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Antagonistic Activity of Endophytic Fungi against *Alternaria triticina* and their Potentials on Growth Promoting of Wheat

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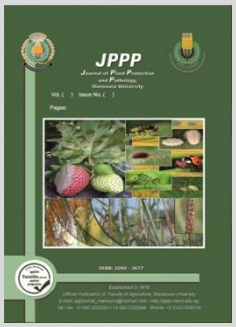

ABSTRACT

The research is concerned with obtaining new endophytic fungi isolates, testing their ability to inhibit the growth of *Alternaria triticina* the causal agent of leaf blight disease of wheat, and also their potential effects as growth promoting factors for wheat plants. In this study, some endophytic fungi isolates were isolated from different wild plant species in Saint Catherine protectorate and evaluated under laboratory conditions using in vitro dual culture technique against *Alternaria triticina*, Overall 72 endophytic fungal isolates have been isolated from inner tissues of wild plants and evaluated for their ability to control the growth of fungal pathogen, 17 isolates of them appeared effective to inhibit Alternaria triticina using dual culture technique. Most effective isolates were evaluated and screened in vitro for confirm potency where 10 isolates belonging different fungal species were high effective and confirmed namely *Aspergillus niger, Chaetomium globosum, Cladosporium cladosporides, Curvularia tunata, Mucor plumbeus, Penicillium brevicompactum, Penicillium sclerotiorum, Penicillium sinaicum, Phoma glomerata* and *Ulocladium atrum* from in vitro results 6 best isolates were selected for greenhouse experiments where showed that among of them 4 enophytic fungal isolates belongs *C. globosum, Mucor plumbeus, Penicillium sinaicum* and *Ulocladium atrum* suppressed *Alternaria triticina*. The endophytic fungi also enhanced the plant growth parameters of wheat plants like plant height, fresh and dry weight as compared to control.

Keywords: endophytes, biocontrol, dual culture assays, plant growth promoters.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most economic and strategic crops in Egypt, which ranks almost fifth among the countries of the world in the level of productivity of the unit grown from wheat. Among cereal diseases, leaf blight disease is one of the most damaging diseases of cereal crops. The most common disease symptoms appear as small chlorotic, oval or elliptical shaped lesions, these lesions become uneven in shape. The chlorotic borders of the lesions become disperse and become dark brown in color. Infection starts on the lower leaves, then symptoms can be detected on all plant parts. *Alternaria triticina* fungus remain as conidia on seed or as mycelia within sporulation and seed on lower leaves (Ahmed *et al*., 1994; Beshir, 1994; Chowdhury and Roy, 1995). High humidity or irrigation, as well as warmer temperatures (20 ºC to 25 ºC) favor infection and disease development which can be very severe if environmental conditions are preferred for disease development. The disease has been detected in many countries worldwide, and it was noticed in different locations of wheat cultivation in Egypt whether in the cultivated lands or the newly reclaimed areas. Due to the nature of spot diseases like the advancement of pathogens, the management of leaf blight disease is very difficult (Dhanbir *et al*., 2009; Khalil *et al*. 2016). Although there are many chemical pesticides that are widely used in the control of spot diseases. However, there are many obstacles to the use of these pesticides, such as environmental conditions that negatively affect pesticides, as well as some pathogens gaining the ability to adapt to pesticides with repeated use (Reis *et al*., 2005), in addition to the environmental and health damage caused by the wrong uses of pesticides. The use of biological control is one of successful approaches in integrated management (Mei and Flinn, 2010; Xiang *et al*., 2016; Manzoor *et. al*., 2019). Biocontrol of A. triticina using different microorganisms could be used as promising alternative approaches and ecofriendly trends. Fungal endophytes are attractive alternative or complementary methods in integrated control programs (Lu *et. al*., 2016). Some of endophytic fungi have been recorded to play an effective role in important functions of host plants due to production of some metabolites and can be applied in plant disease control (Rai *et al*., 2014; Manzoor *et. al*., 2019). Endophytic fungi play an important role in interact with host plants to preventing plant pathogens development (Zeilinger *et. al*., 2016). Endophytic may be isolated from different parts of every plant species. They are important sources of bioactive compounds (Strobel, 2003). Several endophytic fungi were studied to control wheat diseases but leaf blight disease not be controlled completely. Recently, several studies mentioned to evaluate elicitor compounds that are effective in promoting the plant defense mechanisms activation for the control some of fungal pathogens. The application of suspensions of endophytic fungi reduced the severity of some diseases in wheat due to the activation of the plant defense mechanisms, enhancement of the productivity and plant development (Hossain *et al*., 2016). Many types of wild plants have been studied their effects and effectiveness in control of plant diseases, whether using their extracts or through the use of endophytic fungi obtained from the internal tissues of wild plants. Different genus of endophytic fungi have potential in the biological control of plant disease is well known such as fungal species belonging *Chaetomium, Ulocladium, Aspergillus* and *Penicilliu* genera. Their biocontrol mechanisms include producing antibiotics and ergosterol compounds that can reduce the growth of different fungal pathogens, also stimulate growth

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of plants and induce resistance to the diseases (Marwah *et al*., 2007; Zhang and Yang, 2007). This research was carried out to evaluate the effects and antifungal activities of some endophytic fungi under laboratory and greenhouse conditions on *A. triticina* the causal agent of leaf blight disease in wheat, with the purpose of a solution to control crop diseases by using natural microorganisms.

MATERIALS AND METHODS

Isolation and identification of causal agents

Fungal isolates of pathogens were obtained from two different sources, infected grains and plant leaves showing symptoms of the disease. Fungal pathogens were isolated from wheat seeds using nutrient agar plate method. Seeds are surface sterilized for 4 minutes and treated with sterilized water. Four seeds are put on nutrient agar then incubated at 26-28°C for 7 days in dark conditions. The colonies of the fungus are showed with growth of fungal conidia and aerial mycelium. The methodology of isolation technique used for isolation and identification as described in (Mathur and Kongsdal, 2003).

In order to isolate of the pathogens from wheat leaves, fifteen samples from wheat leaves with blight lesions collected from different locations of wheat cultivation. The pathogenic isolates were obtained from wheat leaves Cv., Gemmeiza-9; Misr-1; Sids-11 and Sakha-95, from different Geographic origin such as (New Vally governorate", and Minya governorate during 2020 and 2021 growing seasons. Small pieces of infected leaves were sterilized in 0.5 % NaOCl for 1 min. then washed three times in sterilized distilled water and drained on filter paper then transferred on PDA medium, each contained 4 pieces as 4 replicates. The cultures were incubated at 28 ºC for seven days. Identification of all the isolated fungi was carried out using morphological methods and microscopic examination in Nano-Phytopathology Lab., Desert Research Center, Egypt. The pattern group of sporulation of each culture was examined using stereomicroscope (×20 and ×40 magnification) as described in the method of Simmons and Roberts (1993).

Isolation of endophytes

Healthy leaves and stems of some wild plants were collected from various locations in Saint Catherine protectorate. Plant samples were randomly chosen. Part of branch with leaves was randomly selected from individual plant species. Leaves were treated with tap water and cut into small pieces (5 mm) then surface sterilized by 75% ethanol for 1 min, and rinsed twice in sterile distilled water. Four pieces were put in each Petri dish containing PDA. Dishes were incubated in darkness at 28 ºC for 10 days and checked every 2 or 3 days for growth of endophytic fungi. Isolation of endophytic fungi was carried out as described by (Wiyakrutta *et al*., 2004)

Pathogenicity test

Pathogenicity test were carried out to confirm that the isolated *Alternaria triticina* isolates were pathogenic on wheat. Also the same assay was conducted with endophyte which isolated from different plant species to ensure that the endophytic fungi were nonpathogenic on wheat. An Egyptian cultivar of wheat (Misr-1) was used for pathogenicity test.

The pathogenic fungi were tested for pathogenicity test using 20 day old wheat plants under growth chamber conditions. The inoculum of endophytes were prepared in sterile distilled water, five plants for each three pots were treated and sprayed till run off with suspension on fungal spores (2×10^5 conidia /ml) adjusted by hemocytometer. Control plants were treated with sterile distilled water. All treatments were incubated for 72 h in growth chamber then maintained in greenhouse at 22 ± 2 ºC, were assessed for severity of disease every 4 days for four times (16 days).

Antagonistic effect of endophytic fungi against fungal pathogen isolates

Antagonistic effect of all endophytic fungi was carried out using dual culture method where discs of agar (5 mm diameter of endophytes was taken by cork-borer then placed 3 cm away from disc of pathogenic fungi in 9 cm PDA petri dishes. The discs of both endophytic taxa and pathogens were obtained from cultures of 14 day while the control treatment plants were treated with pathogen alone was the control. Then the cultures were incubated at 26±2 ºC. Pathogenic fungal growth was assessed by calculating of growth inhibition rates % of each endophytic fungi against pathogenic isolate. 3, 7, 10 and 14 days after inoculation. Measurements were stopped when the colonies reached the edge of the petri dishes or stopped growing. Growth inhibition rate (GIR) was calculated as described in the formula of Eksteen *et al.* 2001 as follow: [(C2-C1)/C2]×100, where C1 is the diameter of mycelia colony of pathogen , and C2 is the mycelial growth diameter in control in case of antagonist (Vethavalli and Sudha, 2012). The treatments were replicated 4 times the experiment was carried out two times.

Effect of Endophytic fungi on spore germination of pathogens

In order to evaluate the antagonistic effect of the effective endophytic fungi isolates resulted from antagonistic test against *Alternaria triticina*, The experiment was implemented a mixture suspension of pathogen/endophyte 1:1 v., (50 µl) , then placed into the cavity glass slide. Suspension of pathogen spores alone were used as control. The concentration of fungal conidia were 10^5/ml. the glass slides were maintained moisture box at 26ºC for 48 h and examined using light microscope. The treatment conducted in four replicates, the conodial germination % was calculated compared with control.

Greenhouse experiment

The greenhouse experiment was carried out to study the protective efficiency of endophytes as pre-inoculation of leaves by the most 10 endophytic fungi showed the highly antagonistic performance in dual culture test against *A. triticina* on seedlings of wheat, the experiment in a completely randomized design was designed in greenhouse. Four seeds of wheat cultivar Misr-1 were sown in 16 cm diameter plastic pots each treatment has 4 replicates. Plants were sprayed with suspensions of endophyte using a hand atomizer, while the endophytes were applied to the leaves in distilled water 1 day before the pathogen. Suspension of the pathogen and endophytes were prepared by flooding the 15 day old culture with distilled water. The suspension was filtrated using cheese-cloth. The concentration of pathogens and endophyes were adjusted using hemocytometer as 2×10^5 conidia/ml. Control plants were inoculated with pathogens only. All treatments were incubated for 48 h in plastic bags then maintained in greenhouse. The development of leaf blight disease was assessed by estimating the percentage necrotic leaf area on the first, second and third leaves of each plant/treatment after 15 days (Asif, *et. al.* 2021). Total

chlorophyll and carotenoids were estimated as per Nagata and Yamashita (1992), Growth parameters including fresh and dry weights (g), plant height (cm) were estimated. The root system was tapped out of the pot and was gently washed by tap water, for obtaining fresh and dry weight, the roots were blot-dried to remove excess water then the fresh weight was recorded. Shoots and roots were dried in an oven on 65ºC for several days until constant weight to obtain dry weight.

Statistical analysis

Statistical analysis of obtained data was subjected with procedure "ANOVA". Treatment means were compared by Duncan's multiple Range Test at 0.05 level of probability.

RESULTS AND DISCUSSION

Isolation and identification of causal agents

Totally, 120 fungi isolates were obtained from five wheat growing locations [El-Bostan "Elbeheirah governorate"; Nubariya; Kharja and Dakhla oasis "New Vally governorate", and Minya governorate. in two growing seasons (2020 and 2021). The highest numbers of cultures were obtained from leaves, while the less were from seeds. Isolates obtained from second seasons were more than that obtained from the first one. Cultures from A. alternata were most frequently compared with A. triticina but the most virulent isolates were the A. triticina. The A.alternata and A. triticina were isolated from seeds, and shoot parts of 76% of total samples. The isolates species constituted 82.5% of all fungi obtained from leaves while 17.5 % isolated from seeds. The most frequently isolates were obtained from Minya governorate (35.8%) and the lowest percentage was from Dakhla oasis (8%), while the other locations recorded (22.6% and 18.5% and 13.3%) from El-Bostan , Nubariya and Kharja oasis respectively. All isolates were identified by a light microscope, the pathogenicity test for all isolates was carried out on detached leaves under laboratory conditions. The isolates most capable of causing infections A. triticina was cultured and preserved for use in the experiments of this study.

Isolation of endophytes and their effecacy against fungal pathogen isolates

The seventy two endophytic fungi isolates belong eight different species were isolated from aerial organs of six different wild plant species as mentioned in (Table 1). A wide range of efficacy was detected among endophytic isolates in their ability to inhibit the most effective isolate of A. triticina the causal agent of leaf blight disease using dual culture assay with inhibition rates ranging from 0 to 53.5%. The most effective 10 different endophytic isolates inhibited the pathogen (Table 2) were selected to study their efficacy on the most pathogenic A. triticina isolate.

Antagonistic effect of endophytic fungi against fungal pathogen isolates

The ten selected endophytes were significantly reduced the growth of fungi in range from 9 in control to 2.9 with *C. globosum*. However, all tested endophytes have success- fully inhibited the tested fungal pathogens. Table 2 showed the interaction between endophytes and *A. triticina*, and it was observed that the fungal mycelia were destroyed. *C. globosum*, *Ulocladium atrum* and *Mucor plumbeus* were the most effective endophytes where inhibited A. triticina by (2.23, 2.45 and 2.38 cm) respectively, these endophytes are fungi with a worldwide distribution. Their efficiency in biological control of plant diseases is well known. Their biocontrol mechanisms include producing antibiotics compounds that can suppress the growth pathogens and destroyed the mycelia of fungi (Zhang and Yang, 2007). More than 200 bioactive compounds have been reported from these species. Significant control of many plant diseases especially those soil borne plant pathogenic fungi, were attributed to the presence of these diverse bioactive secondary metabolites but definitive evidence is still lacking (Sibounnayong *et al*., 2005). Secondary metabolites of Chaetomium species were found to degrade cell walls of many plant pathogens such as Fusarium oxysporum, Sclerotinia sclerotiorum, Rhizoctonia solani and Sclerotinia tritici (Liu *et al*., 2008). Chaetomin was known to be produced by C. globosum (Safe and Taylor, 1972; Bai *et al.*, 2015). The same process can be hypothesized when plants are treated with *U. atrum* which was successfully commercialized and is the active ingredient in some commercial products as biocontrol agent product against various pathogens in organic and conventional viticulture is many countries (Berto, *et al.* 2001; Jacometti, *et al.* 2010). The efficiency of *U. atrum* to control of some pathogens has been tested in several crops such as grapevine and tomato against several other pathogens in various crops. For example,, studies on biocontrol of grapevine gray mold were studied by (Schoene *et al.* 2000).

Table 1. endophytic fungi isolated and identified from five different wild plant species that were collected from Saint Catherine protectorate, Egypt.

	Endophyte isolates	Wild plant	Location
1	Ulocladium atrum	Devera turtusa	N: 28, 33, 22.2 E: 33, 56, 14.6 Elev. 1588
2	Penicillium sclerotiorum	Devera turtusa	N: 28, 33, 42.7 E: 33, 55, 55.9 Elev. 1452
3	Phoma glomerata	Centaurea erynzipides	N: 28, 33, 20.3 E: 33, 56, 09.6 Elev. 1609
4	Ulocladium atrum	Centaurea erynzipides	
5	Phoma glomerata	Euphorbia peplis	N: 28, 34, 02.5 E: 33, 55, 51.9 Elev. 1456
6	Penicillium sclerotiorum	Alkanna oriantalis	N: 28, 33, 36.5 E: 33, 55, 57.9 Elev. 1500
7	Aspergillus niger	Alkanna oriantalis	N: 28, 33, 55.8 E: 33, 56, 02.2 Elev. 1503
	Cladosporium cladosporioides	Alkanna oriantalis	N: 28, 33, 55.8 E: 33, 56, 02.2 Elev. 1503
8	Curvularia lunata	Alkanna oriantalis	N: 28, 33, 22.2 E: 33, 56, 14.6 Elev. 1588
9	Chaetomium globosum	Alkanna oriantalis	N: 28, 32, 58.6 E: 33, 56, 58.7 Elev. 1554
10	Penicillium sinaicum	Pulicaria arabica	
11	Penicillium brevicompactum	Phlomis aurea	N: 28, 33, 03.4 E: 33, 57, 49.0 Elev. 1689
12	Ulocladium atrum	Phlomis aurea	N: 28, 33, 42.5 E: 33, 57, 18.8 Elev. 1563
13	Mucor plumbeus	Phlomis aurea	N: 28, 33, 42.5 E: 33, 57, 18.8 Elev. 1563

Effect of Endophytic fungi on spore germination of pathogens

In the experiment to evaluate the ability of endophytes to inhibit spore germination, results indicated

that four endophytes *Chaetomium globosum*, *Mucor plumbeus*, *Ulocladium atrum*, and *Penicillium sinaicum* reduced significantly the percentage of spore germination of A. triticina by (78, 67, 58 and 45%) respectively (Fig.1).

Table 2. Effect of 10 isolates of endophytes on diameters (cm) and growth rate of *A. triticina* at four evaluation times under in vitro test

Treatment	Mean diameter (cm) of growth of *A. Triticina*				
	3	7	10	14	Mean*
Aspergillus niger	2.70	3.90	6.20	6.80	4.90 c
Chaetomium globosum	1.60	1.90	2.50	2.90	2.23 e
Cladosporium cladosporioides	3.50	6.10	6.30	6.90	5.70 b
Curvularia lunata	2.10	3.80	5.00	6.10	4.25 d
Mucor plumbeus	1.80	2.10	2.90	3.00	2.45 e
Penicillium brevicompactum	2.80	5.50	5.90	6.40	5.15 bc
Penicillium sclerotiorum	3.00	5.90	6.10	6.30	5.33 bc
Penicillium sinaicum	3.20	5.07	6.43	7.00	5.43 bc
Phoma glomerata	1.90	3.50	4.70	5.90	4.00 d
Ulocladium atrum	1.50	2.00	2.70	3.30	2.38 e
A.Triticina - Control	4.04	8.00	8.50	9.00	7.39 a

***Mean in the same column with different lowercase letters are significantly different in one group p 0.05**

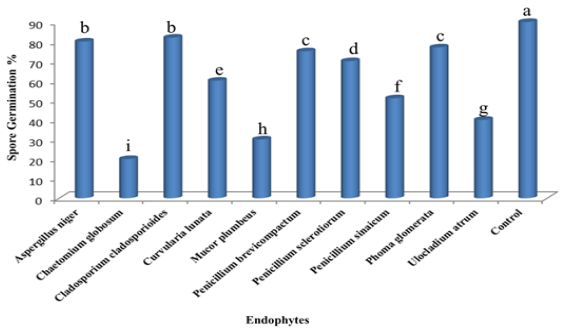

Fig. 1. Spore germination of A. triticina in the presence of each on nine endophytes.

In the same context, the microscopic examination of the spores showed that the endophytes affected the external appearance of the treated spores, and caused the appearance of distortions of the spores swollen and twists in the germination tubes, in addition to the shortening of the germination tubes compared to the untreated spores. All these alterations were not showed in the control cultures.

Greenhouse experiment

The application of endophytes in plant disease management is promising as an alternative to chemical pesticides, the extraction as bicomponant of endophytes suppresses the fungal pathogen growth under laboratory conditions, in this experiment the endophytes were applied on wheat plants under greenhouse conditions to evaluate and ensure the effect of testes endophytes against *A. triticina*. The results in table 3 are in harmony with in vitro antifungal results. The endophytic *Chaetomium globosum* showed the lowest percentage of percentage leaf area diseased (19.78%) followed by *Mucor plumbeus* (23.13%) with a relative increase of about 18.67 and 15.32% respectively as compared to control. There were significant differences between the reduction effects of *Chaetomium globosum* and *Mucor plumbeus* of wheat plants.

Table 3. Effect of endophytes in severity of leaf blight caused by *A. triticina* evaluated on three wheat leaves in greenhouse

	Mean percentage leaf area diseased			
	First leaf	Second leaf	Third leaf	Mean
Chaetomium globosum	44.86	12.23	2.24	19.78 e
Mucor plumbeus	49.55	16.33	3.50	23.13 d
Ulocladium atrum	58.42	22.50	5.50	28.81 c
Penicillium sinaicum	60.00	32.50	5.80	32.77 b
Control	78.25	28.47	8.64	38.45 a

Wheat samples treated with endophytes *Chaetomium globosum*, *Mucor plumbeus*, *Ulocladium atrum*, and *Penicillium sinaicum* showed higher contents of total chlorophyll and carotenoids as shown in fig. 2.

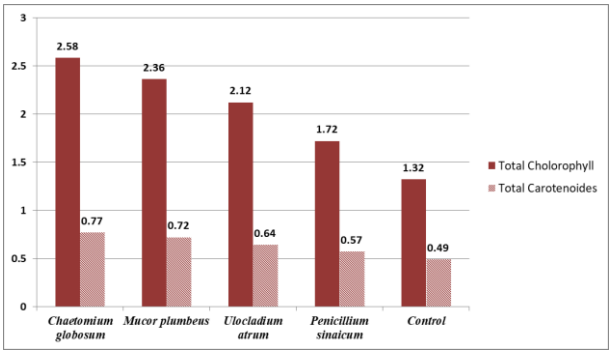

Fig. 2. *In vivo* effect of endophytes on total chlorophyll and total carotenoids

The total chlorophyll content in wheat treated with *Chaetomium globosum* and *Mucor plumbeus* significantly increased with 12.6 % and 10.4% over control. Also the total content of carotenoids were increased by 28% and 23% over control. Various studies stated that *Chaetomium globosum*, *Mucor plumbeus*, endophytes affect the biocomponent of plants and inhance the defense system against pathogens and increased quality parameters and yield of many crops (Schulz and Boyle 2005; Bomke *et al*., 2008). In this study the tested endophytes increased the content of photosynthetic pigments chlorophyll and carotenoids, that may be because plant endophytic fungi produced many natural bioactive compounds with dominant importance in plant health (Schulz and Boyle, 2005;; Khan *et al*., 2012). More than 200 compounds have been reported from *Chaetomium* genus. Endophyte *C. globosum* has been isolated from various economically important plant. From C. globosum a verity of bioactive secondary metabolites have been isolated and identified as reported by Qin *et al*., 2009; Li *et al*., 2011.

Table 4. Effect of endophytes on plant growth parameters in Misr-1 wheat cultivar under greenhouse conditions

Treatment	Plant growth parameters					
	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Shoot length (cm)
Chaetomium globosum	4.00	2.90	19.80	8.63	17.14	77.50
Mucor plumbeus	3.80	2.70	17.50	8.20	15.93	74.35
Ulocladium atrum	3.40	2.23	16.22	7.50	13.84	78.22
Penicillium sinaicum	3.00	1.90	13.22	4.50	12.50	69.45
Control	2.50	1.27	12.14	2.92	11.30	65.00
LSD	0.941	0.23	0.293	0.125	0.116	4.08

As a result of reducing disease incidence and severity by endophytes, a sifnificant increase in all plant growth parameters as shown in table 4. The endophytes *Chaetomium globosum* , *Mucor plumbeus*, *Ulocladium atrum*, and

Penicillium sinaicum significantly increased fresh and dry root weight, fresh and dry shoot weight as well as root and shoot length. The most effective endophytes were *Chaetomium globosum* and *Mucor plumbeus* where recorded Root fresh weight and Root dry weight 4.00 and 3.80 g respectively compared to control 2.50 g. while recorded the values of Root dry weight 2.90 and 2.70 compared to control 1.27 g., in the same direction, *C. globosum* increased shoot fresh weight and shoot dry weight by 7.63 and 5.71 g compared to control.

In current study, the *C. globosum* and and *Mucor plumbeus* have significantly increased allied growth characteristics and the shoot growth of wheat plants. The plant had higher chlorophyll content, shoot biomass compared to control, indicating impacts of growth on plants. In endophyte host symbioses also secondary metabolites may be a contribution of the endophytic fungi. Treated plants with endophytes are healthier than control, these results in harmony with the findings of (Schulz and Boyle, 2005; Hamayun *et al*., 2010; Khan *et al*., 2011), which may be attributed to the endophyte secretion of phytohormones such as IAA and GAs (Bomke *et al*., 2008). (Naz and Bano (2012) reported that, the practical applications of endophytes as prospective sources of bioorganic nutrients and as biocontrol agents can significantly improve yields in ecological friendly method. Understanding the endophytic interactions can help to improve the productivity and quality of economical crops.

CONCLUSION

Endophytic fungi such as *Chaetomium globosum* , *Mucor plumbeus*, *Ulocladium atrum*, and *Penicillium sinaicum* can use against plant pathogenic fungi *A. triticina*, to reduce the using of chemical pesticides which show higher toxicity to environment and humans. It can say that the tested endophytes are beneficial to the health of plants. The tested endophytes in this study are easily obtained from different species of plant as the previous studies reported, so these endophytes also are easily subjugated as fungicides foliar. It can conclude that *Chaetomium globosum* , *Mucor plumbeus* are essential research area that deserves all attention due to their potential application to control of *A. triticina* on wheat after completing the biochemical and environmental studies needed to confirm their efficiency on a larger scale.

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النشاط التضادي للفطريات الداخلية Endophytes ضد فطر *Alternaria triticina* وتأثيراتها المحتملة كمحفزات نمو فى القمح

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يهتم البحث بالحصول على عزلات فطرية داخلية جديدة غير ممرضة، واختبار قدرتها على تثبيط نمو الفطر *Alternaria triticina* المسبب لمرض اللفحة في القمح، ودراسة تأثيرات هذه الفطريات الداخلية كعوامل محفزة لنمو نباتات القمح. في هذه الدراسة تم عزل بعض عزلات من الفطريات الداخلية من أنواع مختلفة من النباتات البرية في محمية سانت كاترين وتم تقييمها تحت ظروف المعمل لاختبار قدرتها التثبيطية لنمو الفطر *Alternaria triticina*، حيث تم الحصول على ٧٢ عزلة من الفطريات الداخلية من الأنسجة الداخلية للنباتات البرية وتقييم قدرتها على تثبيط نمو المسبب المرضي، حيث أظهرت ١٧ عزلة منها فاعلية في تثبيط فطر *Alternaria triticina*. وتم تقييم وفحص العزلات الأكثر فاعلية في المختبر لتأكيد الفاعلية حيث أثبتت ١٠ عزلات تنتمي إلى أنواع فطرية مختلفة فاعلية تضادية لنمو المسبب المرضى، وكانت هذه الفطريات الداخلية هي *Aspergillus niger*، *Chaetomium globosum*، *Cladosporium cladosporides*، *Curvularia tunata*، *Mucor plumbeus*، *Penicillium brevicompactum*، *Penicillium sclerotiorum glomerata*، *Ulocladium atrum* من النتائج المتحصل عليها تم اختيار أفضل ٦ عزلات تحت ظروف الصوبة حيث بينت أن من بينها ٤ عزلات فطرية من النوع *C. globosum*، *Mucor plumbeus*، *Penicillium sinaicum*، *Ulocladium atrum* هى الأكثر فاعلية في تثبيط فطر *Alternaria triticina* والتقليل من الإصابة. كما أوضحت الفطريات الداخلية أيضًا فاعليتها فى تحفيز نمو نباتات القمح مثل ارتفاع النبات والوزن الرطب والجاف مقارنة بالنباتات غير المعاملة.