzianum displays various degrees of antagonism against the tested pathogenic fungi [16], observed differences in antagonism by investigating the antagonistic activity of *T. harzianum* and the pathogenic fungi *Rhizopus* sp., *Asp. niger, Fusarium sp.1* and *Fusarium sp.2*. Physiological differences between these fungi caused differences in the mechanism of antagonism by producing various antifungal substances or due to the direct exploitation of the pathogen [17, 18]. In dual petri plates, *T. harzianum* exhibited a slight clear zone after the 4th day of incubation against *Asp. wentii*, but it could not produce any clear zones against *Asp. flavus* and *Asp. niger* and it was unable to overgrow these pathogens. Furthermore, *T. harzianum* overgrew *Alternaria sp*. and *Penicillium sp.*

As stated by Mukherjee and Raghu [19], the main mechanism of biocontrol agents might not involve metabolite formation as fungitoxic substances. It may be due to other methods like the immediate killing of the pathogen hyphae, inhibition, and competition. In addition, fungal inhibition increased as the incubation period increased, while pathogen aggressive suppression was observed after 12 days of incubation.

Table 3. Growth Percentage inhibition of pathogenic fungi by *T. harzianum*

Fungi	Growth Inhibition Percentage (%)
Alternaria sp	51.53 (45.86)*^{c}
Asp. flavus	60.41 (51.03)^{b}
Asp. niger	52.41 (46.37)^{c}
Asp. wentii	67.91 (55.58)^{a}
Penicillium sp.	54.9 (47.80)^{bc}
Rhizopus sp.	0.0 (0.0)^{d}
LSD (P<0.05)	4.071

* Numbers in parentheses are arcsine-transformed values
LSD= Least Significant Differences

- **In vitro influence of the chemical fungicides on pathogenic fungi growth**

The results in Table 4 indicated that the pathogenic fungi displayed significantly varied sensitivity to chemical fungicide activity. Al Sary fungicide inhibited the mycelial growth of *Asp. niger*, *Asp. wentii* and *Penicillium* completely (100%), followed by *Asp. flavus* (83.82%) and *Alternaria sp*. (26.66%), whereas *Rhizopus sp* showed complete resistance to fungicide

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activity. *Asp. niger* showed high sensitivity to Tachichem fungicide (78.23%) followed by *Aletrnaria sp*. (64.33%), *Asp. wentii* (54.51%) and *Rhizopus sp*. (51.46%), while the inhibition percentage reduced to 16.17% in *Asp. flavus* and 0.0% in *Penicillium* sp. Top Topsin fungicide completely inhibited the growth of *Asp. wentii* and *Penicillium sp.* followed by *Asp. flavus* (94.11%) and *Asp. niger* (46.46%), wherease in *Alternaria* sp. and *Rhizopus sp*. the inhibition percentage decreased by 4.0% and 0.0% respectively.

The efficiency of these fungicides varied according to the tested fungi and fungicides'type. Our results agreed with the findings of [20] through evaluating systemic fungicides (Carbendazim , Propiconazole, Thiophanate-methyl and Tebuconazole. He found Crbendazim caused greater reduction of pathogen radiative growth In vitro. Also, Song et al [21] performed in vitro studies on seven fungicides including Rochloraz, Carbendazim, Thiram, Toclofos-methyl, Hymexazol,Azoxystrobin and Carboxin against Fusarium oxysporum and found that both Prochloraz and Carbendazim were the most effective fungicides in suppressing mycelial growth.

Table 4. Growth Inhibition Percent of fungal pathogens by three chemical fungicides in PDA

Fungi	% Inhibition / Fungicide		
	Al-Sary	Tachichem	Top Topsin
Alternaria sp	26.66 (31.05)*^{f}	64.33 (53.31)^{d}	4.0 (11.54)^{g}
Asp. flavus	83.82 (66.33)^{c}	16.17 (23.54) ^{f}	94.11 (79.95)^{b}
Asp. niger	100 (90.0) ^{a}	78.23 (62.16) ^{c}	46.46 (42.99) ^{e}
Asp. wentii	100 (90.0) ^{a}	54.41 (47.52)^{de}	100 (90.0) ^{a}
Penicillium sp.	100 (90.0) ^{a}	0.0 (0.0) ^{h}	100 (90.0) ^{a}
Rhizopus sp.	0.0 (0.0) ^{h}	51.46 (45.87) ^{de}	0.0 (0.0) ^{h}
LSD (P<0.05)	7.73		

* Numbers in parentheses are arcsine-transformed values

- **The compatibility of *T. harzianum* with the studied fungicides in P.D.A:**

Al Sary (Carbendazim) and Top-Topsin (Thiophanate methyl) fungicides were remarkably lethal to *T. haezianum* (toxic >90%), with a growth inhibition percentage of 100%, as presented in Table 5. Tachichem fungicide showed complete compatibility with *T. harzianum*, resulting in an inhibition percentage of 0.0%. The results are consistent with the study of Priti et al [22] who

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evaluated the compatibility of *T. harzianum* with the fungicides carbendazim, thiophanate methyl, hexacanozole, and propiconazole in vitro. He found that completely inhibited the mycelial radiate growth of *T. harzianum*, whereas the fungicides copper oxide, Captaf, and Thiram were compatible with *T. harzianum*.

Poudel et al, [14] also stated that Carbendazim and Hexaconazole at low concentrations prevented *T. harzianum* mycelial growth by 100%, while the high compatibility was in Copper oxychloride at 100 ppm.

The high inhibitory effect of Carbendazim and thiophanate methyl fungicides is attributed to their impact on the synthesis of DNA by blocking nuclear division and binding with the fungal β-tubulin leading to the prevention of microtubule assembly causing block cell division and dead cells [14]. Whereas, *Trichoderma's* capability to resist elevated concentrations of different natural and artificial poisonous substances (like Tachichem fungicide) is dependent on a complicated membrane flow mechanism that promotes adequate detoxification of the cell processes [23].

Table 5. Growth reduced Percent of *T. harzianum* by three fungicides in PDA

Fungicide	Growth inhibition percent (%)
Al-Sary	100 (90.0)*
Tachichem	0.0 (0.0)
Top Topsin	100 (90.0)

* Numbers in parentheses are arcsine-transformed values

Depending on the International Organization of Biological Control scale, the compatibility of the three fungicides, Al Sary at 2500 ppm and Top-Topsin at 500 ppm were toxic with a growth inhibition percent of <90% . while Tachichem at 1.5 ppm was safe, with a growth inhibition percent was <30%.

- **Effect of the fungicides on the antagonistic capacity of *T. harzianum* in vitro**

The most significant inhibition percent caused by *T. harzianum* previously treated with tested fungicides was 64.16% against *Asp. flavus* (Table 6) , followed by the inhibition percent growth of *Penicillium sp*. and *Asp. wentii* with 57.64% and 54.16% respectively. The inhibition percent was decreased against *Asp. flavus* and *Alternaria* sp. with 46.66% and 34.44% respectively. Whereas *Rhizopus sp.* was not affected by *T. harzianum* antagonistic activity with inhibition percent 0.0%.

On the other hand, *T. harzianum* demonstrated a class II when it interacted with *Alternaria sp*., *Asp. flavus*, *Asp. niger*, *Asp. wentii* because it occupied an area of two-thirds of the

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PDA mid surface. *T. harzianum* and *Penicillium sp.* covered about half of the mid surface, when cultured in dual culture; therefore, *T. harzianum* presented in class III. While *Rhizopus* sp. grew entirely above *T. harzianum* and colonized the entire mid surface, it was present in V class.

The integrated utilization of *T. harzianum* and Tachichem (Hymexazole) fungicide did not affect the antagonistic activity of *T. harzianum* towards fungal pathogens. Similarly, Zhang et al [24] reported that the combination of *T. asperellum* and hymexazol (at the concentration of 50 µg mL−1) enhanced antagonistic activity against *F. oxysporum* . Sabogal-Vargas et al [25] evaluated the antagonistic capacity of three strains of *Trichoderma* integrated with different concentrations of chlorpyrifos insecticide, and they reported that it was not influenced by any chlorpyrifos concentration.

Table 6. Growth Inhibition Percent of fungal pathogens and mycoparasitism on the Bell scale

fungi	Growth inhibition percent (%)	Bell scale
Alternaria sp	34.44 (35.88) [b]	II
Asp. flavus	64.16 (53.22) [a]	II
Asp. niger	46.66 (43.11) [b]	II
Asp. wentii	54.16 (47.41) [ab]	II
Penicillium sp.	57.64 (49.45) [ab]	III
Rhizopus sp.	0.0 (0.0) [c]	V
LSD (P<0.05)	7.794	

* Numbers in parentheses are arcsine-transformed values

CONCLUSION

Combining *T. harzianum* and hymexazol can reduce the need for chemical fungicides, providing a chance for more environmentally friendly methods to control plant diseases caused by fungi.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

المقدمة:

ان استخدام فطر *Trichoderma harzianum* مع بعض المبيدات الكيمياوية الفطرية الحديثة ومعرفة مدى التوافق بينهما من اجل تعزيز برامج المكافحة المتكاملة في السيطرة على مسببات الامراض النباتية ومنع تطور مقاومة الممرضات للمبيدات الكيمياوية

طرق العمل: تم دراسة مدى توافق فطر *Trichoderma harzianum* مع ثلاثة مبيدات فطرية جهازية تجارية: الساري (Carbendazim 50%WP)، وتوب توبسين(Thiophanate-methyl 70%) ، وتاتشيكيم (Hymexazol 30%WP) بالتراكيز الموصى بها (2500 جزء بالمليون من مبيد الفطريات الساري، و 500 جزء بالمليون من التوب توبسين، و1.5 جزء بالمليون من التاتشيكيم) باستخدام تقنية Food poisoned ضد ستة فطريات ممرضة *Alternaria sp.*، و*Asp. flavus*، و*Asp.niger*، و *Asp. wentii*، و*Penicillium sp.*، و Rhizopus sp

النتائج: اظهر فطر *T. harzianum* درجات متفاوتة من الفاعلية المضادة تجاه الفطريات الممرضة المختبرة في المزارع المزدوجة، اذ كان الفطر *Asp. wentii* اكثر حساسية للفعالية المضادة بنسبة تثبيط بلغت 67.91%، بينما أظهر *Rhizopus* sp. مقاومة تامة لتلك الفعالية . كما أظهرت النتائج أن مبيد AL-Sary ثبط نمو الفطريات *Asp. niger* و *Asp. wentii* و *Penicillium* sp. بشكل كامل، وثبط المبيد Top Topsin الفطرين *Asp. wentii* و *Penicillium* sp. بشكل كامل ، بينما لم يتأثر *Rhizops* sp. بفعالية المبيدين .أظهر *Asp. niger* حساسية عالية لمبيد الفطريات Tachichem (87.23) ، بينما كان *Penicillium* sp. مقاومًا للمبيد . (0.0%)أظهر مبيد Tachichemتوافقًا تامًا مع الفطر *T. harzianum* ، إلا أن المبيدين Al- Sary و Top-Topsin كانا شديدي السمية لفطر *T. harzianum*

الاستنتاجات:أظهرت النتائج أن الاستخدام المتكامل لفطر *T. harzianum* مع مبيد الفطريات Tachichem لم يؤثر على فاعليته المضادة للفطريات الممرضة. لذلك، اقترحت الدراسة الحالية إمكانية استخدام المبيد الفطري Tachichem بشكل متوافق مع *T. harzianum*في تقنيات مكافحة الأمراض.

الكلمات المفتاحية: التوافق،ـترايكوديرما هارزيانم، المبيدات الفطرية، مختبريا