Chemical and Biological Control of Pathogenic Fungi Associated with Imported Potato Tubers

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ABSTRACT: Potatoes are a staple crop in Egypt and around the world, contributing significantly to human nutrition. However, during handling, transportation, and storage, potato tubers are particularly vulnerable to a variety of fungal diseases. Two pathogenic aggressive isolates associated with potato tuber seeds (*Solanum tuberosum* L.) fungi namely *Fusarium culmorum* and *Lasiodiplodia theobromae* were obtained from the fungal collection established by the authors and tested in the present study. Their identification was confirmed based on cultural, morphological, and microscopic characteristics and molecular phylogenetic analysis. To control these isolates in Egypt, the research aims to determine the efficacy of some specific chemical fungicides, such as Moncut®, Tazolen®, and Divide®, as well as the efficacy of biocontrol agents, such as Bio Zeid®, Plant Guard®, and Bio Arc®, both *in vitro* and *in vivo*. All the tested fungicides and biocides significantly decreased the *in vitro* growth of *F. culmorum* and *L. theobromae* to different degrees. However, the highest colony growth inhibition was presented by fungicides Tazolen® and bioagent Bio Zeid®. Meanwhile, the results obtained under natural environmental conditions supported the *in vitro* results as the disease severity percentage was decreased, and reduction percentages were decreased due to using each of the tested treatments, but to varying degrees. It was evident that Bio Zeid® was superior to all the other treatments and increased all plant parameters compared to untreated control.

Keywords: *seed potato tubers* **(***Solanum tuberosum L.*), *Lasiodiplodia theobromae*, *Tazolen®*, *Bio Zeid®*

INTRODUCTION

Globally, potatoes are among the most significant crops. A plant that belongs to the Solanaceae family is the potato. Approximately 5,000 different types of potatoes exist in the world **(Moussa and Shama 2019)**. Potato (*Solanum tuberosum* L.) is one of the world's most important non-grain food crops, crucial to human sustenance **(Getu *et al.*, 2023).** Also, are among the most popular and widespread food crops all over the world including in Egypt (**Hamed, 2020). Hamed, (2020)** recorded that in recent years, Egypt's in importing cultivating potato tubers has substantially expanded. While the import coverage time for domestic consumption of potatoes increased from 9.6 days in 1995 to 17.9 days in 2018, the production adequacy period for domestic consumption of potatoes declined from 425 days in 1995 to 406.6 days in 2018. Because potato tubers contain more than 70% water, handling, transportation, and storage during harvest can cause galls, blemishes, and one of the most significant illnesses affecting potatoes is fusarium dry rot, which affects both the seed pieces after planting and the tubers while they are in storage. In temperate regions, *Fusarium sambucinum* and *F. solani* are frequent infections that cause dry rot in stored tubers (**Aydın and İnal 2018).** *F. tricinctum, F. avenaceum, F. oxysporum, F. solani, F. acuminatum, F. equiseti F. solanim* in Northwest of China, Qinghai Province, and *F. moniliform, F. redolens* in South of China Zhejiang Province (**Wang *et al.*, 2020**). "Dry rot" or "stem-end rot" is a disease that potato tubers can contract due to the fungus *Botryodiplodia theobromae*. It is a widespread issue in potato crops across the globe that can result in large yield and quality losses. (**Mello *et al.*, 2020).** Numerous fungus species, including *Fusarium* spp. and *Botryodiplodia theobromae*, have been linked to potato rotting. The tubers become infected with these fungus in the pre-harvest stage when they are still in the soil, as well as through openings and physical damage sustained by the tubers during harvesting. The full manifestation of the infection occurs during storage. **(Salami and Popoola, 2007 and Ogunsola and Aduramigba-Modupe 2014)** Infections of tubers can occur through wounds or natural openings like lenticels, as *Fusarium*

culmorum and *Botryodiplodia theobromae* thrive in soil and plant debris. Growth, harvesting, and storage are all potential times for infection. Warm weather promotes the growth of disease (**Aydin, 2019, Mello *et al*., 2020, and Huda-Shakirah *et al*., 2022 and Xue *et al*., 2023**).Significant losses are seen as a result of a variety of circumstances during potato planting and storage. Given the losses they generate, storage diseases, which are brought on by fungal pathogens, play a significant role in potato production (**Yikilmsoy and Tosun 2021**).

Over the last few decades producers are becoming increasingly reliant on fungicides such as Moncut®, Tazolen® and Divide for disease control (**Rosenzweig *et al*., 2008, Siddique *et.al*., 2016, and Mahmoud *et al*., 2018,).** Fungicide application isn't always effective in addition to the environmental contamination that comes with using agrochemicals to control plant diseases (**Gikas *et al*., 2022).** Creating novel approaches to prevent fungal diseases is crucial to achieving efficient and long-term agricultural productivity **(Garvey *et al*., 2022, and Ilyas *et al*., 2023).**

An alluring substitute for the use of fungicides or the management of plant diseases without the drawbacks of chemical control is biological control, which includes the employment of microbes or their antibiotics. According to **Baysal-Gure and Kabir (2018)** and **Lahlali *et al.* (2022)**, these bioagents competitively colonize plant sections, promote growth, and/or lessen the frequency of plant illness, like Bio Zeid®, Plant Guard® Bio Arc® (**Mohamed and Taha, 2017**, **Mahmoud *et al*., 2018, Sarhan 2020 and Al-Mansoury and Salih 2022**).Biological control is a very helpful strategy for managing diseases, and it is very important for creating an environmentally friendly atmosphere. Biological control is crucial for controlling plant diseases without harming wildlife or flora, and it also improves soil fertility **(Ghorbanpour *et al.*, 2018).** Biological control which relies on using microorganisms to suppress pathogens infecting potato tubers offers an alluring substitute. Numerous fungal biocontrol agents have been employed in the management of plant diseases, with the Trichoderma group showing promising results against tuber pathogens like *F. sambucinum* **(Aydin 2019**). Trichoderma spp. is one of the most commonly used antagonists in biological control, by the source of enzymes that break down cell walls pathogen, as a biocontrol agent boosting crop yield, encouraging plant growth, increasing nutrient availability, and strengthening disease resistance **(Mejdoub-Trabelsi *et al*, 2020).** Several commercial bioagents have been reported to control in laboratories, greenhouses, and fields such as Bio-Zeid® (**Shaaban *et al*., 2022).**

The goal of this paper is to determine the efficacy of certain chemical fungicides, and the efficacy of biocontrol agents (*in vitro* and *in vivo*) for controlling some imported seed potato tubers (*Solanum tuberosum* L.) fungal diseases in Egypt.

MATERIALS AND METHODS

All experiments were conducted in the laboratory and under natural environmental conditions in “Research Branch, Plant Pathology Research Institute, Ornamental, Medicinal and Aromatic Plant Diseases Research Department, El-Sabihia Agricultural Research Station Alexandria” from 2019 to 2022.

1.Morphological and molecular characterization of the tested isolates

Two pathogenic aggressive isolates associated with seed potato tubers (*Solanum tuberosum* L.) fungi namely *Fusarium culmorum* and *Lasiodiplodia theobromae* were obtained from fungal collection established by the authors in the "ARC, Sabihia Agricultural Research Station Alexandria". These two isolates per previously isolated from recovered from imported potato tubers seeds showed dry rot symptoms of the surface of tuber's outside has Brown or black dark depressions, which may evolve and spread inward turn into wrinkle then the dead tissue beneath dries out. Additionally, the fungi may cause tubers to wilt and mummify. Their identification was conducted based on cultural, morphological, microscopic characteristics. Then, in the present study, for the molecular phylogenetic analysis in “Assiut University's Molecular Biology Research Unit”, cultures extracted DNA using a Patho-gene-spin DNA/RNA extraction kit that was donated by the Korean company Intron Biotechnology. After that, fungal DNA samples were sent to SolGent Company in Daejeon, South Korea, for 18S sequencing and polymerase chain reaction (PCR). The reaction mixture contained ITS1 (forward) and ITS4 (reverse) primers, which were used for PCR **(Moore *et al*., 2011).** Primers include ITS1 (5'-TCC GTA GGT GAA CCT GCG G - 3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC -3') that have been using universal primer pairs. The same primers were used to sequence the amplified PCR products (amplicons), but ddNTPs were added to the reaction mixture (**White *et al*., 1990)**. By obtaining sequences of the amplified regions and utilizing "the Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov)", the isolate's identity was verified. The alignments were carried out using MegAlign (DNA Star) version 5.05 for Molecular Evolutionary Genetics Analysis. The fungal strain's ITS sequences were used to identify the phylogenetic tree which was then aligned with

closely related sequences obtained from the GenBank.

2.*In vitro* effect of certain fungicide on *Fusarium culmorum* and *Lasiodiplodia theobromae*

Three fungicides were tested in the laboratory to study their effectiveness against two fungi isolates. Systemic and contact fungicides were used throughout this study as prepared diluted solutions. Names of fungicides (commercial and/or common, and chemical), manufacture source, chemical combinations structure, and application are listed in **Table (1)**. The tests were conducted using three fungicides Moncut®, Tazolen®, and Divide® recommended doses **(Table 1.)** *in vitro* to evaluate their efficacy against the most pathogenic fungi of the seed potato tubers fungi (**Siddique *et.al*., 2016, and Mahmoud *et al*., 2018,)**. Fungicides were tested for their antagonistic potential against *F. culmorum* and *L. theobromae* using a double culture assay with solid PDA plates according to **Rosli *et al*. (2020**). Every PDA plate was split in half, and each half was inoculated on one side with a mycelial disc (5-mm diameter) taken from the margins of the actively growing *F. culmorum* and *L. theobromae* of 7-day-old PDA cultures, keeping a distance of one cm from the plate's edge from opposite sides. Similarly, a 5-mm-diameter filter paper disc impregnated with individual each fungicide separately was placed on the opposite side of each plate, one centimeter from the plate edge (**Rios-Velasco *et al*., 2016 and Abdel-Rahman *et al*., 2023**). It used a negative control by comparing it to the untreated inoculated control. Five replicates of every treatment were created to assess each fungicide. All plates were incubated at 27±1 ºC until untreated control mycelia had just totally covered the plates. At that time, radial growth in plates for each treatment was measured according to **Rosli *et al*. (2020)** and **Abdel-Rahman *et al*. (2023)**. This was done by computing the percentage of radial growth reduction in diameter mycelia of *F. culmorum* and *L. theobromae*.

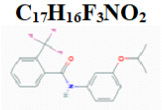
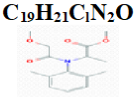
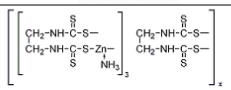
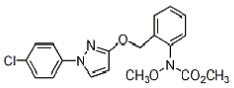
$$\text{Inhibition}\ (\%) = \frac{U-T}{U} \times 100$$

Where :

U= Mean radial growth diameter of pathogen mycelia untreated (cm)

T= Mean radial growth diameter of the treated pathogen mycelia toward the bioagents and/or fungicides (cm)

Table 1. Fungicide, their commercial name, formulation, source, recommended doses, common name, chemical name and chemical structure

Commercial name formulatio (Source)	Recommended doses (Common name)	Chemical name	Chemical structure
Moncut® 25% WP (Shoura chemicals)	2g/l (Flutolanil)	-(3-propan-2-yloxyphenyl)-2-(trifluoromethyl)benzamide	$C_{17}H_{16}F_3NO_2$ 
Tazolen® 72% WP (Elhelb Group)	2.5gm/l (Mancozeb and metaloxyl)	zinc; manganese (2+); N-[2-(sulfidocarbothioylamino)ethyl] carbamodithioate and methyl 2-(N-(2-methoxyacetyl)-2,6-dimethylanilino) propanoate	$C_8H_{12}MnN_4S_8Zn$  and $C_{19}H_{21}C_lN_2O$ 
Divide® (60%) is consisted of metiram and (55%) and pyraclosstrobin (5%) WP (Shoura chemicals)	2g/l metiram (New Zealand, JMAF); métirame zinc (France and pyraclostrobine ((*f*) F-ISO); pyraclostrobin (BSI, E-ISO)	(IUPAC): zinc ammoniate ethylenebis(dithiocarbamate)-poly(ethylenethiuram disulfide) and (IUPAC): methyl *N*-{2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]phenyl}(*N*-methoxy)carbamate	 and 

3. *In vitro* evaluation of the bioagents against *Fusarium culmorum* and *Lasiodiplodia theobromae*

Three biological control were tested in the laboratory to study their effectiveness against two fungal isolates. Biological control solution were preparated to concentrations with sterile distilled water at their recommended doses **Table (2).** The tests were using three biological *i.e.*, Bio Zeid® (*Trichoderma album*), Plant Guard® (*Trichoderma harzianumin*), and Bio Arc® (*Bacillus megaterium*) recommended doses **(Table 2.)** *in vitro* to evaluate their efficacy against the most pathogenic fungi of the seed potato tubers for their antagonistic potential against *F. culmorum* and *L. theobromae* using a double culture assay

with solid PDA plates, and radial growth in plates for each treatment was measured as mentioned above (**Rios-Velasco *et al.*, 2016, Rosli *et al.*, 2020, and Abdel-Rahman *et al.*, 2023**).

Table 2. Biocides, their commercial name, composition and concentration, and source

Commercial name	Composition	Concentration	Source
Bio Zeid®	*Trichoderma album*, 25×10^6 cfu/ml	2.5g/l	Organic Biotechnology, Cairo
Plant Guard®	*Trichoderma harzianum*, 30×10^6 cfu/ml	4ml/l	Agriphar S.A., Belgium
Bio Arc®	*Bacillus megaterium* 25 x 10^6 cell/g	2.5g/l	Organic Biotechnology, Cairo

4.The *in vivo* evaluation of the efficacy of the fungicides and bioagents

Potato tubers seeds varieties Spunta cultivars were obtained from, tuber seeds imported from several different countries To Egypt during the 2018/2019 season The tested tubers were of uniform size from ostensibly healthy tuber seed (Tuber sizes 28/55 mm).

Based on the results of laboratory experiments, the fungicide and biocidal that gives the highest efficiency rate among all treatments is selected and carried out to control aggressive pathogenic isolate of the tested fungal isolates. The tested pathogenic isolate was grown in pure cultures (PDA) at 27±2 ºC to prepare inocula. One 5-mm diameter mycelial disc was removed from the edges of the colony growth on plates after 7 days, and it was placed onto 75 and 25 g of pure sterilized sorghum sand medium, which were made from sorghum and finely washed sand, respectively, and 50 ml of tap water, in 200 ml glass bottles (**Muhanna 2020**) and incubated for 20 days at 27±2 ºC (**Mohamed and Taha, 2017**) 30 cm-diameter plastic pots were used after being sterilized with Clorox (5% sodium hypochlorite), inverted and given two days to dry. Sterilized soil (5 kg/pot) consisting of a 1:1 w:w mixture of sand and clay was placed into the sterilized pots. Before being used, the sand and clay were autoclaved for 30 minutes at 121°C, and they were then allowed to dry for two days (**Abdel-Rahman, 2021**).

All required horticultural precautions were taken before planting, and were placed under natural environmental conditions, and irrigated regularly throughout the entire trial **(Moussa and Shama 2019)**. Then 2 days prior to planting, each soil in a 5-kg plastic pot was pre-infected with pathogenic isolate at a rate of 3% (w/w). Just before planting, potato seeds cv. Spunta cultivars were submerged in each treatment of fungicide and/ or Biocide for 20 minutes at their recommended doses, while control was sterile distilled water. Four weeks after the first sowing, treatments were administered once more using irrigation water. Three replications of each treatment were made, and each replicate consisted of 5 pots.

After 75 days of planting, some results were recorded related to the manifestations of vegetative growth, which include: The height of the plant (in centimeters) from ground level to the highest peak within the shoot, the number of main stems, number of branches, and number of leaflets for each plant. The percentage of infected plants (DI) was also recorded according to (**Thongkantha *et al.*, 2008, and Morang, *et al.*, 2012)**, as follows:

$$\text{Percentage of infection plants} = \frac{\text{No. of infected plants}}{\text{Total No. of examined plants}} \times 100$$

According to **Abd El-Zaher *et al*. (2005), and Raju and Naik (2007)** Disease severity was estimated, with the following ratings: 0= no visual infection (the plants are fully healthy); 1= there are slight infection by a few scattered rotten spots covering less than 25% of the plants area; 2= there are moderately rotten spots covering up to 50% of the plants area; 3= there are heavily rotten spots covering up to 75% of the plants area; and, 4= there are rotten spots covering up to 100% of the plants area (the plants is completely decayed).

$$\text{Percentages of severity (Disease index)} = \frac{\sum (nr)}{4N} \times 100$$

Where: Σnr = Total number of plants under scale degree X scale degree.

4N = Scale degrees (4) X total number of plants tested.

Furthermore, the percentage of reduction of disease by (percentage of disease severity) was calculated (**El-Sersawy *et al.*, 2022).**

$$\text{Reduction (\%)} = \frac{\text{Diseases severity \% of control - Diseases severity \% of treatment}}{\text{Diseases severity \% of control}} \times 100$$

Also, percentage of treatment efficiency (TE %) by (percentage of infected plants "DI %")

was determined as described by **Farag *et al.* (2018),** were computed utilizing the subsequent formula:

$$\text{TE \%} = \frac{\text{DI\% in control} - \text{DI\% in treated}}{\text{DI \% in control}} \times 100$$

Statistical analysis

For every treatment, a randomized full block design was employed with three replications. Using the Statistix programme, the gathered data were statistically examined. and means comparisons, in accordance with **Snedecor and Cochran (1989)**, were carried out at the 5% level using the least significant difference (LSD).

RESULTS

1.Morphological and molecular characterization of the recovered isolates of those associated with Potato tubers seeds **(*Solanum tuberosum* L.)**

The Two tested isolates of *Fusarium culmorum* and *Lasiodiplodia theobromae* were analysed at the molecular level for further identification. The tested strain showed 100% identity and 100% coverage with several strains of *F. culmorum*, AUMC 15122 and *L. theobromae*, AUMC 15123 accessed from the GenBank **(Figure 3. & 4.)**. The fungus was putatively identified as *F. culmorum* (AUMC 15122) and *L. theobromae* (AUMC 15123) "GenBank accession No. OL454806, and MZ715017 respectively". Also, the phylogenetic tree identified showed that based on ITS sequences of rDNA of the fungal sample isolated in the present study (*F. culmorum* AUMC15122, arrowed) aligned with closely related strains accessed from the GenBank. This strain showed 100% identity and 99% -100% coverage with several strains of the same species, GenBank accession no. OL454806 (541 letters) "https://www.ncbi.nlm.nih.gov/nuccore/OL454806" **(Figure 3.)** Also, the Phylogenetic tree based on ITS sequences of rDNA of the fungal strain isolated in the present study (*L. theobromae* AUMC15123) aligned with closely related sequences accessed from the GenBank. *L.* = *Lasiodiplodia*. The tested strain showed 100% identity and 100% coverage with several strains of *L. theobromae* accessed from the GenBank, GenBank accession no. MZ715017 (525 letters) "https://www.ncbi.nlm.nih.gov/nuccore/MZ715017" **(Figure 4.).**

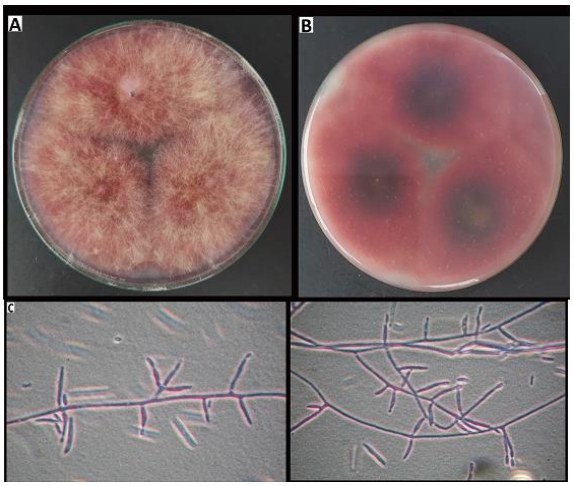


Figure 1. Cultural and morphological characteristics of the tested isolate associated with potato tubers seeds **(*Solanum tuberosum* L.) of *Fusarium culmorum* (AUMC 15122), Colony on PDA after ten days, A. top surface, B. lower surface, and C. conidiophores and Conidia**

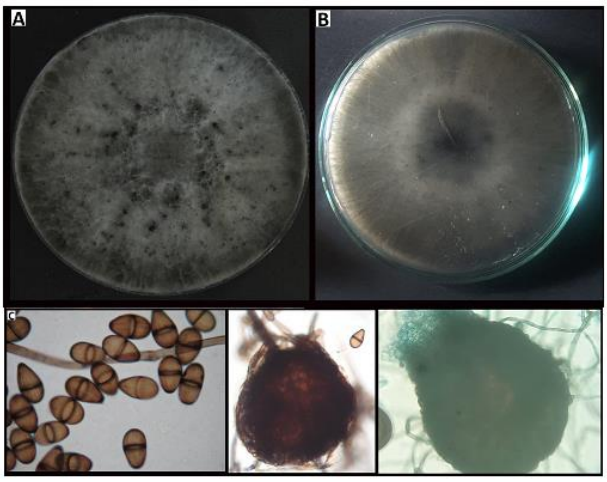


Figure 2. Cultural and morphological characteristics of the tested isolate associated with potato tubers seeds (Solanum tuberosum L.) of Lasiodiplodia theobromae (AUMC 15123): Colony on PDA after ten days, A. top surface, B. lower surface, and C. Conidia

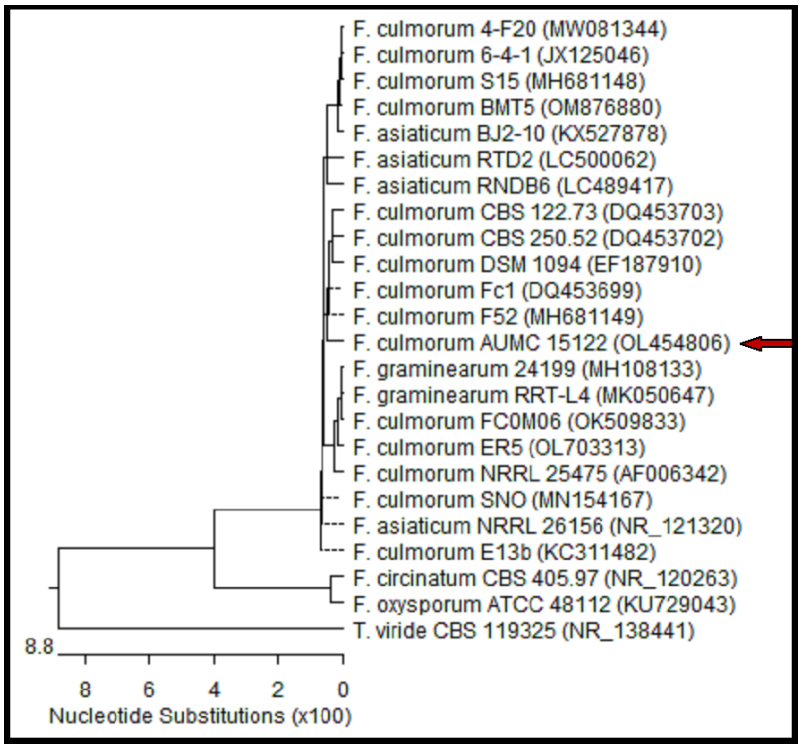


Figure 3. Phylogenetic tree based on ITS sequences of rDNA of the fungal isolate tested in the present study (*Fusarium culmorum* AUMC15122, arrowed) aligned with closely related strains accessed from the GenBank. This strain showed 100% identity and 99% -100% coverage with several strains of the same species, GenBank accession no. OL454806 (541 letters) (https://www.ncbi.nlm.nih.gov/nuccore/ OL454806**)**

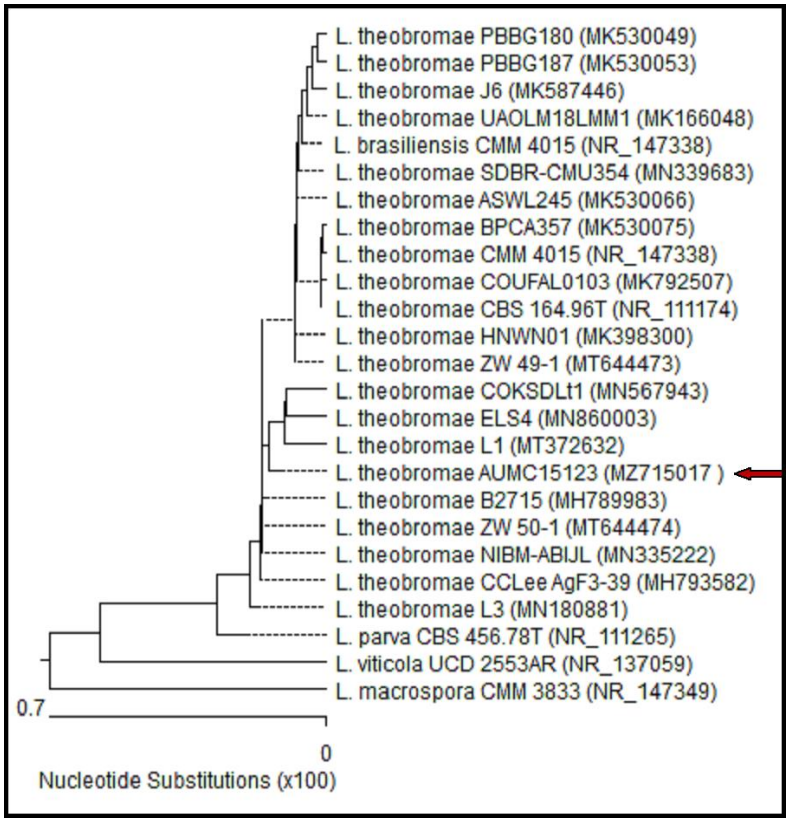


Figure 4. Phylogenetic tree based on ITS sequences of rDNA of the fungal isolate tested in the present study (*Lasiodiplodia theobromae* AUMC15123) aligned with closely related sequences accessed from the GenBank. *L.* = *Lasiodiplodia*. The tested strain showed 100% identity and 100% coverage with several strains of *L. theobromae* accessed from the GenBank, GenBank accession no. MZ715017 (525 letters) (https://www.ncbi.nlm.nih.gov/nuccore/ MZ715017**)**

2.*In vitro* effect of certain fungicide on *Fusarium culmorum* and *Lasiodiplodia theobromae*

In vitro effect of fungicides Moncut® (2g/l), Tazolen® (2.5g/l), and Divide® (2g /l) on colony growth of *F. culmorum* and *L. theobromae* is shown in Table (3). The tested fungicides obviously suppressed the colony diameter of *F. culmorum* and *L. theobromae* the tested to different degrees **(Fig. 5).** Tazolen® fungicide followed by Moncut® (2g/l) proved to be the most effective. Their means of inhibition of colony diameter were (**56.00** & **48.00 %**), and (**35.55** & **28.44 %**) for *F. culmorum* and *L. theobromae* respectively **(Table 3).**

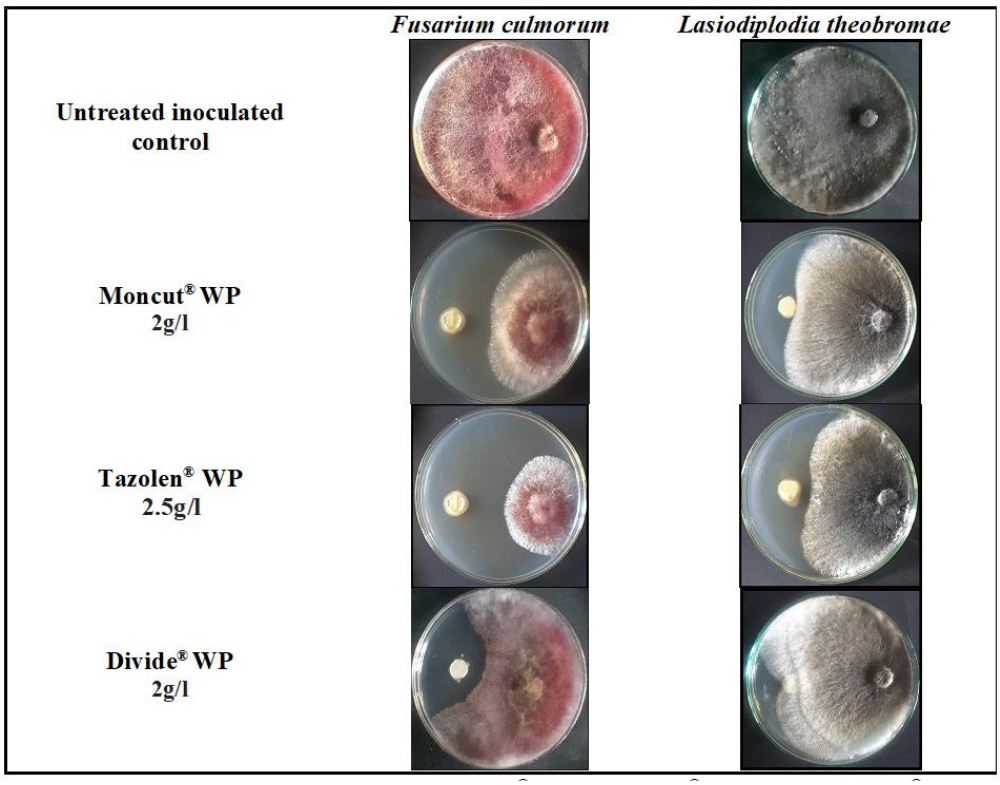


Figure 5. Direct evaluation fungicide Moncut® (2g/l), Tazolen® (2.5g/l), and Divide® (2g/l) antagonistic potential against *Fusarium culmo*rum AUMC15122 and *Lasiodiplodia theobr*omae AUMC15123 *in vitro* using double culture plate assay, Cultures were incubated at 26 ± 2°C for,7 days in darkness.

Table (3): The *in vitro* antifungal inhibition of fungicide Moncut® (2g/l), Tazolen® (2.5g/l), and Divide® (2g/l) to control *Fusarium culmorum* AUMC15122 and *Lasiodiplodia theo*bromae AUMC15123 on PDA medium, incubated at 26 ± 2°C for, 7 days in darkness.

Treatment (Concentrations)	Fungi Inhibition %		Mean
	F. culmorum	*L. theobromae*	
Moncut® WP 2g/l	48.00 B	28.44 D	38.22 A
Tazolen® WP 2.5g/l	56.00 A	35.55 C	45.78 A
Divide® WP 2g/l	29.78 CD	06.44 E	18.11 B
Untreated inoculated control	00.00 E	00.00 E	00.00 C
LSD.at 5%	6.6799		10.393

*The values represent the average of five PDA plates per treatment, for each single column, values followed by different letter(s) are significantly different at p=0.05

3.*In vitro* evaluation of the bioagents against *Fusarium culmorum* and *Lasiodiplodia theobromae*

In vitro three bioagents biological *i.e.*, Bio Zeid® (*Trichoderma album*), Plant Guard® (*T. harzianumin*), and Bio Arc® (*Bacillus megaterium*) were evaluated for their antagonistic potential against *F. culmorum* and *L. theobromae* recovered from potato tubers seeds. It is evident in **(Figure 6.)** that all the biological agents tested (Bio Zeid® (2.5g/l) Plant Guard® (4ml/l) Bio Arc® (2.5g/l) have the ability to suppress the growth of *F. culm*orum and *L. theobromae* to different degrees. However, data in **(Table 4.)** showed that the highest *F. culmorum* and *L. theobromae* colony growth inhibition was presented by bioagent Bio Zeid® Trichoderma album, 25×10^6 cfu/ml (62.67 and 51.78 %, respectively). However, the least growth inhibition was recorded by Bio Arc® *Bacillus megaterium*, 30×10^6 Cells /g with (34.22 and 28.22 %) of inhibition of *F. culmorum* and *L. theobromae*, respectively **(Table 4.)**.

4.The *in vivo* evaluation of the efficacy of the fungicides and bioagents against *Lasiodiplodia theobromae*

This trial was under-placed under natural environmental conditions in plastic pots. the fungicide Moncut® WP (2g/l), and Tazolen® WP (2.5g/l), also, biocidal Bio Zeid® (2.5g/l), and Bio Arc® (2.5g/l), which gave the highest efficiency rate among all treatments *in vitro* selected and carried out to control *Lasiodiplodia theobromae* aggressive pathogenic isolate of the most

pathogenic fungi of the Potato tubers seeds (*S. tuberosum* L.). **Table 5.** displays the data obtained, which indicates that all tested treatments achieved a significant reduction in both disease severity percentages, albeit to differing degrees when compared to untreated plants. Treatment with fungicides Tazolen® WP (2.5g/l) and biocides Bio Zeid® (2.5g/l) resulted in the highest activity against *Lasiodiplodia theobromae* tested fungus, compared to untreated control. On the other hand, data in (**Table 5.)** showed that under the effect of Tazolen® and Bio Zeid®, the mean of disease infection, (20.00 and 46.67%, respectively) and severity percentages were (16.67 and 33.33, respectively). However, the least significant impact was due to treatment by Bio Arc® (2.5g/l) at (73.33, and 71.53% respectively) of the mean of disease infection, and severity percentages.

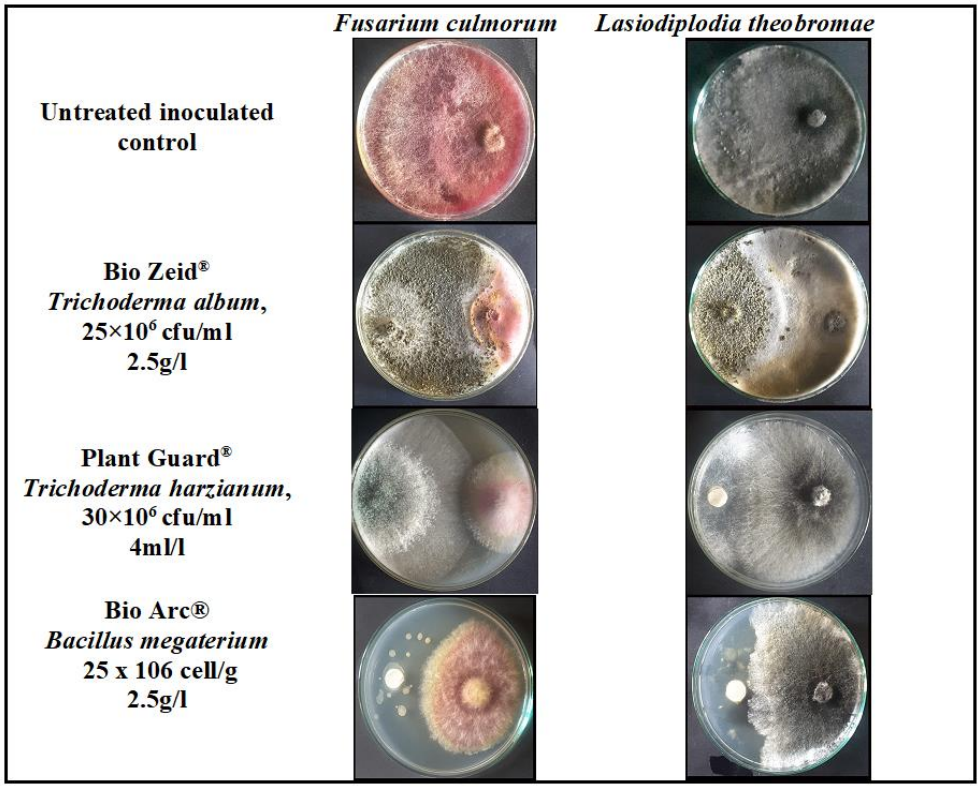


Figure 6. Direct evaluation biological (Bio Zeid® (2.5g/l) Plant Guard® (4ml/l) Bio Arc® (2.5g/l) antagonistic potential against *Fusarium culmorum* AUMC15122, and *Lasiodiplodia theobromae* AUMC15123 *in vitro* using double culture plate assays, Cultures were kept under incubation at 26 ± 2°C for 7 days.

Table (4): The *in vitro* antifungal inhibition of biological by Bio Zeid® Plant Guard®, and Bio Arc® to control *Fusarium culmorum* AUMC15122 and *Lasiodiplodia theobromae* AUMC15123 of potato tubers seeds on PDA medium, incubated in the dark at 27±1 ºC for 7 days

Treatment (Concentrations)	Fungi Inhibition %		Mean
	Fusarium culmorum	*Lasiodiplodia theobromae*	
Bio Zeid® (2.5g/l)	*62.67A	51.78 B	57.22 A
Plant Guard® (4ml/l)	53.78 B	40.00 C	46.89 B
Bio Arc® (2.5g/l)	34.22 D	28.22 E	31.22 C
Untreated inoculated control	00.00 F	00.00 F	00.00 D
LSD.at 5%	**3.8675**		**5.1241**

*The values represent the average of five modified PDA plates per treatment, For each single column, values followed by different letter(s) are significantly different at p=0.05

Table 5. ***In vivo*** **effect of the tested bioagents and certain fungicides and biocides on percentage of disease incidence, and severity caused by artificial infection with** ***Lasiodiplodia theobromae*** **AUMC15123 on potato tubers (*S. tuberosum* L.). cv. Spunta, 77 days after treatment under natural environmental conditions**

Treatment, (Concentrations)	Disease infection (%)	Disease severity %
Moncut® WP (2g/l)	*53.33 BC	47.22 C
Tazolen® WP (2.5g/l)	20.00 D	16.67 D
Bio Zeid® (2.5g/l)	46.67 C	33.33 CD
Bio Arc® (2.5g/l)	73.33 B	71.53 B
Untreated inoculated control	100 A	100 A
LSD.at 5%	26.180	22.962

* The values represent the average of 15 potato tubers /treatment, For each single parameter, values followed by different letter(s) are significantly different at p=0.05

The data presented in **Figure 7.** shows that, all tested treatments resulted in a significant reduction in disease percentages, to differing degrees. Meanwhile, under the effect of Tazolen® followed by Bio Zeid®, the mean of reduction percentage was (83.33 and 66.67%, respectively). However, the least significant impact was due to treatment by Bio Arc® (2.5g/l) at 28.47% of the mean reduction percentages.

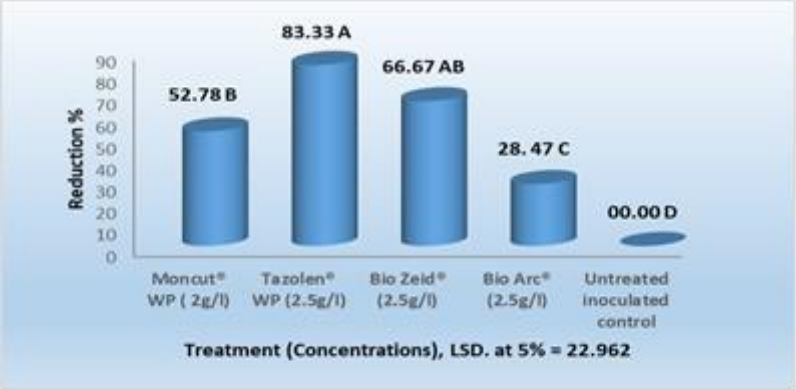


Figure 7. ***In vivo*** **effect of the tested bioagents and certain fungicides and biocides on percentage of reduction of disease severity caused by artificial infection with** ***Lasiodiplodia theobromae*** **AUMC15123 on seeds potato tubers (*S. tuberosum* L.) cv. Spunta, 77 days after inoculation and treatment under were placed under natural environmental conditions**

On the other hand, Fig. 8 showed insignificant differences between treatments Moncut® WP (2g/l), and Bio Arc® (2.5g/l). However, there are significant differences between Tazolen® and Bio Zeid® (2.5g/l) compared to untreated control. Tazolen® WP (2.5g/l) gave the highest effectiveness (80%) followed by Bio Zeid® (53.33%), while Bio Arc® (2.5g/l) was the least effective (26.67%).

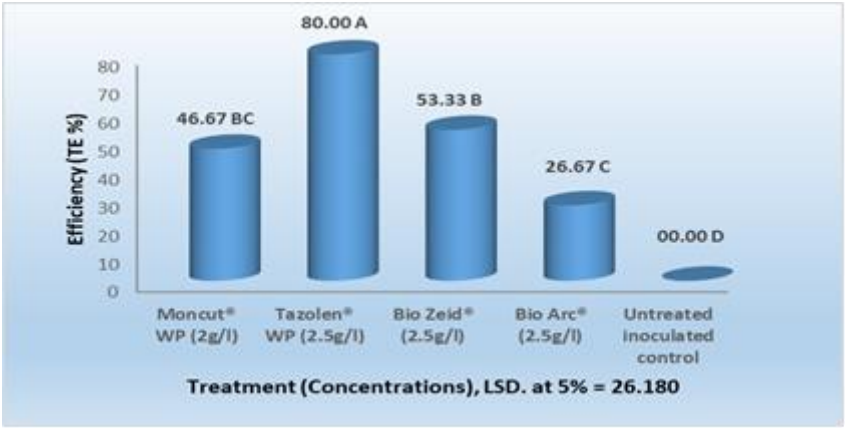


Figure 8. Efficiency percentages of certain fungicides and biocides (applied to seeds potato tubers, and soil) against ***Lasiodiplodia theobromae*** **AUMC15123 placed under natural environmental conditions**

5.Effect on potato growth characteristics

According to data in **Table (6.)**, all the tested fungicides Tazolen® WP (2.5g/l), Moncut® WP (2g/l), and biocides Bio Zeid®, Bio Arc® (2.5g/l) significantly increased all parameters of plants, *i.e.*, Plant height (cm) No. of main stems, No. of

branches, and No. of leaflets, compared to untreated control. It was evident that Bio Zeid® was superior to all the other treatments and increased all plant parameters. Compared to untreated control, the highest values of parameters of plants, *i.e*., Plant height (247, 048 cm) No. of main stems (17, 03), No. of branches (69, 08) and No. of leaflets (119, 020) with Bio Zeid® then untreated control respectively. However, it showed the least effects and insignificant differences between treatments Moncut® WP (2g/l), and Bio Arc® (2.5g/l). Showed the recorded least each all with Plant height (160, 138 cm) No. of main stems (9, 8), No. of branches (30, 27) and No. of leaflets (101, 119), respectively.

Table 6. *In vivo* effect of the tested bioagents, fungicides and biocides on mean percentage of vegetative growth caused by artificial infection with *Lasiodiplodia theobromae* AUMC15123 on seeds potato tubers (*S. tuberosum* L.), cv. Spunta, 77 days after treatment under natural environmental conditions

Treatment, (Concentrations)	Plant height (cm)	No. of main stems	No.of branches	No. of leaflets
Moncut® WP (2g/l)	***160 C**	**09 C**	**30 C**	**101C**
Tazolen® WP (2.5g/l)	**202 B**	**12 B**	**49 B**	**140 B**
Bio Zeid® (2.5g/l)	**247 A**	**17 A**	**69 A**	**231 A**
Bio Arc® (2.5g/l)	**138 C**	**08 C**	**27 C**	**119 BC**
Untreated inoculated control	**048 D**	**03 D**	**08 D**	**020 D**
LSD.at 5%	**27.395**	**3.5724**	**13.651**	**26.162**

***** The values represent the average of 15 plants/treatment, The values represent the average of 15 plants/treatment, For each single parameter, values followed by different letter(s) are significantly different at p=0.05

DISCUSSION

The research aims to at evaluating the efficacy of certain chemical fungicides *i.e.*, Moncut®, Tazolen®, and Divide® and biocontrol agents viz, Bio Zeid® (*Trichoderma album*), Plant Guard® (*Trichoderma harzianumin*), and Bio Arc® (*Bacillus megaterium*) *in vitro* and *in vivo*, for controlling two of the most pathogenic aggressive isolates of those associated with imported potato tubers seeds (**Siddique *et.al*., 2016, and Mahmoud *et al*., 2018, Aydin 2019, Gikas *et al*., 2022, and Shaaban *et al*., 2022)** of fungi namely *Fusarium culmorum* and *Lasiodiplodia theobromae* obtained from fungal collection established by the authors in the "ARC, Sabihia Agricultural Research Station Alexandria".

Potatoes (*S. tuberosum* L.) are considered one of the most important popular crops to the Egyptian people. Pathogen-caused storage diseases are an important and economic serious problem causing severe losses are potato production in Egypt, numerous conditions during the importation, cultivation and storage of potatoes can lead to significant losses. **(Yikilmsoy and Tosun 2021).** Results in the present study showed that it has been determined, to identify and confirm *F. culmorum* and *L. theobromae* isolates based on morphological and microscopic characteristics and Molecular characterization which were matched to strains that were obtained from GenBank and closely related. Several investigators have been reported that *F. culmorum* and *L. theobromae* are a soil-borne phytopathogen fungus and attack seed potato tubers during storage and wide spread in the world including Egypt (**Hammam, and El Damrawy, 2022, and Xue *et al*., 2023).** Soil-borne storage infections, the most dangerous of which are *Fusarium* species, including *F. culmorum* are a problem for post-harvest potato storage. If more than one *Fusarium* species are involved, the damage is always greater **(Tiwari *et al*, 2021)**. Dry patches are formed as a result of spoiling, and while subsequent symptoms might not be seen during a visual inspection, they are far more dangerous. Among these are the rise in sucrose and total soluble sugars and the decrease in the amount of starch and amylose. **(Tiwari *et al*, 2021, and Xue *et al*., 2023).** Also, the fungus *Botryodiplodia theobromae* (*Lasiodiplodia theobromae*) is responsible for the "dry rot" or "stem-end rot" that affects potato tubers. It can result in considerable losses in yield and quality and is a widespread issue with potato crop around the world **(Mello *et al*., 2020).** When potato tubers are infected with *B. theobromae*, they can develop brown or black sunken lesions on their surface. These lesions can begin at the tuber's stem end and progress inside. Additionally, the fungus can cause the tubers to shrivel and mummify **(Mello *et al*., 2020, and Huda-Shakirah *et al*., 2022).** These results are in agreement with some other reports about the effects of different both *F. culmorum* and *Botryodiplodia theobromae* can infect tubers through wounds or naturally occurring openings like lenticels. They can also survive in soil and plant debris. During cultivation, harvesting, or storage, infections can happen. Warm, humid weather encourages the growth of disease **(Aydin, 2019, Mello *et al*., 2020, and Huda-Shakirah *et al*., 2022 and Xue *et al*., 2023).**

For controlling *F. culmorum* and *B. theobromae*, six common fungicides and bio were investigated *in vitro* and *in vivo*. All the tested fungicides and bio significantly decreased the *in vitro* growth of

F. culmorum and *L. theobromae* to different degrees. However, the highest colony growth inhibition was presented by fungicides Tazolen® (2.5g/l) bioagent Bio Zeid® (*Trichoderma album*, 25×10^6 cfu/ml). Meanwhile, the results obtained under natural environmental conditions supported the *in vitro* results as disease severity percentages were decreased, and reduction percentages were decreased due to using each of the tested treatments, but to varying degrees, compared with untreated plants. Also, the highest effect was presented by fungicides Tazolen® (2.5g/l) bioagent Bio Zeid® (*Trichoderma album*, 25×10^6 cfu/ml). Such results are in harmony with those obtained by several foreign authors (**Siddique *et al*., 2016, Mahmoud *et al.,* 2018, Guzmán-Guzmán *et al*., 2018 and Halifu *et al.,* 2019).** Tazolen consisting of Mancozeb functions as a multi-site inhibitor, interfering with several enzymes and fungal metabolic processes **(Siddique *et al*., 2016)**. Metalaxyl functions by obstructing the growth of fungi and preventing the formation of fungal cell walls **(Mahmoud *et al.,* 2018).** Mancozeb and Metalaxyl both provide potent curative effects against a variety of fungal diseases in seed potato tubers, guarding against a broad spectrum of pathogens. **(Siddique *et al*., 2016, and Mahmoud *et al.,* 2018).**

Mancozeb is a contact and protective non-systemic fungicide. Mancozeb affects fungi at several different sites, interfering with their lipid metabolism. It is effective against a variety of pathogens, such as rusts, blights, scabs, and leaf spots on crops like potatoes, because of its multi-site activity. It functions to control fungal infections that are already present on the seed potato tubers because of its curative mode of action. (**Huang *et al*., 2021**). Conversely, metalaxyl functions as both a preventative and a therapeutic systemic fungicide. It works very well against Oomycete fungi, which include the pathogens that cause potato tuber root and collar rot and seedling damping-off. With its ascending system, high lipophilicity, and rapid penetration, Metalaxyl can easily cross waxy membranes, move up the xylem, enter the plant in less than an hour, and remain active for two weeks inside the plant. Because Metalaxyl is absorbed by the leaves, stems, and roots due to its translaminar properties, it is a good option for managing fungal diseases such as mildew. Its fungicide features (**Kankwatsa *et al*., 2003**).

Trichoderma spp. is a type of fungus that provides protection for plant roots by forming a barrier against pathogen attack by removing the used by pathogen nutrients. Meanwhile, secretion of chitinases dissolves the cell wall and creates holes in the pathogen, causing cell wall damage and lysis through the production of chitinase and extracellular-(1-4) glucanase, causing pathogen cell wall damage and lysis. **(Oraghi *et al*., 2011, Leelavathi *et al*., 2014, Guzmán-Guzmán *et al*., 2018, and Halifu *et al.,* 2019)**. The fact that plant bioprotection is accomplished by the synthesis of plant growth regulators, such as gibberellic acid, indole-3-acetic acid, and abscisic acid, by bioagents, explains the tested bioagents' activities. The production of phytohormones has been directly linked to the availability of bioagents, which increase plant growth and lowers disease parameters. **(Mohamed and Taha, 2017, and Mahmoud *et al.,* 2018).** *Trichoderma album* worked by competing with fungi for nutrients and available space, exhibiting mycoparasitism towards the pathogen, and possibly secreting antibiosis. The pathogen's growth was impeded by the antagonist's quick development and competition for nutrients and available space. Due to their high rate of reproduction, effective nutrition uptake, strong aggressiveness against other pathogens, and quick and efficient colonization of wound sites against invasive pathogens, *Trichoderma* species have been used successfully as biological control agents **(Sarhan 2020 and Al-Mansoury and Salih 2022).**

CONCLUSIONS

Potatoes are an important crop worldwide, including in Egypt, and play a significant role in human nutrition. However, potato tubers are susceptible to various fungal diseases, especially during handling, transportation, and storage. We recommend dipping seed potato tubers for 20 minutes. Then four weeks after sowing, treatments were administered once more with irrigation water in the soil by one of the treatments *i.e*., Tazolen® WP (2.5g/l), and Bio Zeid® (2.5g/l).

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الملخص العربى

المكافحة الكيميائية والبيولوجية للفطريات المسببة للأمراض المصاحبة لدرنات البطاطس المستوردة

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تعد البطاطس محصولًا أساسيًا في مصر وفي جميع أنحاء العالم، حيث تساهم بشكل كبير في تغذية الإنسان. ومع ذلك، أثناء النقل والتخزين، تتعرض درنات البطاطس بشكل خاص لمجموعة متنوعة من المسببات المرضية. تم الحصول على اثنتين من أكثر العزلات العدوانية المسببة للأمراض من تلك المرتبطة بدرنات البطاطس (*Solanum tuberosum* L.) وهي *Fusarium culmorum* و *Lasiodiplodia theobromae* من المجموعة الفطرية المعزوله. تم تأكيد هويتهم بناءً على الخصائص المورفولوجية والمجهرية والتحليل الجزيئي. يهدف البحث إلى تحديد كفاءة بعض المبيدات الفطرية الكيميائية مثل مون كت، تازولين، ديفايد، وكذلك كفاءة عوامل المكافحة الحيوية مثل بيو زيد، بلانت جارد، وبيو آرك، وتم دراستهم في المختبر وفي الظروف الطبيعية. جميع المبيدات الفطرية والحيوية التي تم اختبارها أدت إلى انخفاض معنوى بتثبيط نمو *F. culmorum* و *L. theobromae* بدرجات معنوية متفاوتة. ومع ذلك، فإن أعلى تثبيط معنوى للمستعمرة الفطرية بواسطة المبيد الفطرى التازولين والمبيد الحيوي البيو زيد وفى الوقت نفسة أكدت التجربة في ظل الظروف البيئية الطبيعية هذة النتائج باتجاه مشابة للأختبارات المعملية بتاثيرات متفاوتة للنسبة المئوية لكفاءة جميع المعاملات فى مكافحة المرض، وقد ترافق مع ذلك تسجيل البيو زيد ا أعلى قيم معنوية فى معاملات نمو النبات من أرتفاع النبات، عدد السيقان الرئيسية، عدد الفروع وعدد الوريقات مقارنة بالكنترول غير المعامل.