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Antifungal Activities of Endophytic Fungus *Trichoderma viride* against *Fusarium oxysporum*

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Abstract: Endophytic fungi have drawn a lot of attention as biological control agents against numerous plant diseases and as stimulators of plant growth. This work used an *in vitro* dual culture experiment to assess endophytic fungal species isolated from different plant types against the phytopathogen *Fusarium oxysporum*. *Trichoderma spp.* are significant biocontrol agents that have been successfully used to address a number of plant diseases. The goal of the current investigation was to assess *Trichoderma viride's* ability to be antagonistic to *F. oxysporum.* The findings of the current investigation unmistakably demonstrated that the dual culture approach was used to evaluate the antagonistic activity of each of the 24 endophytic fungal isolates against *F. oxysporum*. We noticed significant variation in the 24 isolates' inhibitory efficacy against the pathogen, with inhibition ranging from 90% to 63.75%. In dual culture investigations, the endophytic *Trichoderma* isolates generally inhibited *F. oxysporum* more potently than the control isolate. One of the most promising endophytic isolates was chosen, and its potential for producing soluble inhibitory metabolites for *F. oxysporum* was further evaluated. Overall, based on the *in vitro* tests, *Trichoderma viride* was discovered to be the most promising, with dual culture studies indicating a 90% inhibition of *F. oxysporum* growth

keywords: Endophytic,*Trichoderma viride*, antagonist, dual culture.

1.Introduction

The physical environmental circumstances that

fungus encounters determine the activities of biological control agents. Advantages of biological management utilizing antagonistic fungus include ease of adaptation due to their natural occurrence in soil, typical role as organic matter decomposers, and lack of environmental pollution. Through a variety of mechanisms, including competition for nutrients and space, the production of antibiotics in the form of chemical compounds, and parasitism by entanglement of harmful hyphae, antagonistic fungi can prevent the spread of disease-causing pathogens. There are a number of fungi that can act as antigonistics against each other. Pathogenic fungi, including *Trichoderma viride,* as an example of the antagonist fungi that create antibiotic substances as secondary metabolites to inhibit the development of bacteria [1]. *Trichoderma viride*, a parasitic fungus with an antibiosis mechanism, can attack and rob resources from other fungi, as well as kill or prevent the growth of other fungi. In order to compete with pathogens, the fungus *Trichoderma viride* secretes antibiotics from the viridiol phytotoxin complex, which can prevent pathogen growth, directly parasitize pathogens, and more quickly consume oxygen, water, and nutrients[2]. Oppositional fungus *Trichoderma viride* can prevent the growth of other microbes by creating secondary metabolites that take the form of antibiotic substances. Consequently, this study was performed to prevent the establishment of the pathogen *Fusarium oxysporum*, which damages some cultivated plants, by using an antagonist fungus. The dual culture approach is frequently used when screening fungal strains for disease biocontrol.

To choose the strains with the best control efficacy on the target disease, the strains with better antagonistic activity toward the target pathogen are further examined for antifungal activity[3,4,5]. The shortcomings of this screening strategy, however, have been noted in certain papers [6,7,8]. Examining the link between the results of dual culture tests and assessing the inhibitory effects of endophytic fungi against pathogenic fungi are the main goals of this study. In order to better manage plant diseases, we hope that this paper will be useful in adopting a practical and efficient screening approach for biocontrol fungi as well as in exploring and utilizing endophytic fungi.

2. Materials and methods

Collection of plant samples

In clean labelled sterilized bags,22 samples of healthy (showing no visual disease) plants were obtained randomly from different places of agricultural farmlands of Mansoura university, Dakahlia governorate, Egypt and were identified according to [9,10]. . Three replicates of each plant sample were taken and mixed to prepare one composite sample . Plants were identified and authenticated for entophytes isolation as well as *Fusarium oxysporum* had been isolated from wilted cucumber plants.

Isolation and identification of fungal endophytes

Endophytic fungi were isolated from plant following the procedure given by[11].The collected plants were subjected to surface sterilization procedures. Briefly, Plant materials were first washed several times under running tap water, followed by washing in distilled water. Surface sterilization was then done by sequentially rinsing the plant materials (Table 1), with 70% ethanol for 30 s, followed by 0.5% sodium hypochlorite (NaOCl) for 2–3 min, and then rinsing in 70% ethanol for nearly 2 min, and finally with sterile distilled water 2–3 times. Plant materials were then dried in between folds of sterile filter papers, and each sample was then dried under aseptic conditions. The efficiency of surface sterilization procedure was ascertained for every segment of tissue, After sterilization, the plant materials were further cut (aseptically) to expose the interior surface to the PDA media. For each plant, three segments were placed in petri dishes containing PDA amended with chloramphenicol 500 mg/l The dishes were sealed with parafilm and incubated at 27°C for 3–6 days [12]. The plates were monitored continuously for spore formation. Fungal specimens were stained and studied under Leitz microscopes, using the identification key of [13].

In vitro antagonistic activity in dual culture

In this experiment, the endophytic fungal isolates were chosen to assess the antagonistic activity against cucumber vascular wilt pathogen *Fusarium oxysporum*.The Interactions between the isolates and *Fusarium oxysporum* were determined by the method described by[13]. In this assay, a 5 mm diameter, mycelial disc from the growing edge of one week old fungal isolates and one week old *F.oxysporum* culture were placed on the opposite of the PDA petri dish (Size – 90 × 15 mm) and equal distance apart distance. In control plates (without endophytic fungal isolates), a sterile agar disc was place at the opposite side of the pathogen inoculated disc. The plates were incubated at 28 ± 2 C for 5 days in the dark. Experiments were performed in triplicate (three plates for each replicate). After the incubation period, the inhibition zone was measured and used to determine percentage of inhibition by using the formula:

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

I-percent of inhibition (inhibition rate), C – growth of pathogen in control plate, T – growth of pathogen in dual plate culture[14,15,16].

Antifungal activity of endophytic fungus *trichoderma viride*

Agar diffusion assay was used to determine antifungal activity of *Trichoderma viride* extracts. The pathogenic *F. oxysporum* was cultured on PDA for 10 days to induce sporulation. The distilled sterile water was added to wash off spores from the culture plate. The spore solution was adjusted to 1×10^6 spores/ml using a hemocytometer and 100µl of spore suspension was transferred using sterile micropipette to the center of the PDA medium plate and spread by sterile glass spreader. Then 4mm diameter of PDA medium spreading with *F.oxysporum* disc was cut using a sterile cork borer then, about 200 µl of ethyl acetate of culture filtrate and methanolic extract of mycelium were added in agar well separately in each plate with a total of three well in each plate. The plates were incubated at 28 ◦C for 3 days. After the incubation period, antifungal activity was assessed by measuring the inhibition diameter zones (mm).The experiment was performed in triplicates. The inhibition zones were measured as the diameter of the fungal and was expressed as the percentage of growth inhibition. Inhibition zone = average diameter(mm) of the colony [17,18,19].

Evaluation of antifungal activity of extracts of *Trichoderma viride* on radial growth of pathogenic fungus *F. oxysporum*

The extracts of endophytic fungus that showed promise in mycelium growth inhibition of *F. oxysporum*[20,21,22], were further investigated using Linear growth assay. PDA plates containing final concentrations of (10,20,30,40,50,60,70 and 80%) culture filtrate, (0.25,0.5,1,2,4,6,8and 10 mg/ml) fungal ethyl acetate and (0.5,1,2,4,6,8and 10 mg/ml) of mycelial methanolic extracts were prepared. Fungal extracts were mixed with media and 9 ml of mixed PDA were poured into Petri dish plates (60 × 15 mm). Petri dishes were allowed to cool and solidify under a laminar flow hood. Sterilized water was used as negative controls. Experiments were performed in triplicate (three plates for each replicate). Antifungal activity was quantified by the percentage of inhibition of the growth of pathogenic isolate[23,25].

Determination of minimum inhibitory concentrations(MICs)

The MICs were determined using agar diffusion method according to the method described by[20]. The microbe's inoculum $10\hat{}^8$ CFU/ml was swabbed onto surface of PDA agar media in sterile petri dishes. Filter paper discs were sterilized in autoclave at 121 °C,1.5 atm for 20 min,After cooling the sterile filter paper disc were placed in different concentrations of culture filtrate (10,20,30,40

50,60,70&80%)
ethylacetae(0.25,0.5,1,2,4,6,8 &10mg/ml) and methanolic (0.5,1,2,4,6,8 &10

mg/ml) extracts overnight to be saturated. Filter paper discs impregnated with a different concentration of extracts were applied to the agar surface, flame-sterilized forceps was used to gently press each disc onto the agar and ensure it was attached[24,25,26]. The plates were incubated at 28˚c for 3-5 days. The MICs were determined as the lowest concentration of extract inhibiting the visible growth of microbe on agar plate . The test was carried out in triplicate and the mean was recorded[27,28].

Statistical analysis

In the present study, all obtained data were statistically analyzed using one-way analysis of variance (ANOVA) with Post Hoc Duncan test. * p value ≤ 0.05 was accepted statistically significant and performed using COSTAT software version 6.3.

3.Results and Disscusion

Isolation and identification of endophytic fungi from various host species.

A total of 25 plant samples (Table1),were screened for the presence of endophytic fungi. 499 isolates belonging to 42 species and 16 genera were obtained. The isolates were identified as follows: five species of *Alternaria* from 94 isolates, seven species of *Aspergillus* from 152 isolates, two species of *Botrytis* from 9 isolates, four species of *Fusarium* from 9 isolates, two species of *Mucor* from 30 isolates, five species of *Trichoderma* from 81 isolates, eight species of *Pencillium* from 82 isolates, one species of *Cercospora* from one isolate, one species of *Circinella* from three isolates , one species of *Cladosporium* from one isolates , one species of *Cunninghamella* from eight isolates, one species of *Drechslera* from one isolates , one species of *Gliocladium* from four isolates, one species of *Nigrospora* from three isolates , one species of *Rhizopus* from 17 isolates, one species of *Stachybotrys* from four isolates. The most commonly isolated species were *Aspergillus* with an overall occurrence frequency of 44% followed by *Alternaria* with occurrence frequency of 36%(Table 2) and Fig(1&2).

***In vitro* antagonistic activity in dual culture**

Antagonistic activity of all 24 different species of endophytic fungi isolated from different host species was tested against

F.oxysporum causing wilt of cucumber under in vitro conditions. The 5-day-old mycelia culture of the endophytic fungi and *F.oxysporum* pathogen by using dual culture techniques on PDA medium and by using the diameter of the growth inhibition % was calculated against each endophyte vis-a-vis pathogen. In the present study, fungal endophytes isolated from all host species showed considerable antagonistic activity against *F.oxysporum*. The result showed that all the 24 endophytic fungal species were capable of significant inhibition on the mycelia colony growth in culture with control values 14.37-90% inhibition noted after 5-8 days of inoculation and incubated at 25°C as compared to untreated control, but the effects were the highest to the lowest depended upon each endophyte species and tested pathogen(Table 2). The mycelial colony growth of *F.oxysporum* was significantly different within the 24 endophytes but the most potent endophytic fungi against *F.oxysporum* were *Trichoderma* spp. Table (3), illustrated that maximum inhibition of mycelial growth of FOC was observed against *Trichoderma viride* (90%). According to this, *Trichoderma viride* isolated from Radish plant was the most potential strain which was selected for testing their broad antifungal activities toward important fungal plant pathogen *F.oxysporum* under in vitro conditions as shown in Fig(3,4) Our results accord with those of [30], who confirmed the antagonistic potency of *Trichoderma viride* strain against F. oxysporum, with a mycelial inhibition rate of 45.69%. In addition, [31] reported that *Trichoderma viride* strain suppressed the mycelial growth of *F. oxysporum* strains demonstrating inhibition rates in the range of 62.50%–71.00%. Furthermore, the antagonistic potential of *Trichoderma viride* strain was evaluated by [32], who stated that *Trichoderma viride* suppressed the mycelial growth of Fusarium moniliforme strain, recording an inhibition rate of 58.70%.

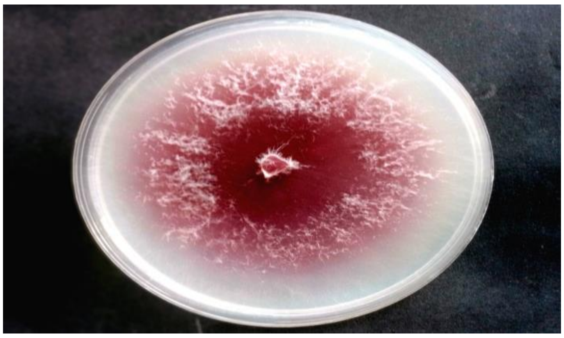
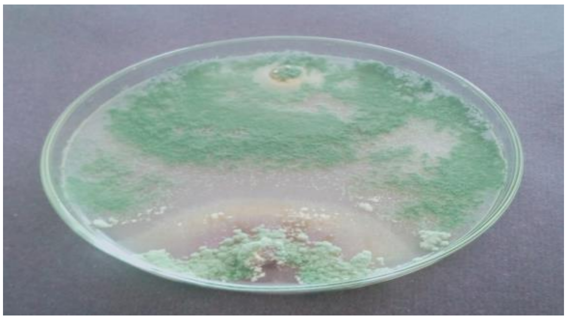


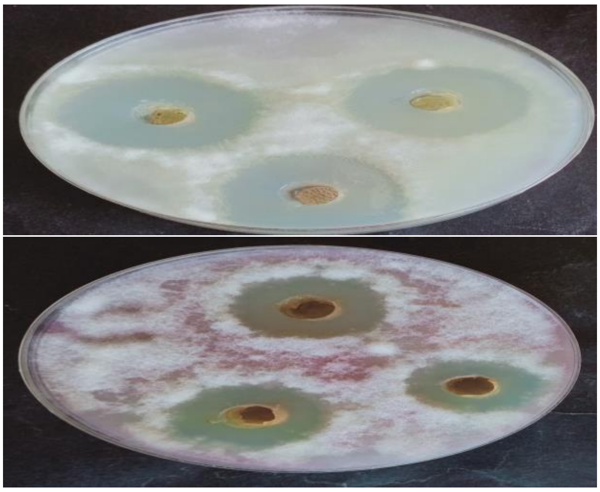
Fig (3): The growth of Fusarium oxysporum in absence of Trichoderma viride(control)



Fig(4): Effect of Trichoderma viride on Fusarium oxysporum in a dual culture test

Antifungal activity of endophytic fungus *Trichoderma viride*

Antimicrobial test by agar diffusion assay of the ethyl acetate and methanolic extract of the selected endophytic isolate based on the result of dual culture test was *Trichoderma viride* against phytopathogenic fungus *F.oxysporum* with DMSO as -ve control showed that, the tested isolate was found to exhibit antagonistic effect against FOC and this was evidenced by clear values of inhibition zones (mm) around the mycelium of tested pathogen as apparent in Fig(5) measured using Vernier calipers [33]. Our findings are consistent with those of [34], who reported the antimicrobial efficiency of culture filtrates of T. viride strain at a concentration of 5% v/v against *F. oxysporum* strain, recording mycelial inhibition rates of 51.53% and 24.71%, respectively. [35] confirmed the antifungal potency of culture filtrates of *Trichoderma* isolates against *F. oxysporum* strains and attributed the potent activity of these filtrates to the production of active secondary metabolites.



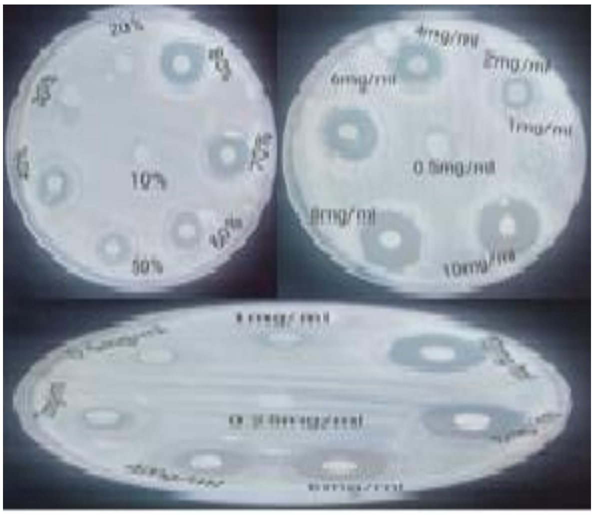
Fig(5): Effect of A:ethyl acetate & B:methanolic extract of *Trichoderma viride* on growth of Fusarium oxysporum

Effect of endophytic fungi *Trichoderma viride* on radial growth of pathogenic fungus *F.oxysporum*

The obtained result listed in table(4) revealed that,all treatments were efficiently inhibitors to pathogen radial growth when compared to control. The results were indicated that ethyl acetate extract was the most significantly effective on the growth of pathogen followed by methanolic extract of mycelium, whereas culture filtrate was the least significantly effective on the pathogen radial growth. It was found that, *Trichoderma viride* ethyl acetate extract was the most significant effective extract on radial growth of *F.oxysporum* at different concentrations that gives(8-84.5mm) of colony growth as compared with control. The highest concentration was recorded at 10mgmL^{-1} which completely suppressed *F.oxysporum* growth by 91.2% yielding(8mm) of average colony diameter. It was also pronounced that, different concentrations of *Trichoderma viride* methanolic extract caused higher significant inhibitory effect on radial growth of *F.oxysporum* that gives (19-75mm) of colony growth as compared with control. Highest inhibition displayed at concentration 10mgmL^{-1} which reduced mycelium growth by 78.88% yielding(19mm) of colony growth. The results illustrated in table(3) also revealed that, the cell free culture filtrate showed higher significant reduction on growth at a concentration dependent manner where a highest inhibition was achieved at concentration 70 and 80% in which growth was declined by 61.2 and 72.3 % respectively encountering 28 and 19mm of colony growth. Many reports demonstrated that *Trichoderma* culture filtrates had strong competition for nutrition or space [36]or secreted some metabolites [37] to suppress the growth of Fusarium.

Determination of minimum inhibitory concentration (MIC) of extracts of *Trichoderma viride* against tested pathogen

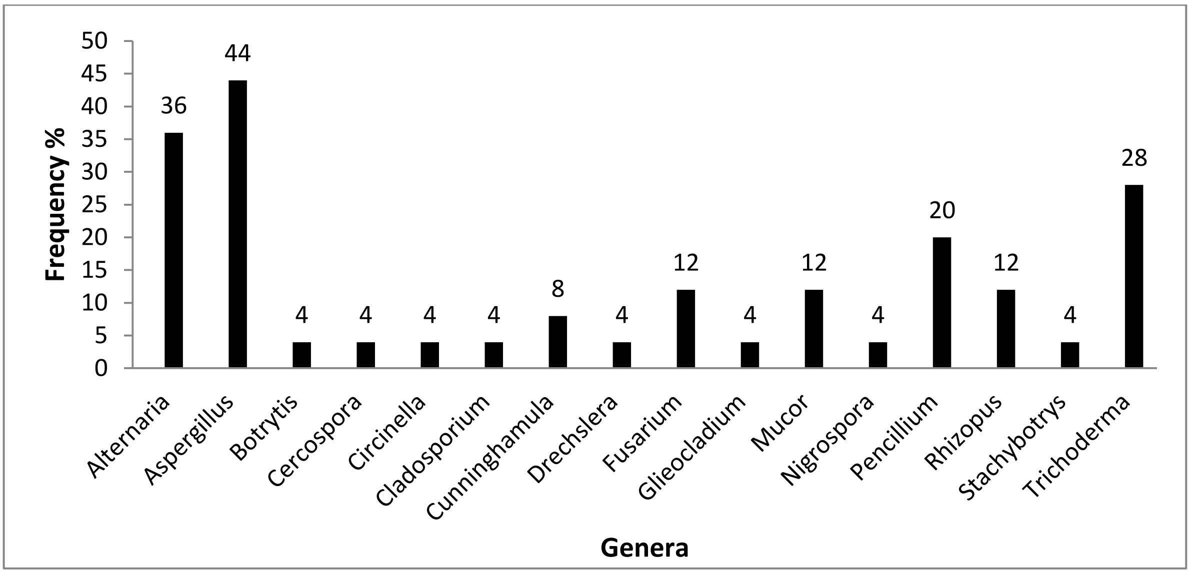
The minimum inhibitory concentration values were determined by using agar well diffusion method on potato dextrose agar (PDA) medium. The MIC values were taken as the minimum concentration of extracts of the endophytic fungus *Trichoderma viride* at which no microbial growth of tested pathogen was observed and it is carried out by measuring average of inhibtion zone diameter(mm) . As illustrated in table(5) and Fig(6), ethyl acetate extracts had the lowest MIC that ranged between 0.25mg/ml to10mg/ml. *F. oxysporum* was observed to be sensitive(1mm) at MIC (0.5mg/ml). ,whereas methanolic extracts exhibited MIC values that ranged between (0.5mg/ml to 10 mg/ml). Methanolic extracts affected the growth of *F. oxysporum* (1mm) with MIC value(1mg/ml). Moreover, CF of *Trichoderma viride* had the lowest MIC that ranged between (10%to 80%) and appeared to be sensitive on growth (1mm) at MIC (40%). Inhibition zones of *F.oxysporum* was higher (17.5 mm) in case of ethyl acetate than that of methanolic (17mm) treated at the same concentration(10mg/ml) ,whereas *F. oxysporum* had higher inhibition zone (14.5mm) at concentration 8mg/ml. It can be concluded from results that; ethyl acetate was more efficient on *F.oxysporum* even at (0.5mg/ml) followed by methanolic and culture filtrate with the same MIC value (1mg/ml). The MIC data contradicted those reported in a previous study, which indicated that the ethyl acetate extract of *T. viride* showed antifusarial potency against *Fusarium oxysporum* strains with an MIC value of 100 mg/ml. [38] reported that the MIC value of the ethyl acetate extracts of *Trichoderma* isolates against A. flavus strain was 1.0 mg/ml, recording suppressive zones ranging from 6 to 13.8 mm in diameter. The difference in MIC values between our findings and the previous studies is attributed to the variation in sensitivity of different fungal strains.



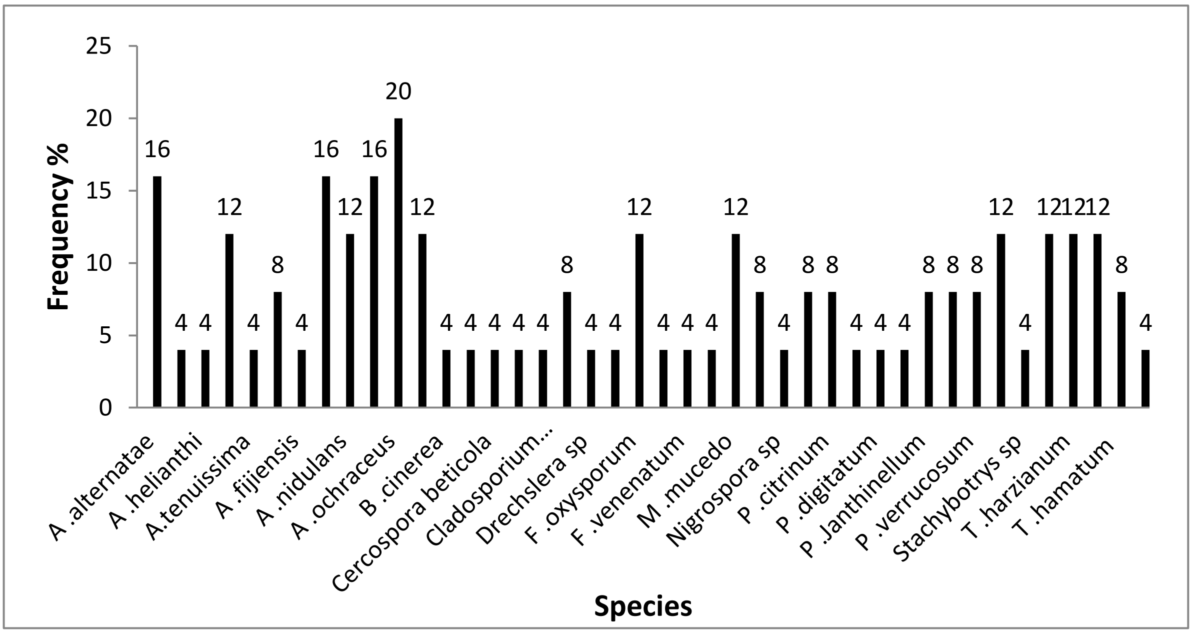
Fig(6): A, MIC of CF ,B MIC of MET , C MIC of ETH of *T.viride*

4.Conclusion

Potential antimycotic action against fusarial phytopathogen was seen in the antagonistic strains of *Trichoderma viride*. *Fusarium oxysporum* is a fungal pathogen, and the powerful antagonistic efficacy of culture filtrates and organic solvent extracts against it highlights the opportunity to use unique and secure bioactive chemicals to prevent the negative effects of chemical fungicides on the environment and human health. The best antifungal efficacy was demonstrated by the ethyl acetate of culture filtrate, followed by methanolic extracts of mycelium, underlining the possibility for employing these bioagents to manage resistant fungal phytopathogens.



Fig(1): Frequency of isolated fungal genera



Fig(2): Frequency of isolated fungal species

Table (1): List of plant species used in this study

N0	Scientific Name	Common Name	Family	Arabic name	Part of Plant Used
1	*Cucumis Sativus L.*	Cucumber	Cucurbitaceae	خيار	Root
2	*Pelargonium graveolens L.*	Crane-bill	Geraniaceae	العتر	Leave
3	*Pimpinella anisum L.*	Anise	Apiaceae	يانسون	Seed
4	*Solanum Lycopersicum L.*	Tomato	Solanaceae	طماطم	Root
5	*Cucurbita Pepo L.*	Pumpkin	Cucurbitaceae	يقطين	Leaves
6	*Capsium L.*	Pepper	Solanaceae	فلفل	Stem
7	*Phaseolus vulgaris L.*	Bean	Leguminosae	الفاصوليا	Root
8	*Raphanus sativus L.*	Radish	Brassicaceae	فجل	Leave
9	*Coriandrum sativum L.*	Coriander	Apiaceae	كزبرة	Leave
10	*Thymus vulgaris L.*	Thyme	Labiatea	زعتر	Leave
11	*Zea mays L.*	Corn	Poaceae	ذرة	Leave
12	*Helianthus annuus L.*	Sunflower	Asteraceae	عباد الشمس	Leave
13	*Apium graveolens L.*	Celery	Apiaceae	كرفس	Stem
14	*Anethum graveolens L.*	Dill	Apiaceae	شبت	Stem
15	*Chrysanthemums indicum L.*	Chrysanthemums	Asteraceae	الاقحوان	Stem
16	*Mentha spicata L.*	Mentha	Labiatae	النعناع	Leave
17	*Cuminum cyminum L.*	Cumin	Apiaceae	كمون	Seed
18	*Eruca sativa L.*	Arugula	Brassicaceae	جرجير	Root
19	*Eugenia carophyllus Bullock& S.G Harrison*	Clove	Myrtaceae	قرنفل	Fruits
20	*Ricinus communis L.*	Ricinus	Euphorbiaceae	خروع	Seed
21	*Rosmarinusofficinnalis L.*	Rosemary	Labiatae	روزماري	Leave
22	*Solanum tuberosum L.*	Potato	Solanaceae	بطاطس	Stem
23	*Ocimum basilicum L.*	Basil	Labiatae	ريحان	Leave
24	*Allium sativum L.*	Garlic	Amaryllidaceae	ثوم	Bulb
25	*Allium cepa L.*	Onion	Alliaceae	بصل	Bulb

Table(2) : Occurrence frequency of endophytic fungi isolated from different plant Species on PDA medium at 26±1˚C

Fungal genera and species	Total count	NCI	% Frequency of occurrence
Alternaria	94	9M	36
A .alternatae	20	4L	16
A .brassicicola	7	1R	4
A .helianthi	10	1R	4
A .solani	45	3L	12
A.tenuissima	12	1R	4
Aspergillus	152	11M	44
A .flavus	25	2R	8
A .fijiensis	3	1R	4
A .fumigatus	21	4L	16
A .nidulans	16	3L	12
A .niger	28	4L	16
A .ochraceus	37	5L	20
A .tubingensis	22	3L	12
Botrytis	9	1R	4
B .cinerea	5	1R	4
B .fabae	4	1R	4
Cercospora beticola	1	1R	4
Circinella simplex	3	1R	4
Cladosporium oxysporum	1	1R	4
Cunninghamula elegans	8	2R	8
Drechslera spp	1	1R	4
Fusarium	9	3L	12
F .poae	1	1R	4
F .oxysporum	5	3L	12
F .solani	2	1R	4
F .venenatum	1	1R	4
Glieocladium penicillioides	4	1R	4
Mucor	30	3L	12
M .mucedo	17	3L	12
M .ramosissimus	13	2R	8

Nigrospora spp	3	1R	4
Rhizopus oryzae	17	3L	12
Stachybotrys spp	4	1R	4
Trichoderma	81	7M	28
T .viride	20	3L	12
T .harzianum	43	3L	12
T .kongngii	10	3L	12
T .hamatum	6	2R	8
T .reesei	2	1R	4
Pencillium	82	5L	20
P .chrysogenum	17	2R	8
P .citrinum	14	2R	8
P .camemberti	1	1R	4
P .digitatum	6	1R	4
P .italicum	5	1R	4
P .Janthinellum	11	2R	8
P .notatum	15	2R	8
P .verrucosum	13	2R	8

Number of cases of isolation(NCI) or Occurrence Remarks (OR):H = High occurrence(more than 12 cases); M = Moderate occurrence(between 6-12 cases); L =Low occurrence(between 3-5 cases); R = Rare occurrence(less than 3 cases).

Table(3): In vitro antagonistic assay of endophytic fungi and *Fusarium oxysporum*. Each value represents the mean of three replicates(Mean±SD). The same letters in each column represents insignificant difference where LSD at P≤0.05 using Post Hoc. Duncan test

NO.	Endophytes	pathogen(C)	Dual pathogen(T)	Growth Inhibition (%)
1	Alternaria alternatae	80.00^{a}±0.01	29.00^{j}±0.01	63.75^{h}±0.87
2	*Alternaria brassicicola*	80.00^{a} ±0.01	59.50^{de}±.05	25.62^{o}±0.13
3	*Alternaria tenuissima*	80.00^{a} ±0.01	57.00^{ef}±0.30	28.75^{n}±0.24
4	*Aspergillus fijiensis*	80.00^{a} ±0.01	62.00^{cd}±0.23	22.50^{p}±0.29
5	*Aspergillus flavus*	80.00^{a}±0.01	25.00^{k}±0.34	68.75^{g}±0.18
6	*Aspergillus fumigatus*	80.00^{a}±0.01	64.40^{bc}±0.11	19.50^{q}±0.35
7	*Aspergillus niger*	80.00^{a}±0.01	65.00^{bc}±0.12	18.75^{q}±0.40
8	*Aspergillus ochraceus*	80.00^{a}±0.01	19.00^{l}±0.03	76.25^{f}±0.31
9	*Aspergillus terreus*	80.00^{a}±0.01	60.00^{de}±0.95	25.00^{o}±1.2
10	*Cercospora beticola*	80.00^{a}±0.01	47.00^{hi}±0.75	41.25^{j}±1.9
11	*Circinella simplex*	80.00^{a}±0.01	67.00^{ab}±0.14	16.25^{r}±0.45
12	*Cladosporium oxysporumi*	80.00^{a}±0.01	54.50^{fg}±0.85	31.87^{m}±0.87
13	*Cunninghamula elegans*	80.00^{a}±0.01	17.60^{l}±0.38	78.00^{e}±0.98
14	*Glieocladium penicillioides*	80.00^{a}±0.01	45.00^{i}±0.09	43.75^{j}±1.9
15	*Mucor mucedo*	80.00^{a}±0.01	47.70^{hi}±0.08	40.25^{j}±1.5
16	*Nigrospora sp*	80.00^{a}±0.01	68.50^{a}±0.41	14.37^{s}±0.66
17	*Pencillium digitatum*	80.00^{a}±0.01	53.00^{g}±0.29	33.75^{l}±0.39
18	*Pencillium notatum*	80.00^{a}±0.01	48.00^{hi}±0.75	40.00^{j}±0.70
19	*Pencillium verrucosum*	80.00^{a}±0.01	50.00^{h}±0.25	37.50^{k}±0.43
20	*Trichoderma hamatum*	80.00^{a}±0.01	14.50^{mn}±0.19	81.87^{d}±0.12
21	*Trichoderma harzianum*	80.00^{a}±0.01	10.00^{o}±0.15	87.00^{b}±0.15
22	*Trichoderma viride*	80.00^{a}±0.01	$8.00^{o}0.02$	90.00^{a}±.17
23	*Trichoderma kongngii*	80.00^{a}±0.01	13.00^{n}±0.06	83.75^{c}±.22
24	*Trichoderma reesei*	80.00^{a}±0.01	16.80^{lm}±0.11	79.00^{e}±010

Table(4):Effect of endophytic fungus *Trichoderma viride* extracts on radial growth of *F. oxysporum*. Each value represents the mean of three replicates(Mean±SD). The same letters in each column represents insignificant difference where LSD at *P*≤0.05 using Post Hoc. Duncan test

Endophytic fungus	**Average of colony diameter (mm) inhibition(%) Conc % CF Conc mg/ml MET ETH CF MET ETH**							
Trichoderma viride	10	80.20^{a}±0.23	0.25	75.00^{a}±0.12	84.50^{a}±0.94	10.90^{h}±0.78	16.66^{h}±0.34	6.20^{h}±0.43
	20	78.50^{b}±0.20	0.5	70.00^{b}±0.16	82.00^{b}±0.90	12.80^{g}±0.65	22.23^{g}±0.9722.22^{g}±0.27	8.90^{g}±0.06
	30	74.40^{c}±0.18	1	32.00^{c}±62.00	78.8^{c}±0.83	17.40^{f}±0.63	31.11^{f}±2	12.50^{f}±0.37
	40	62.9^{d}±0.19	2	45.00^{d}±0.41	40.50^{d}±0.75	30.20^{e}±0.38	50.00^{e}±3.1	55.00^{e}±0.29
	50	61.00^{e}±0.13	4	39.00^{e}± 0.19	39.00^{e}±0.48	32.30^{d}±1.5	56.66^{d}±0.70	56.70^{d}±0.19
	60	60.00^{f}±0.10	6	38.00^{f}± 0.29	37.5^{f}±0.38	33.40^{c}±0.95	57.77^{c}±0.41	58.40^{c}±1.3
	70	35.00^{g}±0.09	8	28.00^{g}± 0.21	15.00^{g}± 0.30	61.20^{b}±0.66	68.88^{b}±0.44	83.40^{b}±0.09
	80	25.00^{h}±0.05	10	19.00^{h}±0.34	8.00^{h}±0.28	72.30^{a}±2.6	78.88^{a}±0.31	91.20^{a}±0.08
control	90.00							

CF: culture filtrate, MET: methanol, ETH: ethyl acetate

Table(5): Determination of minimum inhibitory concentration (MIC) of extracts of *Trichoderma viride* against *F. oxysporum*. Each value represents the mean of three replicates(Mean±SD). The same letters in each column represents insignificant difference where LSD at *P*≤0.05 using Post Hoc. Duncan test

Minimum inhibitory concentration				
Inhibition zone diameter(mm)				
Conc %	**CF Conc(mgmL^{-1}) ETH MET**			
10	visible growth	0.25	visible growth	-
20	visible growth	0.5	1^{g}±0.05	visible growth
30	visible growth	1	2.5^{f}±0.33	1^{f}±0.04
40	10^{b}±0.11	2	4^{e}±0.10	3^{e}±0.01
50	11^{b}±0.32	4	10^{d}±0.02	14^{d}±0.05
60	12^{ab}±0.15	6	15^{c}±0.03	15^{c}±0.03
70	13^{ab}±0.19	8	15.6^{b}±0.13	16.3^{b}±0.02
80	14.5^{a}±0.88	10	17.5^{a}±0.23	17^{a}±0.08

CF: culture filtrate, MET: methanol, ETH: ethyl acetate

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