SMTCT
Society of Land Measurements and Cadastre from Transylvania

Ahmed HAM *et al.* (2024)
Notulae Scientia Biologicae
Volume 16, Issue 2, Article number 11854
DOI:10.15835/nsb16211854
Research Article

NSB
Notulae Scientia Biologicae

Efficiency of bio-agents and green-synthesized silver nanoparticles in controlling purple blotch disease caused by *Alternaria porri* in onion plants

Hoda A.M. AHMED[1]*, Mohamed A.E. HASSAN[2], Mohamed A. HUSSEIN[3], Osama A.M. AL-BEDAK[4]*

[1]*Plant Pathology Research Institute, Agricultural Research Center, Giza, 12511, Egypt; hudafatah@arc.sci.eg (*corresponding author)*
[2]*Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut 71511, Egypt; habatalasamar@agr.aun.edu.eg*
[3]*Department of Plant Pathology, Faculty of Agriculture, New Valley University, New Valley, Egypt; Mmon3m@gmail.com*
[4]*ERU Science & Innovation Center of Excellence, Egyptian Russian University, Badr City, 11829, Cairo, Egypt; osama-albedak@eru.edu.eg (*corresponding author)*

Abstract

One of the most fatal diseases that harm *Allium* species is purple blotch, which is brought on by *Alternaria porri*. As the disease's severity rises, crop production may decrease. In this study, 12 isolates of *A. porri* exhibiting purple blotch symptoms have been isolated from diseased onion plants. Sequencing of the internal transcribed spacer region (ITS) allowed for the identification of the isolate that was the most virulent and caused a disease severity of 85.93%. Under greenhouse and field growing conditions, the efficiency of two bio-agents, *Trichoderma asperellum* T34 and *Saccharomyces cerevisiae* AUMC 10203, as well as three doses (50, 25, and 12.5 ppm) of their green-synthesized silver nanoparticles (AgNPs) against purple blotch disease was assessed. For avoiding or treating purple blotch disease in onion plants, it has been demonstrated that the *T. asperellum* T34 spore suspension was the most effective resulting in a 76% reduction in disease severity. The spore suspension of *S. cerevisiae* and a 50-ppm dosage of AgNPs were the top contenders for *T. asperellum*. When compared to the control plants, the plants treated with bio-agents and AgNPs showed a significant decline in disease incidence and disease severity. Additionally, improvements were made to the broadness of inflorescences, the number of flowers and seeds, the weight of seeds per inflorescence, and the seed productivity. Therefore, it is recommended in this study to use *T. asperellum* T34 spore suspension as a potent bio-agent to manage the symptoms of purple blotch on onions.

Keywords: *Alternaria*; fungicides; onion; *Saccharomyces*; nanoparticles; *Trichoderma*

Introduction

The onion (*Allium cepa* L.), a plant belonging to the Liliaceae family, is one of the most noteworthy and historically significant vegetable crops (Sagar *et al.,* 2021). It is also a highly valuable export commodity that

Received: 27 Dec 2023. Received in revised form: 15 Apr 2024. Accepted: 22 Apr 2024. Published online: 14 Apr 2024.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

generates a sizable income. It is a widely consumed and one of the principal vegetable crops grown in a variety of locations (Schwartz and Mohan, 2007; Kim *et al*., 2022).

A total of 5,192,651 hectares are used to produce 99,968,016 tons of onions worldwide (Oecd, 2022). With an average yield of 13.74 tons per feddan and a total production of 1875.74 thousand tonnes between 2000 and 2018, this crop was grown on a total of around 154.17 thousand feddan in Egypt (Fangary and Adam, 2020). The explanation for the poor output of onions worldwide is complex. The major causes of base rot, purple blotch, downy mildew, *Stemphylium* blight, and storage rots are a variety of diseases (Abdel-Hafez *et al.,* 2014; Meena *et al.,* 2017). There were over 60 fungal infections in onions and garlic, 14 bacterial infections, one yeast infection, six nematode infections, three viral infections, and one infection with a phytoplasma-like organism (Schwartz and Mohan, 2007). In addition to the biggest yearly production and storage losses, the majority of fungal infections, which are widespread throughout the world's onion and garlic-producing regions, may result in considerable crop losses (Abdel-Hafez *et al.,* 2014).

The most damaging of all those diseases to *Allium* spp. (onions, garlic, shallots, leeks, scallions, and chives) is purple blotch, which is brought on by *Alternaria porri*. The production of host-specific or nonspecific toxins by the fungus, which is primarily secondary metabolites, can induce disease and leaf necrosis in cultivars that are vulnerable to it (Mamgain *et al.,* 2013). A plant's leaves and bulbs are usually affected by the disease, which can decrease output by up to 97% (Kareem *et al.,* 2012). Purple blotch disease is exacerbated by high humidity levels of 80-90% and moderate temperatures of 25-30 °C. As a result, the output of onions has been significantly reduced, which has affected exports and increased local prices (Mandal *et al.,* 2022).

Chemical fungicides are widely used to treat plant diseases. On the other hand, the efficiency of chemical control is primarily determined by how often it is administered (Faraz *et al.,* 2022; Rhouma *et al.,* 2023). Additionally, more frequent chemical use results in the emergence of disease-resistant strains and the buildup of residues in products as well as the environment, posing risks to human health, the environment, and non-target species (Gao *et al.,* 2022; Taylor and Cunniffe, 2023). These concerns led to a downturn in pesticide use, which sparked interest in sustainable, eco-friendly alternatives.

Nanotechnology is an entirely novel and fascinating area of science that enables more in-depth research in a variety of fields (Rawat *et al.,* 2022). Nanotechnology advancements might also have dramatic effects on biotechnology and agriculture (Saritha *et al.,* 2022; Shelar *et al.,* 2023). It has long been known that silver and silver ions have strong antimicrobial and antibiofilm characteristics in addition to a variety of antibacterial actions (Huang *et al.,* 2022; Owaid *et al.,* 2022; Tripathi and Goshisht, 2022). Large specific surface area and a large fraction of surface atoms in silver nanoparticles compared to bulk silver metal are expected to improve antimicrobial activity.

Numerous studies have demonstrated that the use of bio-agents, such as *Trichoderma* spp., can inhibit several foliar fungal diseases that harm onions, including *Stemphylium vesicarium* (Özer and Köycü, 2004; Zapata-Sarmiento *et al.,* 2020), *Alternaria porri* (Camacho-Luna *et al.,* 2021), *Alternaria palandui* (Karthikeyan *et al.,* 2008), as well as *Alternaria alternata* and *Botrytis* spp. They have been correlated with several mechanisms for their biocontrol actions, which may be categorized as direct and indirect impacts on the plant pathogen. The synthesis of lytic and antibiotic enzymes, inactivation of the pathogen's enzymes, and parasitism are examples of direct impacts. Other indirect effects include competition for resources or space. Indirect impacts are any factors that cause the host plant to undergo morphological or biochemical changes that result in the development of resistance (Charoenporn *et al.,* 2010).

Currently, yeasts have been used as biocontrol agents for controlling many diseases, including powdery mildews (Ziedan and Farrag, 2011), postharvest decay of tomato caused by *Botrytis cinerea* (Abdel-Rahim *et al.,* 2017), bacterial fruit blotch (de Melo *et al.,* 2015), brown rot in apple fruits (Madbouly *et al.,* 2020), and *Botrytis cinerea* of Snap Beans (Feng *et al.,* 2021). However, it has not previously been documented that yeasts

were used to treat onions with the purple blotch disease. Consequently, *Saccharomyces cerevisiae* was applied, for the first time, with *Trichoderma asperellum* T34, in addition to their biologically synthesized silver nanoparticles (AgNPs) for purple blotch disease management, to provide an environmentally acceptable management approach for onion purple blotch disease compared to some commonly used fungicides.

Materials and Methods

Sampling and isolation of the purple blotch's causal pathogen

In certain fields, near to Assiut University campus, Assiut Governorate, Egypt, symptoms of the purple blotch disease on onion (*Allium cepa*) were observed. For the isolation of the causal pathogen from the infected leaves, the direct plating method described by Golden *et al.* (1988) was employed. The diseased samples were cut into 1.0 cm^2 pieces, washed three times in sterile distilled water, and surface-sterilized with 5.0% aqueous sodium hypochlorite for 5 min. and then with 70% ethanol for 30 seconds. Five segments were then plated on a 9 cm diameter Petri plate containing 20 mL potato dextrose agar (PDA; (Smith and Onions, 1994). Three replicates were used for each sample. The plates were then incubated at 25 °C until the development of the fungal growth. The developed colonies were purified on PDA and preserved, as pure culture, in the culture collection of Assiut University Mycological Centre (AUMC).

Molecular identification of the pathogen

The DNA extraction of *Alternaria* sp. AUMC 16012 was performed according to the method described by Moubasher *et al.* (2019), in which a 0.2 g of 7-day-old fungal mycelia grown on PDA, was grounded and transferred to a 1.5-mL microfuge tube. The isolation of the DNA was performed using the CTAB method (800 µL CTAB buffer composed of 3% CTAB, 1.4 M NaCl, 0.2% Mercaptoethanol, 20 mM EDTA, 100 mM TRIS-HCl pH 8.0 and 1% PVP-40). The PCR reaction was carried out at SolGent Co., Ltd (Yuseong-Gu, Daejeon, South Korea) using ITS1 and ITS4 primer pair (White *et al.,* 1990) and SolGent EF–Taq, as described by Al-Bedak and Moubasher (2020) and Al-Bedak *et al.* (2020). A contiguous sequence of *Alternaria* sp. AUMC 16012 isolate was produced by using the DNASTAR (version 5.05). Sequence of *Alternaria* sp. AUMC 16012 was uploaded to GenBank as OQ283744. Sequences of the closely similar species belonging to the genus *Alternaria* were downloaded from GenBank. Sequence of *Curvularia lunata* CBS 730.96 (NR_138223) was used as an outgroup. All sequences were aligned together using MUSCLE with the default options (Edgar, 2004). Alignment gaps and parsimony of uninformative characters were optimized manually. Maximum-likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed using MEGA X version 10.2.6 (Kumar *et al.,* 2018). The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Felsenstein, 1985). The best optimal model of nucleotide substitution for the ML analyses was determined using the Akaike information criterion (AIC) as implemented in Modeltest 3.7 (Posada and Crandall, 1998). The phylogenetic tree was drawn and visualized using MEGA X (Kumar *et al.,* 2018).

Pathogenicity test

The pathogenicity test was carried out, on a 110-day-old onion cultivar ('Giza-6'), at the Department of Plant Pathology's experimental farm in Arab-El-Awamer Research Station, Assiut Governorate, Egypt, during the growing season 2019-2020. Four sets of plastic pots (25 cm in diameter) were used, of which one set was used as negative control. Before planting two onion seedlings, each pot was separately filled with 3 kg of pre-sterilized clay soil. A spore suspension that contained 1.2×10^5 spore/mL of the *Alternaria* sp. AUMC 16012's culture, which was 7–day–old, was utilized in spraying the 'Giza-6' onion cultivar's leaves. The infected

seedlings were covered with sterile polyethylene bags after the pathogen was applied, to maintain a high humidity level. After 48 hours of pathogen's application, the bags were then taken off and the onion plants were kept in a greenhouse environment. The severity of the disease was noted fifteen days after the infection.

Estimation of Percent Disease Index (PDI)

For each single plot under each treatment, the percentage of the disease index (PDI) was estimated using the following scale (Guo *et al.,* 2021):

0 = no disease symptoms.

1 = 1-25%: several dark purplish brown patches covering the leaf area.

2 = 26-50%: streaks covering leaf area.

3 = 51-75%: streaks covering leaf area.

4 = 76-100%: streaks covering leaf area.

The disease severity index (DSI) was calculated using the following formula. The disease severity index was calculated according to the following equation:

$$\text{DSI} = \frac{\text{Total sum of numerical ratings}}{\text{No.of observation x Maximum disease rating in the scale}} \times 100$$

Preparation of silver nanoparticles (AgNPs)

The Assiut University Mycological Centre (AUMC), Assiut Governorate, Egypt, provided the *Saccharomyces cerevisiae* AUMC 10203 and *Trichoderma asperellum* T34 (= EPA 87301-1; Environmental Protection Agency, Pennsylvania Avenue, NW Washington, D.C.). Fungi were grown in 500-mL Erlenmeyer conical flasks with 100 mL of potato dextrose broth in each flask under shacked conditions (150 rpm) for 7 days at 25 °C. After the incubation period, the cell-free supernatants were collected by centrifugation of the fungal growth (10,000 rpm for 10 min at 4 °C). The cell-free supernatants of *S. cerevisiae* AUMC 10203 and *T. asperellum* T34 were separately combined with a 100 mM solution of $AgNO_3$ to produce $AgNO_3$ concentrations of 10, 20, 30, 40, and 50 ppm. The flasks were then incubated at 25 °C under shaking at 150 rpm until the solution's color changed. Both a visual examination of the solution and TEM analysis were regularly used to track the reduction of silver ions to silver nanoparticles.

Characterization of AgNPs by Transmission Electron Microscopy (TEM)

For transmission electron microscope (TEM) measurements (Kumar and Gopidas, 2010), a10–fold diluted sample of the synthesized AgNPs was mounted on carbon-coated copper grids and held in a vacuum for the night before being loaded into the specimen holder. The size of the AgNPs was determined using JEM100CX11 operating at 100KV (0.23 nm resolution), Electron Microscopy Unit, Assiut University, Assiut, Egypt.

Impact of bioagents and AgNPs on disease development by A. porri under greenhouse conditions

The experiment was conducted on a 110–day–old 'Giza–6' onion cultivar in a greenhouse setting at the Plant Pathology Department Experimental Farm, Arab–El–Awamer Research Station, Assiut Governorate, Egypt during the 2020-2021 growing season. Each 25-cm-diameter container contained two onion seedlings that were planted one by one in autoclaved clay soil (3 kg each). For each treatment, three replicas of four pots were employed. As a control, 4 pots were not infected. To maintain a high humidity level, the infected plants were covered with polyethylene bags for 48 hours after the pathogen application. Following that, the bags were taken off and the onion plants were maintained in their regular environment. The severity of the disease was measured fifteen days after the spraying. After being cultured on PDA at 25 °C for 72 hours with *S. cerevisiae* AUMC 10203 and *T. asperellum* T34, respectively, spore suspension from each fungus was collected in sterile

distilled water to obtain spore concentrations of 1×10^6 spore/mL of each fungus. AgNPs were produced using the two fungi at concentrations of 50, 25, and 12.5 mM, and the three concentrations were applied foliarly to onion seedlings. Uninfected seedlings served as the negative control, while the fungicide Ridomil–M 72% (Syngenta Egypt) served as the positive control. In this experiment, 100 mL of each treatment was utilized. As previously noted, disease severity was recorded after 14 days after spraying. Three replicates of the experiment were run.

Experiment treatments

According to the experiment's design, a total of 10 treatments from the following tested treatments were administered to the plots that were assigned to them:

T1 = Control (untreated)
T2 = 50 mM – T34 – AgNPs
T3 = 25 mM – T34 – AgNPs
T4 = 12.5 mM – T34 – AgNPs
T5 = 50 mM – *S. cerevisiae* – AgNPs
T6 = 25 mM – *S. cerevisiae* – AgNPs
T7 = 12.5 mM – *S. cerevisiae* – AgNPs
T8 = *T. asperellum* T34
T9 = *S. cerevisiae*
T10 = Ridomil – M 72%

Field experiment

Using Randomized Complete Block Design (RCBD) with three replications, the field experiment (plot size 2 $m^2 \times 2$ m^2) was conducted during the growing seasons 2019/2020 and 2020/2021. Before planting the onion seedlings, the soil was prepared by upholding appropriate agronomic practices and adding the essential fertilizers. There were two methods used for the treatment; the first was carried out after the crop was transplanted, and the second was carried out after the disease's initial symptoms started to appear in the field. Every treatment was applied three times, 15 days apart, and each application was contrasted with the untreated control group without the use of any bioagents. Observations on the prevalence and severity of the condition were made before each spray treatment and then again 15 days afterward. The proportion of disease incidence was calculated as previously declared after counting the number of plants affected by the purple blotch disease according to treatment. Data on the prevalence and severity of the disease has been collected from the field using 25 randomly selected plants.

Effect of various treatments on seed production

The inflorescence diameter (cm), flower and seed counts per inflorescence, weight of seeds per inflorescence (g), and number of seeds per plot (g), were all estimated for each experimental unit.

Statistical analysis

The Msatatc software was used to analyze variance (ANOVA). To find changes between treatments, the least significant difference (LSD) at $P \leq 0.05$ was used (Gomez and Gomez, 1984).

Results

Isolation of the pathogen

The symptoms started as water-soaked lesions that were 2-3 mm in diameter. They were fast growing and became concentric dark and bright zones on leaves and/or inflorescence. Later, these lesions clumped together and their color changed from purple to brown, and they expanded upwards and downwards (Figure 1). Onion-affected leaves were checked for signs of purple blotch disease. Deep, oval-shaped lesions could be seen on the infected leaves. The light brown tissue around the lesions surrounds the lesions' cores, which range in hue from brown to purple. Onion-infected leaves were used to isolate the pathogen, which was then cultured on PDA at 25 °C.

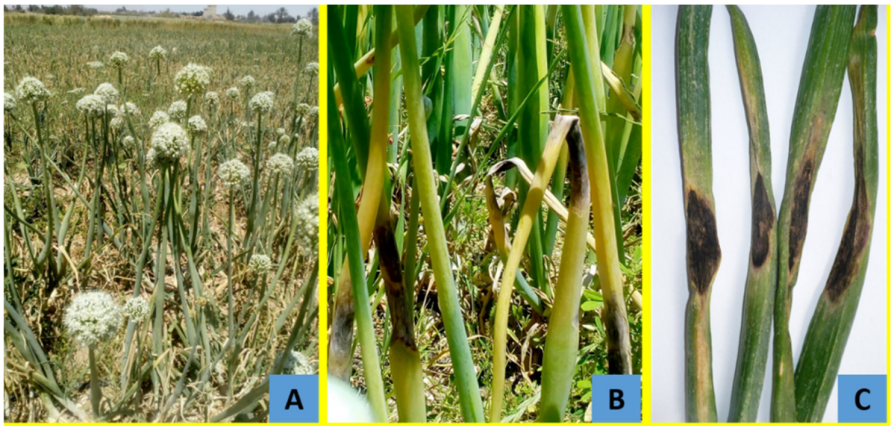

Figure 1. A–B, Infected onion plants in the field ('Giza-6' cultivar). C, Symptoms of purple blotch as sunken purple lesions on onion's leaves.

Pathogenicity test

During this experiment, twelve pure *Alternaria* isolates were discovered on onion leaves with purple blotch symptoms. Since all of the isolates had morphological characteristics and effectively manifested purple blotch symptoms, *A. porri* was identified in each one. Table 1 showed the disease incidence ranged from 35.15 to 85.93%. The pathogenicity test was conducted on all isolates (Figure 2), and the most virulent isolate, that had an 85.93% disease incidence, was then selected for further investigation. This isolate was deposited in the culture collection of the Assiut University Mycological Centre as AUMC 16012.

Table 1. Pathogenicity test of *A. porri* isolates on onion (Giza–6 cultivar)

Isolate No.	Disease incidence (%)
1	35.15
2	62.49
3	44.53
4	**85.93**
5	53.46
6	64.58
7	50.77
8	62.23
9	49.43
10	59.56
11	77.21
12	48.56
L.S.D. at 5%	**10.03**

Figure 2. Pathogenicity test showing: A. infected onion seedlings. B-C. The development of the purple blotch disease

Molecular identification of the pathogen

The most severe isolate, with a disease severity of 85.93%, was re-isolated from the diseased samples and identified using sequencing of the internal transcribed spacer region (ITS). Phylogenetic analysis of the ITS dataset was employed to determine the taxonomic status of our strain relative to other members of *Alternaria*. The ITS final data set was made up of 18 ITS sequences that generated 570 characters, 447 of which could be successfully matched. *Alternaria porri* isolate V5 (MW794319) and *A. porri* isolate G (MT550031) were both 99.79% and 100% comparable to the isolate used in this investigation. Kimura 2-parameter (K2) was the best model to express the phylogenetic relationship of our fungus with the most similar *Alternaria* species. The dataset for maximum parsimony (MP) yielded 10 trees, the most parsimonious of which with a final ML optimization value of -1246.08, a tree length of 95 steps, is shown (Figure 3). ITS sequence of *A. porri* in this study was uploaded to GenBank as OQ283744. The *A. porri* in this study consistently located with *A. porri* V5 and *A. porri* G in the same clade.

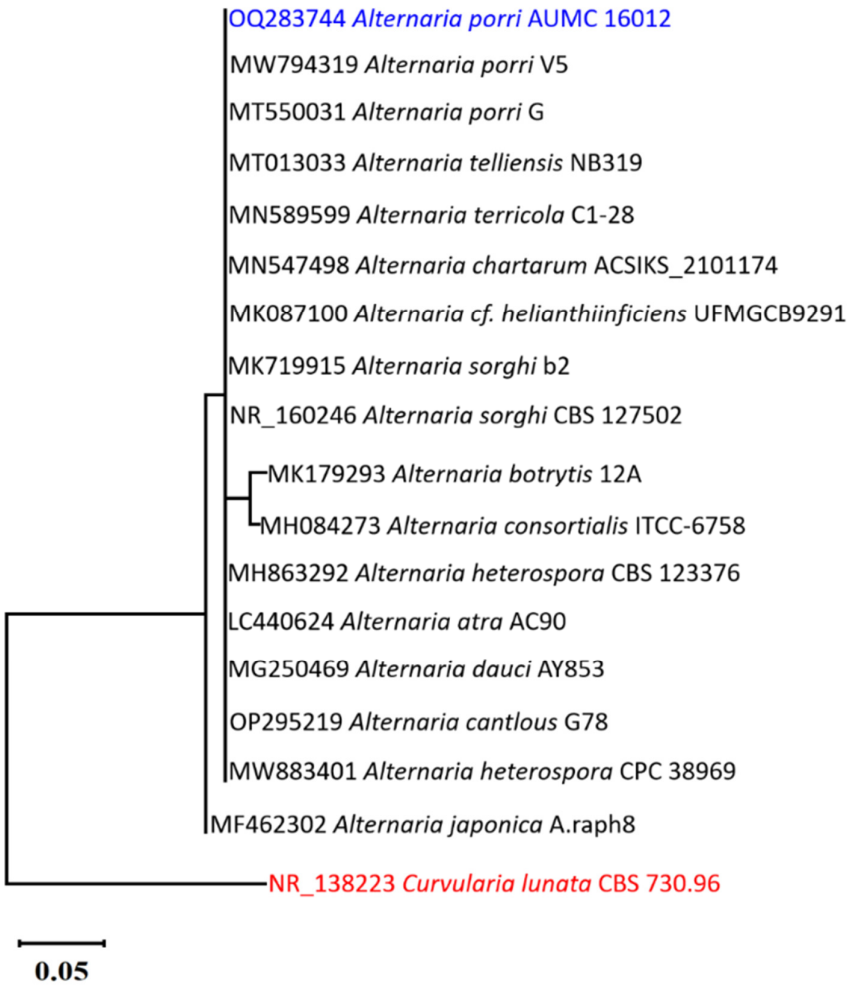

Figure 3. Maximum likelihood phylogenetic tree obtained from a heuristic search (1000 replications) of *Alternaria porri* in this study (in blue color) compared to other closely similar ITS sequences belonging to *Alternaria* in GenBank. The tree is rooted to *Curvularia lunata* CBS 730.96 as an outgroup (in red color)

Synthesis of AgNPs

The most recent findings demonstrated that the color of the fungal supernatant/AgNO3 solution changed when $AgNO_3$ was reduced to AgNPs by mixing it with fungal supernatants at a concentration of 50 mM. The ability of each of the fungi to reduce $AgNO_3$ and induce a color change in the medium from light yellow to dark purple, signifying the creation of AgNPs, was demonstrated in Figure 4.

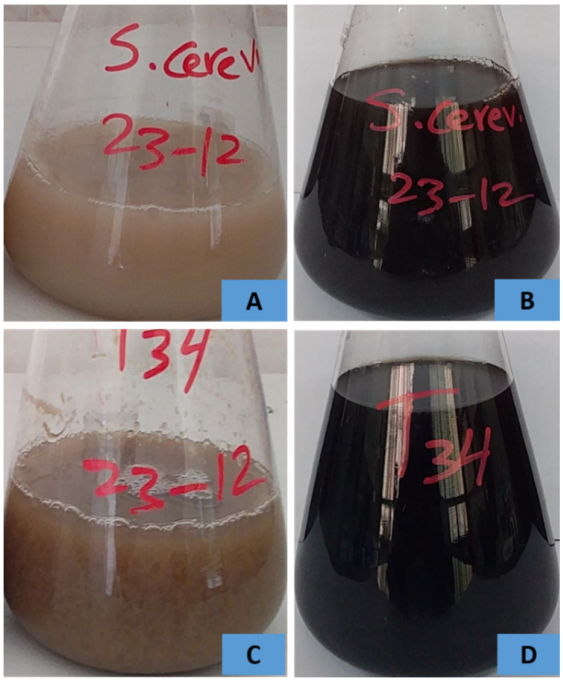

Figure 4. Development of AgNPs showing **(A&C)**, Cultural filtrate of *S. cerevisiae* AUMC 10203, and *T. asperellum* AUMC 15967 (T34) **(B&D)**, Color change as AgNPs were formed

Characterization of AgNPs by TEM

According to TEM photographs, the AgNPs generated by *S. cerevisiae* AUMC 10203 and *T. asperellum* AUMC 15967 (T34) ranged in size from 25.3 to 46.1 nm and from 12.7 to 43.6 nm, respectively. The AgNPs were spherical to subspherical (Figure 5).

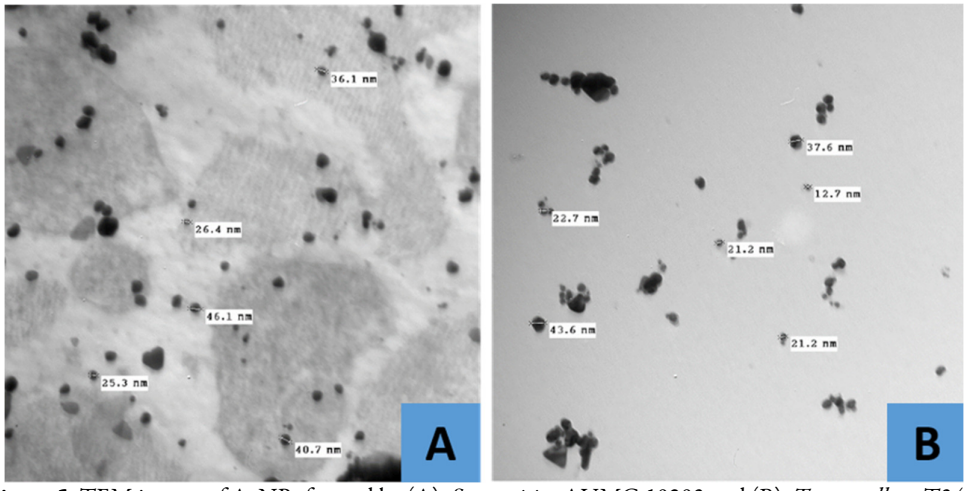

Figure 5. TEM images of AgNPs formed by (A), *S. cerevisiae* AUMC 10203 and (B), *T. asperellum* T34

Greenhouse experiments

Under greenhouse conditions, the effect of two bioagents - *S. cerevisiae* AUMC 10203 and *T. asperellum* T34-and three concentrations of their AgNPs on the appearance of purple blotch on onions was assessed as a preventative and/or therapeutic strategy. According to the current study, as compared to controls, all therapies

significantly reduced purple blotch, but at different rates. Following *S. cerevisiae*, which exhibited a greater decrease in disease severity at 13.88 and 15.13%, both before and after infection, was the application of *T. asperellum* T34 spore suspension. When T34-AgNPs were administered before and after infection, the severity of the disease decreased by 16.93 and 18.42%, respectively, whereas *S. cerevisiae*-AgNPs were provided before and after infection, and the severity of the disease decreased by 20.00 and 23.38%, respectively (Table 2).

Table 2. Effect of different treatments against disease severity of onion's purple blotch under greenhouse conditions

Treatments	(DSI) disease severity	
	Before infection	After infection
S. cerevisiae		
Spore suspension	16.93^{cd}	20.76^{efg}
50 mM AgNPs	20.0^{cd}	23.38^{ef}
25 mM AgNPs	24.17^{bc}	30.62^{bcd}
12.5 mM AgNPs	29.62^{b}	36.35^{b}
***T. asperellum* T34**		
Spore suspension	13.88^{d}	15.13^{g}
50 mM AgNPs	16.93^{cd}	18.42^{fg}
25 mM AgNPs	18.51^{cd}	26.59^{cde}
12.5 mM AgNPs	22.68^{bc}	31.72^{bc}
Ridomil-M 72%	28.33^{b}	25.34^{de}
Control	85.5^{a}	85.5^{a}
L.S.D. at 5%	**7.92**	**5.95**

Field experiment

The field experiment showed that the treatment using all bioagents and contemporaneous AgNPs differed from the control group statistically in a similar way. The use of *T. asperellum* T34 significantly reduced the frequency and severity of purple blotch disease from 88.31 and 89.16% to 12.25 and 12.83% over the course of the 2019–2020 growing season and to 11.66 and 14.0 over the course of the 2020-2021 growing season, respectively. With 50 mM of T34-AgNPs therapy, disease incidence, and severity decreased dramatically to 19.34 and 20.95% during the 2019–2020 growth season and 16.83 and 20.5% during the 2020-2021 growing season (Table 3).

Table 3. Effect of different treatments against disease incidence and disease severity of purple blotch of onion under field conditions

Treatments	Disease incidence		Disease severity	
	2019/2020	2020/2021	2019/2020	2020/2021
S. cerevisiae				
Spore suspension	20.54	20.00	20.00	21.11
50 mM AgNPs	17.22	18.00	26.71	28.75
25 mM AgNPs	30.66	30.33	36.48	35.32
12.5 mM AgNPs	31.87	32.11	37.33	38.32
***T. asperellum* T34**				
Spore suspension	12.25	11.66	12.83	14.00
50 mM AgNPs	19.34	16.83	20.95	20.50
25 mM AgNPs	21.25	20.76	30.38	26.93
12.5 mM AgNPs	25.92	27.74	34.45	36.24
Ridomil-M 72%	32.05	31.33	33.33	31.50
Control	88.31	89.16	88.31	89.16

Treatments	Disease incidence		Disease severity	
	2019/2020	2020/2021	2019/2020	2020/2021
L.S.D. at 5%	**5.62**	**4.90**	**4.77**	**4.78**

Effect of T. asperellum T34 and AgNPs on onion production

During the growth seasons 2019/2020 and 2020/2021, the application of T34 spore suspension and various concentrations of its AgNPs demonstrated the greatest improvement in all investigated parameters (inflorescence diameter, number of flowers and seeds, seed weight per inflorescence, and seed yield) over the control and *S. cerevisiae* treatment or its AgNPs (Tables 4 and 5).

Table 4. Effect of different treatments on flowering and seed production of onion plant (Giza 6 cultivar) during the 2019/2020 growing season

Treatments	Before infection					After infection				
	Inflorescence diameter (cm)	No. of flowers /Inflorescence	No. of seeds/ Inflorescence	Seed weight/ Inflorescence	Seed yield/ plot (g)	Inflorescence diameter (cm)	No. of flowers /Inflorescence	No. of Seed/ Inflorescence	Seed weight/ Inflorescence	Seed yield/ plot (g)
S. cerevisiae										
Spore suspension	6.1	393.30	465.26	1.97	36.20	6.00	389.63	445.23	1.93	36.15
50 mM AgNPs	5.3	384.73	470.80	2.16	31.77	5.4	302.60	452.41	2.00	30.30
25 mM AgNPs	5.2	344.84	420.86	1.86	23.41	5.00	294.63	398.30	1.85	21.79
12.5 mM AgNPs	5.1	308.50	405.96	1.81	20.49	5.00	275.26	383.50	1.78	19.43
***T. asperellum* T34**										
Spore suspension	6.4	415.53	528.23	2.21	42.05	6.1	412.43	500.30	2.00	42.00
50 mM AgNPs	5.7	402.66	481.76	2.27	35.15	5.5	390.13	461.36	2.01	32.53
25 mM AgNPs	5.4	378.66	433.26	2.12	28.48	5.3	301.03	411.23	1.91	25.30
12.5 mM AgNPs	5.2	320.33	412.33	1.92	21.53	5.2	284.80	389.26	1.86	20.00
Ridomil-M 72%	5.5	355.26	459.26	1.96	31.11	5.4	330.80	444.26	1.90	30.50
Control	4.1	202.83	212.26	0.81	17.73	4.1	202.83	212.26	0.81	17.73
L.S.D. at 5%	**0.95**	**8.39**	**6.94**	**0.86**	**2.13**	**1.36**	**4.31**	**2.71**	**0.35**	**1.76**

Table 5. Effect of different treatments on flowering and seed production of onion plant (Giza 6 cultivar) during the 2020/2021 growing season

	Before infection					After infection				
	Inflorescence diameter (cm)	No. of flowers /Inflorescence	No. of Seed/ Inflorescence	Seed weight/ Inflorescence	Seed yield/plot (g)	Inflorescence diameter (cm)	No. of flowers /Inflorescence	No. of Seed/ Inflorescence	Seed weight/ Inflorescence	Seed yield/plot (g)
S. cerevisiae										
Spore suspension	6.3	390.33	461.23	1.99	36.66	6.1	385.26	446.26	1.94	35.84
50 mM AgNPs	5.4	380.20	465.23	2.12	32.15	5.4	305.60	455.26	2.00	29.15
25 mM AgNPs	5.2	350.21	415.30	1.87	22.96	5.0	294.26	390.16	1.85	20.20
12.5 mM AgNPs	5.1	311.36	400.16	1.81	20.66	5.1	270.53	381.96	1.78	19.50
***T. asperellum* T34**										
Spore suspension	6.5	410.41	520.60	2.10	43.35	6.3	400.33	505.16	2.03	43.11
50 mM AgNPs	5.8	403.20	476.46	2.40	35.35	5.6	391.16	458.20	2.00	33.00
25 mM AgNPs	5.4	370.60	425.13	2.15	28.50	5.3	300.33	402.26	1.92	24.94
12.5 mM AgNPs	5.1	315.46	405.30	1.96	21.93	5.1	280.26	380.26	1.89	20.00
Ridomil–M 72 %	5.6	350.33	451.53	1.96	31.51	5.4	320.36	440.63	1.90	30.00
Control	4.2	205.33	220.16	0.77	15.46	4.2	205.33	220.16	0.77	15.46
L.S.D. at 5%	**1.50**	**2.19**	**3.20**	**0.62**	**1.31**	**1.34**	**1.41**	**2.94**	**0.39**	**1.14**

Discussion

Purple blotch disease is capable of having an impact on how crops are grown, harvested, and distributed, which lowers yield and quality and therefore raises production costs and export potential. During this investigation, twelve isolates from *Alternaria porri* were found to be the source of the purple Blotch symptoms on the onion cultivar 'Giza-6'. Onion cultivar 'Giza-6' was subjected to a pathogenicity test in a greenhouse. The observations reported here demonstrated that each isolate tested was capable of infecting onion plants and

causing disease, albeit to variable degrees. Numerous studies have identified *A. porri* and *Stemphylium vesicarium* as the causative agents of purple blotch disease (Datar, 1993; Suheri and Price, 2000; Schwartz, 2004; Razdan *et al.,* 2011; Mishra and Gupta, 2012; Abdel-Hafez *et al.,* 2014; Mohsin *et al.,* 2016; Abdel-Rahim *et al.,* 2017; Ahmed *et al.,* 2017; Yadav *et al.,* 2017; Kim *et al.,* 2022).

Due to the high reported levels of morphological similarity, observing the morphological diversity of fungi at the genus and species level is challenging. Therefore, the most current advances in molecular biology are required to use molecular markers to generate vast amounts of genetic data. Within the same group of fungi, distinct physiological and morphological diversities might be strongly associated with molecular genetic data (Chattopadhyay *et al.,* 2017). Internal transcribed spacer (ITS) region of rDNA sequencing and blasting using all-purpose ITS primers were the first steps in the molecular characterization of fungal isolates (White *et al.,* 1990; Chethana *et al.,* 2018). In this study, the most severe *A. porri* isolate, causing the highest disease severity, was genetically identified by sequencing the ITS region, and the isolate was clustered with *A. porri* isolates at the same branch in the phylogenetic tree.

There have been several secondary metabolites found in *A. porri* that may contribute to its pathogenicity, including altersolanol A, alterporriol, macrosporin, erythroglaucin, and tentoxin (Andersen *et al.,* 2008). The genes responsible for *Alternaria*'s pathogenicity code for a variety of physiological factors, including toxins and enzymes involved in signal transduction and cell wall disintegration.

Some *Alternaria* species share similar toxin precursors during biosynthesis, although distinct *Alternaria* species generate various kinds of toxins (Mamgain *et al.,* 2013; Dar *et al.,* 2020). Due to the significant crop damage and output reduction caused by *A. porri*, purple blotch disease of *Allium* spp. crops has continued to be a key issue in agriculture for both farmers and the scientific community. Therefore, effective management is necessary to sustain high crop output by avoiding such significant yield losses. Many researchers have experimented with chemical methods to control onion purple blotch (Deshmukh *et al.,* 2007; Ali, 2008; Gupta *et al.,* 2014; Priya *et al.,* 2015; Ali *et al.,* 2016; Dar *et al.,* 2020; Sofy *et al.,* 2020; Gore *et al.,* 2021; Younas *et al.,* 2021). The drawbacks of fungicides include the persistence of carcinogens, toxicity issues, environmental contamination, and the emergence of chemically resistant disease strains. Additionally, it has been demonstrated that the use of synthetic fungicides contributes to the emergence of fungicide-resistant strains and diseases, the severity of which is increased by the use of certain chemical compounds (Rial-Otero *et al.,* 2005; Madhavi *et al.,* 2012; Dar *et al.,* 2020).

The current findings revealed a 76% reduction in the disease severity when *T. asperellum* T34 spores were applied for the treatment. In the past decades, several investigations have been carried out in many different parts of the world to eradicate purple blotch disease. For example, by combining isolates of *Aureobasidium pullulans*, *Cryptococcus luteolus*, *Sporobolomyces roseus*, and *Penicillium* spp., Tyagi *et al.* (2008) found an intriguing observation of a 79% reduction in *A. porri* infection. It additionally reported that *Trichoderma harzianum* by itself considerably decreased purple blotch disease in onion crop yields to between 60 and 80% (Prakasam and Sharma, 2012; Abo-Elyousr *et al.,* 2014; Hasna, 2021). Gothandapani *et al.* (2015) investigated the effectiveness of *Metarhizium anisopliae*, *Beauveria bassiana*, and *Verticillium lecanii* as entomopathogenic fungi against *A. porri*, and they concluded that all three fungal strains had notable inhibitory effect on mycelial development and conidial germination, which significantly reduced pathogen invasion.

Many plant diseases have been treated with various yeast species. For example, on a damaged 'Golden Delicious' apple, spore suspension of *Pichia anomala* or *Candida sake* was used to combat *Penicillium* sp. and *Botrytis cinerea* (Jijakli *et al.*, 1993). *Rhodotorula rubra* and *Candida pelliculosa* were found to be strongly antagonistic reducing the postharvest decay of tomato caused by *Botrytis cinerea* (Dal Bello *et al.*, 2008). Bacterial fruit blotch (BFB), caused by *Acidovorax citrulli* was controlled by *Rhodotorula aurantiaca* LMA 1, *Pichia anomala* CC-2, and *Rhodotorula glutinis* LMS (de Melo *et al.*, 2015). The pathogen *Monilinia fructicola*,

which causes brown rot in apple fruits, was controlled biologically using the yeasts *Debaryomyces hansenii* and *Wickerhamomyces anomalus* (Czarnecka *et al.*, 2019). *Cryptococcus albidus* (Ca63), *Cryptococcus albidus* (Ca64), and *Candida parapsilosis* (Yett1006) and their combinations were applied to control *Botrytis cinerea* of Snap Beans (Feng *et al.*, 2021).

A biocontrol product's active ingredient must be effective against the disease it is intended to treat, but secondary characteristics like biosafety and registration concerns, production needs and conditions, formulation options, and the equipment needed for application are equally or even more crucial. Although the lack of invasive, filamentous development in the majority of yeasts may appear to be a drawback, the yeast-like shape is what allows for flexible cultivation in fermenters, beneficial formulation properties, and a wide range of application possibilities. Similar to bacteria, yeasts' single-celled morphology encourages adhesion and biofilm development, which directly affects environmental persistence, competitiveness, and ultimately improved biocontrol activity (Verstrepen and Klis, 2006; Pandin *et al.*, 2017; Rossouw *et al.*, 2018; Freimoser *et al.*, 2019).

The high-copy 2 µm plasmid is present in many *S. cerevisiae* strains. As a result, bacteria and yeasts share growth characteristics and biocontrol mechanisms without incurring the risk of picking up or passing on genes for pathogenicity, toxin production, or antibiotic resistance based on plasmids. Horizontal gene transfer is also significantly less frequent in yeasts than in their prokaryotic counterparts, even though it occurs in fungi more frequently than was previously thought due to their more complicated genome structure (Richards *et al.*, 2011; Fitzpatrick, 2012; Moriguchi *et al.*, 2013; Freimoser *et al.*, 2019).

T. asperellum T34 and *Saccharomyces cerevisiae* produced green synthesized AgNPs, which were used in this investigation at three different doses (50, 25, and 12.5 mM). Following the effects of *T. asperellum* and *S. cerevisiae*, the three doses were effective with the 50 mM concentration having the most significant effects on the severity and incidence of *A. porri* disease. "Green synthesis" refers to the use of plant extracts as reducing and stabilizing agents during the nanoparticle creation process. This process can create nanoparticles with special features while being economical and environmentally benign (Ashraf *et al.* 2022). Silver nanoparticles (AgNPs), which have a wide range of antifungal effects, have been the subject of several investigations (Gautam *et al.* 2020).

Silver nanoparticles are among the most promising nanomaterials (Huy *et al.*, 2017; Ghareeb *et al.*, 2022) due to their mechanical, optical, antibacterial, and antiviral (Ansari *et al.* 2018), as well as their electrical conductivity and thermal conductivity characteristics. Applications of nanomaterials depend on how they were created. Many methods, including physical, chemical, and biological ones, have been devised to create stable nanomaterials with regulated size and form (Singh *et al.*, 2020). Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzyme activity is increased in plants by the use of silver nanoparticles, increasing the plant's ability to withstand oxidative stress and maintain cellular homeostasis under adverse conditions (Azeez *et al.*, 2022). AgNPs application boosts the quantity of chlorophyll in leaves, which improves photosynthetic efficiency (Gao *et al.*, 2021; Zhang *et al.*, 2021), as well as photosynthetic activity by reducing oxidative stress and enhancing the absorption of essential nutrients including nitrogen, phosphorus, and potassium (Rastogi *et al.*, 2019). Additionally, they support the safeguarding of the firmness of the chloroplasts and other photosynthesis-related cellular elements (Lalau *et al.*, 2020). When resources are more easily accessible, the production of various secondary metabolites, such as sugar, protein, alkaloids, flavonoids, and phenolic compounds, is enhanced (Abbas *et al.*, 2019).

Conclusions

The current study offers convincing evidence for the potential of two bio-agents, *T. asperellum,* and *S. cerevisiae*, along with their green-synthesized AgNPs, as an economical and environmentally friendly method for controlling the purple blotch disease on onions, as well as enhancing the growth and yield of onion plants and safeguarding them against the disease. The *T. asperellum* T34 spore suspension was shown to be the most effective for treating or preventing purple blotch disease affecting onion plants. The *S. cerevisiae* spore solution was *T. asperellum*'s front-runner, followed by a 50 mM dose of AgNPs. The plants treated with both bio-agents and AgNPs showed a substantial decrease in disease incidence and severity when compared to the control plants. Additionally, the width of inflorescences, the quantity of flowers and seeds, the weight of seeds per inflorescence, and the yield of seeds were all improved. These results emphasize the possibility of employing these two bio-agents, along with the environmentally friendly-synthesized AgNPs, as a secure, efficient, and sustainable substitute for traditional fungicides for crop protection and yield improvement.

Authors' Contributions

All authors participated equally in data analysis, authoring, and revising the article.
All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abbas Q, Liu G, Yousaf B, Ali MU, Ullah H, Ahmed R (2019). Effects of biochar on uptake, acquisition and translocation of silver nanoparticles in rice (*Oryza sativa* l.) in relation to growth, photosynthetic traits and nutrients displacement. Environmental Pollution 250:728-736. *https://doi.org/10.1016/j.envpol.2019.04.083*

Abdel-Hafez SI, Abo-Elyousr KA, Abdel-Rahim IR (2014). Effectiveness of plant extracts to control purple blotch and *Stemphylium* blight diseases of onion (*Allium cepa* l.) in Assiut, Egypt. Archives of Phytopathology and Plant Protection 47(3):377-387. *https://doi.org/10.1080/03235408.2013.809926*

Abdel-Rahim IR, Abdel-Hafez SI, Abo-Elyousr KA (2017). Onion purple blotch symptoms, at Assiut governorate (Egypt), caused by synergistic association between *Alternaria porri* and *Stemphylium vesicarium*. Journal of Plant Diseases and Protection 124:195-200. *https://doi.org/10.1007/s41348-016-0057-5*

Abo-Elyousr KA, Abdel-Hafez SI, Abdel-Rahim IR (2014). Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. Journal of Phytopathology 162(9):567-574. *https://doi.org/10.1111/jph.12228*

Ahmed M, Amin M, El-Fiki I (2017). Efficacy of bioagents against *Alternaria porri* incitant of purple blotch of onion in Egypt. Egyptian Journal of Phytopathology 45(1):17-29. *https://doi.org/10.21608/ejp.2017.89518*

Al-Bedak OA, Moubasher AH (2020). *Aspergillus gaarensis*, a new addition to section Circumdati from soil of Lake El-Gaar in Wadi-El-Natron, Egypt. Studies in Fungi 5(1):59-65. *https://doi.org/10.5943/sif/5/1/5*

Al-Bedak OA, Teama EA, Ali E, Said M, Shalaby E, Moharram ZA (2020). Impact of fumigation with phosphine on viability of wheat grains stored for six months at two levels of moisture content, in addition to description of four new records of associated fungi and assessment of their potential for enzymatic production. Journal of Basic & Applied Mycology (Egypt) 11:77-97.

Ali H, Nisha HaC, Hossain MB, Islam MR (2016). Evaluation of combined effect of micronutrients (ZnSO4 + Borax) and fungicides to control the purple blotch complex of onion (*Allium cepa*). American Journal of Plant Sciences 7(05):715. *https://doi.org/10.4236/ajps.2016.75065*

Ali MH (2008). Control of purple blotch complex of onion through fertilizer and fungicide application. Dept. of Plant Pathology.

Andersen B, Dongo A, Pryor BM (2008). Secondary metabolite profiling of *Alternaria dauci*, *A. porri*, *A. solani*, and *A. tomatophila*. Mycological Research 112(2):241-250. *https://doi.org/10.1016/j.mycres.2007.09.004*

Ansari A, Pervez S, Javed U, Abro MI, Nawaz MA, Qader SAU, Aman A (2018). Characterization and interplay of bacteriocin and exopolysaccharide-mediated silver nanoparticles as an antibacterial agent. International Journal of Biological Macromolecules 115:643-650. *https://doi.org/10.1016/j.ijbiomac.2018.04.104*

Ashraf H, Batool T, Anjum T, Illyas A, Li G, Naseem S, Riaz S (2022). Antifungal potential of green synthesized magnetite nanoparticles black coffee–magnetite nanoparticles against wilt infection by ameliorating enzymatic activity and gene expression in *Solanum lycopersicum* l. Frontiers in Microbiology 13:31. *https://doi.org/10.3389/fmicb.2022.754292*

Azeez L, Adebisi SA, Adetoro RO, Oyedeji AO, Agbaje WB, Olabode OA (2022). Foliar application of silver nanoparticles differentially intervenes remediation statuses and oxidative stress indicators in *Abelmoschus esculentus* planted on gold-mined soil. International Journal of Phytoremediation 24(4):384-393. *https://doi.org/10.1080/15226514.2021.1949578*

Camacho-Luna V, Flores-Moctezuma HE, Rodríguez-Monroy M, Montes-Belmont R, Sepúlveda-Jiménez G (2021). Induction of the defense response of onion plants in interaction with *Trichoderma asperellum* and *Alternaria porri*. Revista Mexicana de Ciencias Agrícolas 12(4):685-698. *https://doi.org/10.29312/remexca.v12i4.2683*

Charoenporn C, Kanokmedhakul S, Lin F, Poeaim S, Soytong K (2010). Evaluation of bio-agent formulations to control *Fusarium* wilt of tomato. African Journal of Biotechnology 9(36). *https://doi.org/10.5897/AJB10.260*

Chattopadhyay A, Tiwari KK, Chaudhary K, Pratap D (2017). Genic molecular markers in fungi: Availability and utility for bioprospection. Molecular Markers in Mycology: Diagnostics and Marker Developments 151-176. *https://doi.org/10.1007/978-3-319-34106-4_18*

Chethana B, Girija G, Archana SR, Bellishree K (2018). Morphological and molecular characterization of *Alternaria* isolates causing purple blotch disease of onion. International Journal of Current Microbiology and Applied Sciences 7(4):3478-3493. *https://doi.org/10.20546/ijcmas.2018.704.394*

Czarnecka M, Żarowska B, Połomska X, Restuccia C, Cirvilleri G (2019). Role of biocontrol yeasts *Debaryomyces hansenii* and *Wickerhamomyces anomalus* in plants' defence mechanisms against *Monilinia fructicola* in apple fruits. Food Microbiology 83:1-8. *https://doi.org/10.1016/j.fm.2019.04.004*

Dal Bello G, Monaco C, Rollan M, Lampugnani G, Arteta N, Abramoff C, Ronco L, Stocco M (2008). Biocontrol of postharvest grey mould on tomato by yeasts. Journal of Phytopathology 156(5):257-263. *https://doi.org/10.1111/j.1439-0434.2007.01351.x*

Dar AA, Sharma S, Mahajan R, Mushtaq M, Salathia A, Ahamad S, Sharma JP (2020). Overview of purple blotch disease and understanding its management through chemical, biological and genetic approaches. Journal of Integrative Agriculture 19(12):3013-3024. *https://doi.org/10.1016/S2095-3119(20)63285-3*

Datar VV (1993). Investigation on purple blotch of onion in India. In: Proceedings of the International Symposium on Alliums for the Tropics 358:259-264. *https://doi.org/10.17660/ActaHortic.1994.358.42*

De Melo EA, De Lima R, Mariano R, Laranjeira D, Dos Santos LA, De Omena Gusmão L, Barbosa De Souza E (2015). Efficacy of yeast in the biocontrol of bacterial fruit blotch in melon plants. Tropical Plant Pathology 40:56-64. *https://doi.org/10.1007/s40858-015-0008-x*

Deshmukh V, Dhruj I, Chavan R (2007). Chemical control of purple blotch (*Alternaria porri*) (Ellis) cif. of onion. Plant Disease Research 22(1):34-36.

Edgar RC (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5):1792-1797. *https://doi.org/10.1093/nar/gkh340*

Fangary A, Adam H (2020). Analytical study of the onion crop in Egypt. Scientific Journal of Agricultural Sciences 2(2):216-239. *https://10.21608/sjas.2020.48635.1051*

Faraz A, Haq IU, Ijaz S, Sahi ST, Khan I (2022). Antimycotic potential assessment of *Trichoderma* species and fungicides for sustainable management of *Sclerotinia trifoliorum* causing stem and crown rot of *Trifolium alexandrinum* l. International Journal of Phytopathology 11(2):195-205. *https://doi.org/10.33687/phytopath.011.02.4271*

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39(4):783-791. *https://doi.org/10.1111/j.1558-5646.1985.tb00420.x*

Feng M, Lv Y, Li T, Li X, Liu J, Chen X, Zhang Y, Chen X, Wang A (2021). Postharvest treatments with three yeast strains and their combinations to control *Botrytis cinerea* of snap beans. Foods 10(11):2736. *https://doi.org/10.3390/foods10112736*

Fitzpatrick DA (2012). Horizontal gene transfer in fungi. FEMS Microbiology Letters 329(1):1-8. *https://doi.org/10.1111/j.1574-6968.2011.02465.x*

Freimoser FM, Rueda-Mejia MP, Tilocca B, Migheli Q (2019). Biocontrol yeasts: Mechanisms and applications. World Journal of Microbiology and Biotechnology 35:1-19. *https://doi.org/10.1007/s11274-019-2728-4*

Gao D, Li M, Zhang J, Song D, Sun H, Qiao L, Zhao R (2021). Improvement of chlorophyll content estimation on maize leaf by vein removal in hyperspectral image. Computers and Electronics in Agriculture 184:106077. *https://doi.org/10.1016/j.compag.2021.106077*

Gao X, Hu S, Liu Z, Zhu H, Yang J, Han Q, Fu Y, Miao J, Gu B, Liu X (2022). Analysis of resistance risk and resistance-related point mutations in cyt b of qioi fungicide ametoctradin in *Phytophthora litchii*. Pest Management Science 78(7):2921-2930. *https://doi.org/10.1002/ps.6916*

Gautam N, Salaria N, Thakur K, Kukreja S, Yadav N, Yadav R, Goutam U (2020). Green silver nanoparticles for phytopathogen control. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 90:439-446. *https://doi.org/10.1007/s40011-019-01115-8*

Ghareeb RY, Shams El-Din NGE-D, Maghraby DME, Ibrahim DS, Abdel-Megeed A, Abdelsalam NR (2022). Nematicidal activity of seaweed-synthesized silver nanoparticles and extracts against *Meloidogyne incognita* on tomato plants. Scientific Reports 12(1):3841. *https://doi.org/10.1038/s41598-022-06600-1*

Golden D, Beuchat L, Brackett R (1988). Direct plating technique for enumeration of *Listeria monocytogenes* in foods. Journal-Association of Official Analytical Chemists 71(3):647-650. *https://doi.org/10.1093/jaoac/71.3.647*

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research John Wiley & Sons.

Gore P, Ingle R, Rakhonde P (2021). Management of purple blotch of onion caused by *Alternaria porri*. Journal of Plant Disease Science 16(1):47-51. *https://doi.org/10.48165/*

Gothandapani S, Boopalakrishnan G, Prabhakaran N, Chethana B, Aravindhan M, Saravanakumar M, Ganeshan G (2015). Evaluation of entomopathogenic fungus against *Alternaria porri* (Ellis) causing purple blotch disease of onion. Archives of Phytopathology and Plant Protection 48(2):135-144. *https://doi.org/10.1080/03235408.2014.884532*

Guo W, Yang Y, Zhao H, Song R, Dong P, Jin Q, Baig MHA, Liu Z, Yang Z (2021). Winter wheat take-all disease index estimation model based on hyperspectral data. Applied Sciences 11(19):9230. *https://doi.org/10.3390/app11199230*

Gupta R, Pandey N, Gupta R (2014). Management of purple blotch (*Alternaria porri*) disease of garlic (*Allium sativum* l.). In: Proceedings of the Abstract, IV National Symposium on Plant protection in horticultural crops: Emerging challenges and sustainable pest management organized at Indian Institute of Horticultural Research, Bengaluru held on, pp 25-28.

Hasna M (2021). Study on efficacy of Trichoderma in biological control against purple blotch of onion. Bangladesh Journal of Nuclear Agriculture 35:135-143.

Huang Y, Bai L, Yang Y, Yin Z, Guo B (2022). Biodegradable gelatin/silver nanoparticle composite cryogel with excellent antibacterial and antibiofilm activity and hemostasis for Pseudomonas aeruginosa-infected burn wound healing. Journal of Colloid and Interface Science 608:2278-2289. *https://doi.org/10.1016/j.jcis.2021.10.131*

Huy TQ, Thanh NTH, Thuy NT, Van Chung P, Hung PN, Le A-T, Hanh NTH (2017). Cytotoxicity and antiviral activity of electrochemical–synthesized silver nanoparticles against Poliovirus. Journal of Virological Methods 241:52-57. *https://doi.org/10.1016/j.jviromet.2016.12.015*

Jijakli H, Choutka C, Lepoivre P (1993). Formulation and integrated use of two antagonistic yeasts to postharvest treatments against diseases on apples. In: Proceedings of the Proceedings of an EC Workshop: Biological control of fruit and Foliar diseases.

Kareem MA, Murthy K, Hasansab AN, Waseem M (2012). Effect of temperature, relative humidity and light on lesion length due to *Alternaria porri* in onion. BIOINFOLET-A Quarterly Journal of Life Sciences 9(3):264-266.

Karthikeyan M, Radhika K, Bhaskaran R, Mathiyazhagan S, Sandosskumar R, Velazhahan R, Alice D (2008). Biological control of onion leaf blight disease by bulb and foliar application of powder formulation of antagonist mixture. Archives of Phytopathology and Plant Protection 41(6):407-417. *https://doi.org/10.1080/03235400600813474*

Kim MY, Han JW, Dang QL, Kim J-C, Kim H, Choi GJ (2022). Characterization of *Alternaria porri* causing onion purple blotch and its antifungal compound magnolol identified from *Caryodaphnopsis baviensis*. Plos One 17(1):e0262836. *https://doi.org/10.1371/journal.pone.0262836*

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6):1547. *https://doi.org/10.1093/molbev/msy096*

Kumar VKR, Gopidas KR (2010). Synthesis and characterization of gold-nanoparticle-cored dendrimers stabilized by metal–carbon bonds. Chemistry–An Asian Journal 5(4):887-896. *https://doi.org/10.1002/asia.200900388*

Lalau CM, Simioni C, Vicentini DS, Ouriques LC, Mohedano RA, Puerari RC, Matias WG (2020). Toxicological effects of AgNPs on duckweed (*Landoltia punctata*). Science of the Total Environment 710:136318. *https://doi.org/10.1016/j.scitotenv.2019.136318*

Madbouly AK, Elyousr KaA, Ismail IM (2020). Biocontrol of *Monilinia fructigena*, causal agent of brown rot of apple fruit, by using endophytic yeasts. Biological Control 144:104239. *http://dx.doi.org/10.1016/j.biocontrol.2020.104239*

Madhavi M, Kavitha A, Vijayalakshmi M (2012). Studies on *Alternaria porri* (Ellis) ciferri pathogenic to onion (*Allium cepa* l.). Archives of Applied Science Research 4(1):1-9.

Mamgain A, Roychowdhury R, Tah J (2013). Alternaria pathogenicity and its strategic controls. Research Journal of Biology 1:1-9.

Mandal D, Pal R, Kar I (2022). Effect of weather parameters on the progression and development of purple blotch of onion. Journal of Agrometeorology 24(3):325-327. *http://dx.doi.org/10.54386/jam.v24i3.1676*

Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, ... Gupta VK (2017). Abiotic stress responses and microbe-mediated mitigation in plants: The omics strategies. Frontiers in Plant Science 8:172. *https://doi.org/10.3389/fpls.2017.00172*

Mishra R, Gupta R (2012). *In vitro* evaluation of plant extracts, bio-agents and fungicides against purple blotch and Stemphylium blight of onion. Journal of Medicinal Plants Research 6(48):5840-5843. *https://doi.org/10.5897/JMPR10.246*

Mohsin SM, Islam MR, Ahmmed ANF, Nisha HaC, Hasanuzzaman M (2016). Cultural, morphological and pathogenic characterization of *Alternaria porr*i causing purple blotch of onion. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 44(1):222-227. *https://doi.org/10.15835/nbha44110110*

Moriguchi K, Yamamoto S, Tanaka K, Kurata N, Suzuki K (2013). Trans-kingdom horizontal DNA transfer from bacteria to yeast is highly plastic due to natural polymorphisms in auxiliary nonessential recipient genes. PLoS One 8(9):e74590. *https://doi.org/10.1371%2Fjournal.pone.0074590*

Moubasher AH, Ismail MA, Al-Bedak OA, Mohamed RA (2019). *Ramophialophora chlamydospora*, a new species from an alkaline Lake of Wadi-El-Natron, Egypt. Asian Journal of Mycology 2(1):110-117.

Oecd FAO (2022). Oecd-fao agricultural outlook 2022-2031.

Owaid MN, Rabeea MA, Aziz AA, Jameel MS, Dheyab MA (2022). Mycogenic fabrication of silver nanoparticles using picoa, pezizales, characterization and their antifungal activity. Environmental Nanotechnology, Monitoring & Management 17:100612. *http://dx.doi.org/10.1016/j.enmm.2021.100612*

Özer N, Köycü ND (2004). Seed-borne fungal diseases of onion, and their control. Fruit and Vegetable Diseases 281-306. *http://dx.doi.org/10.1007/0-306-48575-3_8*

Pandin C, Le Coq D, Canette A, Aymerich S, Briandet R (2017). Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? Microbial Biotechnology 10(4):719-734. *https://doi.org/10.1111/1751-7915.12693*

Posada D, Crandall KA (1998). Modeltest: Testing the model of DNA substitution. Bioinformatics (Oxford, England) 14(9):817-818. *https://doi.org/10.1093/bioinformatics/14.9.817*

Prakasam V, Sharma P (2012). *Trichoderma harzianum* (Th-3) a potential strain to manage the purple blotch of onion (*Allium cepa* l.) caused by *Alternaria porri* under north Indian plains. Journal of Agricultural Science 4(10):266. *http://dx.doi.org/10.5539/jas.v4n10p266*

Priya R, Sataraddi A, Darshan S (2015). Efficacy of non-systemic and systemic fungicides against purple blotch of onion (*Allium cepa* l.) caused by *Alternaria porri* (Ellis) cif. International Journal of Recent Scientific Research 6(9):6519-6521.

Rastogi A, Zivcak M, Tripathi D, Yadav S, Kalaji H, Brestic M (2019). Phytotoxic effect of silver nanoparticles in *Triticum aestivum*: Improper regulation of photosystem I activity as the reason for oxidative damage in the chloroplast. Photosynthetica 57(1):209-216. *http://dx.doi.org/10.32615/ps.2019.019*

Rawat K, Kurechia N, Vandre R, Jogi J, Tandia N, Shivhare M, Shakkarpude J, Patil A, Shrivastava N, Caesar D (2022). Application of nanotechnology in veterinary science. Annals of Forest Research 65(1):7047-7076.

Razdan V, Shahnaz E, Kumar S (2011). Influence of weather parameters on purple blotch of onion. Indian Phytopathology 61(1):90-94.

Rhouma A, Mehaoua MS, Mougou I, Rhouma H, Shah KK, Bedjaoui H (2023). Combining melon varieties with chemical fungicides for integrated powdery mildew control in Tunisia. European Journal of Plant Pathology 165(1):189-201. *http://dx.doi.org/10.1007/s10658-022-02599-3*

Rial-Otero R, Arias-Estévez M, López-Periago E, Cancho-Grande B, Simal-Gándara J (2005). Variation in concentrations of the fungicides tebuconazole and dichlofluanid following successive applications to greenhouse-grown lettuces. Journal of Agricultural and Food chemistry 53(11):4471-4475. *https://doi.org/10.1021/jf047848h*

Richards T, Leonard G, Soanes D, Talbot N (2011). Gene transfer into the fungi. Fungal Biology Reviews 25:98-110. *http://dx.doi.org/10.1016/j.fbr.2011.04.003*

Rossouw D, Meiring SP, Bauer FF (2018). Modifying *Saccharomyces cerevisiae* adhesion properties regulates yeast ecosystem dynamics. Msphere 3(5):e00383-18. *https://doi.org/10.1128%2FmSphere.00383-18*

Sagar NA, Khar A, Tarafdar A, Pareek S (2021). Physicochemical and thermal characteristics of onion skin from fifteen Indian cultivars for possible food applications. Journal of Food Quality 2021:1-11. *https://doi.org/10.1155/2021/7178618*

Saritha GNG, Anju T, Kumar A (2022). Nanotechnology-big impact: How nanotechnology is changing the future of agriculture? Journal of Agriculture and Food Research 100457. *http://dx.doi.org/10.1016/j.jafr.2022.100457*

Schwartz HF (2004). Botrytis, downy mildew, and purple blotch of onion. Colorado State University Cooperative Extension.

Schwartz HF, Mohan SK (2007). Compendium of onion and garlic diseases and pests. Compendium of onion and garlic diseases and pests. (Ed. 2).

Shelar A, Nile SH, Singh AV, Rothenstein D, Bill J, Xiao J, Chaskar M, Kai G, Patil R (2023). Recent advances in nano-enabled seed treatment strategies for sustainable agriculture: Challenges, risk assessment, and future perspectives. Nano-Micro Letters 15(1):54. *https://doi.org/10.1007/s40820-023-01025-5*

Singh A, Gautam PK, Verma A, Singh V, Shivapriya PM, Shivalkar S, Sahoo AK, Samanta SK (2020). Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. Biotechnology Reports 25:e00427. *https://doi.org/10.1016/j.btre.2020.e00427*

Smith D, Onions AH (1994). The preservation and maintenance of living fungi. CAB international.

Sofy HL, Abd-El-Moneim M, Hassan M (2020). Effect of potassium salts on onion purple blotch incidence and some physiological and yield parameters in onion seed plants. Fayoum Journal of Agricultural Research and Development 34(2):158-168. *https://dx.doi.org/10.21608/fjard.2020.189889*

Suheri H, Price T (2000). Infection of onion leaves by *Alternaria porri* and *Stemphylium vesicarium* and disease development in controlled environments. Plant Pathology 49(3):375-382. *https://doi.org/10.1046/j.1365-3059.2000.00458.x*

Taylor NP, Cunniffe NJ (2023). Optimal resistance management for mixtures of high-risk fungicides: Robustness to the initial frequency of resistance and pathogen sexual reproduction. Phytopathology 113(1):55-69. *https://doi.org/10.1094/phyto-02-22-0050-r*

Tripathi N, Goshisht MK (2022). Recent advances and mechanistic insights into antibacterial activity, antibiofilm activity, and cytotoxicity of silver nanoparticles. ACS Applied Bio Materials 5(4):1391-1463. *https://doi.org/10.1021/acsabm.2c00014*

Tyagi S, Dube V, Charaya M (1990). Biological control of the purple blotch of onion caused by *Alternaria porri* (Ellis) *ciferri*. International Journal of Pest Management 36(4):384-386. *https://doi.org/10.1080/09670879009371517*

Verstrepen KJ, Klis FM (2006). Flocculation, adhesion and biofilm formation in yeasts. Molecular Microbiology 60(1):5-15. *https://doi.org/10.1111/j.1365-2958.2006.05072.x*

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1):315-322.

Yadav RK, Singh A, Jain S, Dhatt AS (2017). Management of purple blotch complex of onion in Indian Punjab. International Journal of Applied Sciences and Biotechnology 5(4):454-465. *http://dx.doi.org/10.3126/ijasbt.v5i4.18632*

Younas M, Atiq M, Rajput NA, Abbas W, Rizwan Bashir M, Ahmad S, ... Ahmad I (2021). Induction of resistance in onion against purple leaf blotch disease through chemicals. Asian Journal of Agriculture and Biology 3. *http://dx.doi.org/10.35495/ajab.2021.01.039*

Zapata-Sarmiento DH, Palacios-Pala EF, Rodríguez-Hernández AA, Melchor DLM, Rodriguez-Monroy M, Sepulveda-Jimenez G (2020). *Trichoderma asperellum*, a potential biological control agent of *Stemphylium vesicarium*, on onion (*Allium cepa* l.). Biological Control 140:104105. *https://doi.org/10.1016/j.biocontrol.2019.104105*

Zhang Y, Hui J, Qin Q, Sun Y, Zhang T, Sun H, Li M (2021). Transfer-learning-based approach for leaf chlorophyll content estimation of winter wheat from hyperspectral data. Remote Sensing of Environment 267:112724. *http://dx.doi.org/10.1016/j.rse.2021.112724*

Ziedan E, Farrag ES (2011). Application of yeasts as biocontrol agents for controlling foliar diseases on sugar beet plants. International Journal of Agricultural Technology 2011:1789-1799.

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.