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

Metwally, O.A.O. 1  Abdel-Rahman, T.F.M. 2,  SHAMA, S.2, and AMER, M.3

1. Egyptian Central Administration of plant quarantine (CAPQ) omarquarantine2022@outlook.com
2. Ornamental, Medicinal & Aromatic Plants Div. Res. Dept., Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt. E-mail: tagheeb fekry@ar.scie.eg https://orcid.org/0000-0003-3668-7337
3. Faculty of Agriculture, Saba Basha. University of Alexandria, P.O. Box 21531 Egypt

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 Moncur®, 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 (Aydin and İnal 2018). F. tricinctum, F. avenaceum, F. oxysporum, F. solani, F. acuminatum, F. equiseti F. solani 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

Journal Article © 2023 by (JAAR) is licensed under CC BY-NC 4.0
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®, Tazol® 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 Sallih 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-Trabesi 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-genespin 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 GGA TGC 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 °C 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 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

<table>
<thead>
<tr>
<th>Commercial formulation (Source)</th>
<th>Recommended doses (Common name)</th>
<th>Chemical name</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moncut® 25% WP (Shoura chemicals)</td>
<td>2g/l (Flutolanil)</td>
<td>-(3-propan-2-ylxylophenyl)-2-(trifluoromethyl)benzamide</td>
<td>[Chemical structure]</td>
</tr>
<tr>
<td>Tazolen® 72% WP (El Helb Group)</td>
<td>2.5gm/l (Mancorez and metaloxy)</td>
<td>zinc; manganese (2+); N-[2-(sulfurcarbothioylamino)ethyl]carbamodithioate and methyl 2-[(N-(2-methoxacyetyl)-2,6-dimethylaminolino)propanoate</td>
<td>[Chemical structure]</td>
</tr>
<tr>
<td>Divide® (60%) is consisted of metiram (New Zealand, JMAF); metiram zinc (France) and pyraclostrobin (5%) WP (Shoura chemicals)</td>
<td>2g/l (IUPAC): zinc ammoniate ethylenebis(dithiocarbamate)-poly(ethylene thiuriam disulfide) and methyl N-(2-[1-(4-chlorophenyl)]pyrazol-3-yl)ethyl)methyl]phenyl[N-(methoxy)carbamate</td>
<td>[Chemical structure]</td>
<td></td>
</tr>
</tbody>
</table>

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 prepared 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 harzianum), 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

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Composition</th>
<th>Concentration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio Zed®</td>
<td>Trichoderma album, 25×10⁶ cfu/ml</td>
<td>2.5g/l</td>
<td>Organic Biotechnology, Cairo</td>
</tr>
<tr>
<td>Plant Guard®</td>
<td>Trichoderma harzianum, 30×10⁶ cfu/ml</td>
<td>4ml/l</td>
<td>Agriphar S.A., Belgium</td>
</tr>
<tr>
<td>Bio Arc®</td>
<td>Bacillus megaterium 25 x 10⁹ cell/ml</td>
<td>2.5g/l</td>
<td>Organic Biotechnology, Cairo</td>
</tr>
</tbody>
</table>

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 °C 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 °C (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 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).

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

Where: \( \Sigma \text{nr} = \text{Total number of plants under scale degree X scale degree} \)

\( 4N = \text{Scale degrees (4) X total number of plants tested} \)

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:

\[
\text{TE} \% = \frac{\text{DF}\% \text{ in control} - \text{DF}\% \text{ in treated}}{\text{DF}\% \text{ in control}} \times 100
\]

**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. 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](image-url)
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 culmorum* AUMC15122 and *Lasiodiplodia theobromae* 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 theobromae* AUMC15123 on PDA medium, incubated at 26 ± 2°C for 7 days in darkness.

<table>
<thead>
<tr>
<th>Treatment (Concentrations)</th>
<th><em>F. culmorum</em> Inhibition %</th>
<th><em>L. theobromae</em> Inhibition %</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moncut® WP 2g/l</td>
<td>48.00 B</td>
<td>28.44 D</td>
<td>38.22 A</td>
</tr>
<tr>
<td>Tazolen® WP 2.5g/l</td>
<td>56.00 A</td>
<td>35.55 C</td>
<td>45.78 A</td>
</tr>
<tr>
<td>Divide® WP 2g/l</td>
<td>29.78 CD</td>
<td>06.44 E</td>
<td>18.11 B</td>
</tr>
<tr>
<td>Untreated inoculated control</td>
<td>00.00 E</td>
<td>00.00 E</td>
<td>00.00 C</td>
</tr>
</tbody>
</table>

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. harzianum*), 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. culmorum* 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, 25×10⁶ cfu/ml (62.67 and 51.78 %, respectively). However, the least growth inhibition was recorded by Bio Arc® *Bacillus megaterium, 30×10⁶ 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.

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 °C for 7 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fusarium culmorum</th>
<th>Lasiodiplodia theobromae</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio Zeid® (2.5g/l)</td>
<td>*62.67A</td>
<td>51.78 B</td>
<td>57.22 A</td>
</tr>
<tr>
<td>Plant Guard® (4ml/l)</td>
<td>53.78 B</td>
<td>40.00 C</td>
<td>46.89 B</td>
</tr>
<tr>
<td>Bio Arc® (2.5g/l)</td>
<td>34.22 D</td>
<td>28.22 E</td>
<td>31.22 C</td>
</tr>
<tr>
<td>Untreated inoculated control</td>
<td>00.00 F</td>
<td>00.00 F</td>
<td>00.00 D</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>3.8675</td>
<td>5.1241</td>
<td></td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Treatment, (Concentrations)</th>
<th>Disease infection (%)</th>
<th>Disease severity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moncut® WP (2g/l)</td>
<td><em>53.33 BC</em></td>
<td>47.22 C</td>
</tr>
<tr>
<td>Tazolen® WP (2.5g/l)</td>
<td>20.00 D</td>
<td>16.67 D</td>
</tr>
<tr>
<td>Bio Zeid® (2.5g/l)</td>
<td>46.67 C</td>
<td>33.33 CD</td>
</tr>
<tr>
<td>Bio Arc® (2.5g/l)</td>
<td>73.33 B</td>
<td>71.53 B</td>
</tr>
<tr>
<td>Untreated inoculated control</td>
<td>100 A</td>
<td>100 A</td>
</tr>
<tr>
<td><strong>LSD at 5%</strong></td>
<td><strong>26.180</strong></td>
<td><strong>22.962</strong></td>
</tr>
</tbody>
</table>

* 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
branched, 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 treatment under natural environmental conditions

<table>
<thead>
<tr>
<th>Treatment, (Concentrations)</th>
<th>Plant height (cm)</th>
<th>No. of main stems</th>
<th>No. of branches</th>
<th>No. of leaflets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moncut® WP (2g/l)</td>
<td>*160 C</td>
<td>09 C</td>
<td>30 C</td>
<td>101C</td>
</tr>
<tr>
<td>Tazolen® WP (2.5g/l)</td>
<td>202 B</td>
<td>12 B</td>
<td>49 B</td>
<td>140 B</td>
</tr>
<tr>
<td>Bio Zeid® (2.5g/l)</td>
<td>247 A</td>
<td>17 A</td>
<td>69 A</td>
<td>231 A</td>
</tr>
<tr>
<td>Bio Arc® (2.5g/l)</td>
<td>138 C</td>
<td>08 C</td>
<td>27 C</td>
<td>119 BC</td>
</tr>
<tr>
<td>Untreated inoculated control</td>
<td>048 D</td>
<td>03 D</td>
<td>08 D</td>
<td>020 D</td>
</tr>
</tbody>
</table>

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 harzianum), 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 (Hamnam, 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 amyllose. (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 Tazolene® (2.5g/l) bioagent Bio Zeid® (Trichoderma album, 25x10^6 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 Tazolene® (2.5g/l) bioagent Bio Zeid® (Trichoderma album, 25x10^6 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). Tazolene 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 Oomycte 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 transamplinary 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., Tazolene® WP (2.5g/l), and Bio Zeid® (2.5g/l).

REFERENCES


Al-Mansoury, B.A.R.; and Salih. Y.A. 2022. Evaluation of the efficiency of bio agents...
Trichoderma harzianum and T. longibrachiatum and some fungicides and a chemical compound against the fungus Rhizoctonia sp. that causes eggplant root rot disease in vitro. Euphrates Journal of Agriculture Science. 13 (3): 210-231


Siddique, N.A.;Aktar, M.S.; and Swapon, N.H. 2016. Comparative efficacy of different fungicides against late blight diseases of potato incited by phytophthora infestans (Mont.) de bary and its management. Journal of Plant Pathology & Microbiology. 7 (7):1000364. DOI: 10.4172/2157-7471.1000364


الملخص العربي

المكافحة الكيميائية والبيولوجية للفطريات المسببة للأمراض المصاحبة لدرنات البطاطس المستورة

عمر أحمد عمر متولي 1 تغريد فكري محمد عبد الرحمن 2 سعد محمود شمة 3 مصطفى عبد العظيم 3

الحجر الزراعى المصرى - فريق بحوث أمراض نباتات الزينة والطبية والعطرية - معهد بحوث أمراض النباتات - مركز البحوث الزراعية - جنوب مصر - فريق البحوث الزراعى - كلية زراعة ساباباشا - جامعة الأسكندرية

تعد البطاطس محصولاً أساسياً 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 الأمراض المرضية. تم الحصول على اثنين من أكثر العزلات العدوانية المسببة للأمراض من تلك المرتبطة بدرنات البطاطس (Solanum tuberosum L.) وهي Fusarium culmorum و Lasiodiplodia theobromae، وبعض المبيدات الفطرية الكيميائية مثل مون كت، تازولين، ديفايد، وكذلك كفاءة عوامل المكافحة الحيوية مثل بيو زيد، بلانت جارد، وبيو آرك، وتدرستهم في المختبر وفي الظروف الطبيعية. جميع المبيدات الفطرية والحيوية التي تم اختبارها أدت إلى انخفاض معنوي بدرجات معنوية متفاوتة. ومع ذلك، فإن أعلى تثبيط معنوي للمستعمرة الفطرية بواسطة المبيدات المذكورة في الظروف البيئية، والبيو زيد إجمالاً، وفي الوقت نفسه أكدت التجربة في ظل الظروف البيئية الطبيعية هذه النتائج بإتجاه مشابه، إلا أن اختبارات المبيدات الفطرية في كل من الظروف المختبرية والطبيعية لم تؤدي إلى تثبيط معنوي متفاوت للمستعمرة الفطرية. تم توثيق هذه النتائج بشدة، وتم ملاحظة تأثيرات متفاوتة للفطريات على نمو النباتات، ورغم ذلك، فقد تحقق مع ذلك تسجيل البيو زيد إجمالاً أقل رقم معنوي في معاملات نمو النباتات من أرتفاع النباتات، عند السيناق الرئيسية، عند الفروع في معاملات مقارنة بالكترول غير المعامل.