

The Future of Agriculture

FSA

Article

Comparative Efficacy of *Trichoderma* spp. and *Bacillus* spp. versus CuO and ZnO Nanoparticles as First Report in Egypt of Eco-Friendly Management of *Botrytis cinerea* Fruit Rot on Blueberries (*Vaccinium corymbosum*)

Mabrouk S. S. Hassan[1] and Ibrahim M. K. Elmaghraby[2,*]

[1]Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, Egypt.

[2]Central Lab. of Organic Agriculture, Agricultural Research Center, Giza 12619, Egypt.

https://doi.org/10.37229/fsa.fja.2026.10.17

[*]**Corresponding author:** ibrahim_elmaghraby@yahoo.com

Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2687-8151

Online ISSN: 2687-8216

Received: 17 August 2026
Accepted: 25 September 2026
Published: 17 October 2026

Publisher's Note: FA stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Blueberry fruit rot was surveyed on cv. Eureka Sunrise and cv. Eureka Dawn in plastic greenhouses in Menoufia and Beheira, Egypt, during 2024 and 2025. Disease incidence and severity were significantly higher on cv. Eureka Sunrise. Four fungi, *Botrytis cinerea*, *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp. were isolated from symptomatic fruits; *B. cinerea* was recovered most frequently, whereas *Penicillium* spp. was least frequent. Pathogenicity assays identified *B. cinerea* as the most aggressive pathogen on both cultivars. *In vitro* assays showed that *Trichoderma album* and *Bacillus subtilis* antagonized *B. cinerea*, with *B. subtilis* and its culture filtrate producing the strongest inhibition. Gas chromatography–mass spectrometry (GC–MS) profiling detected metabolites in both bioagents that may contribute to their antifungal activity. Biological products (Bio Zeid 2.5%, Biocontrol T34 12%, Bio Arc 6.0% and Fungisei 1%) and zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs) were assessed *in vitro*, on detached fruits *in vivo* and under greenhouse conditions. All treatments suppressed mycelial growth relative to the untreated control. The Bio Arc 6.0% and Fungisei 1% were the most effective products, followed by Bio Zeid 2.5% and Biocontrol T34 12%. ZnO and CuO NPs inhibited colony growth by 75.55% and 80%, respectively, but generally provided weaker control than the biological products. Greenhouse applications reduced fruit rot severity in both cultivars across both seasons. Bio Arc 6.0% and Fungisei 1% consistently showed the best performance, whereas the NPs were the least effective. These findings support biological products as components of blueberry fruit rot management. To our knowledge, this study represents the first documented report of *B. cinerea*-associated blueberry fruit rot in Egypt.

Key words: Blueberries, Fruit rot**,** *Trichoderma* spp.**,** *Bacillus* spp.**,** Nanoparticles, Eco-management.

1. Introduction

Blueberry (*Vaccinium corymbosum* L.) is an economically important crop valued for its nutritional quality and phenolic compounds, particularly anthocyanins, which contribute to its antioxidant properties (**Moyer *et al*., 2002**; **Kim *et al*., 2009**). Commercial production spans North America and other regions, and consumer interest has increased because of the fruit's potential health benefits (**Cassidy *et al*., 2013; Evans and Ballen, 2014).** Blueberry production is affected by several diseases, and accurate diagnosis is essential for selecting appropriate management measures (**Scherm *et al*., 2001**). Fruit is particularly perishable because of its delicate skin, high sugar content and susceptibility to damage. Fungal rots can reduce market quality both before and after harvest. Botrytis fruit rot, commonly known as gray mold, is primarily associated with *Botrytis cinerea*, which can infect flowers and fruits and remain latent until symptoms develop during storage or transport. Symptoms commonly include soft rot and gray mycelial growth (**Rivera *et al*., 2013; Bell *et al*., 2021**). Other important fruit diseases include anthracnose, caused by *Colletotrichum* species, and rots associated with *Alternaria*, while *Exobasidium maculosum* and *Monilinia vaccinii-corymbosi* cause fruit spots and mummy berry, respectively (**Wharton and Schilder, 2008; Polashock *et al*., 2017; Ingram *et al*., 2019**). Disease occurrence and severity vary with environmental conditions and cultivar susceptibility. Synthetic fungicides remain widely used against *B. cinerea*, but repeated applications can select for resistant populations and may pose environmental concerns (**Lu *et al*., 2016; Kim *et al*., 2017**). Biological control using antagonistic microorganisms and their metabolites offers an alternative approach. *Bacillus species* can produce antifungal secondary metabolites, while *Trichoderma species* suppress plant pathogens through competition, mycoparasitism and the production of cell-wall-degrading enzymes (**Herrera-Parra *et al*., 2017; Fira *et al*., 2018**). These organisms have shown activity against *B. cinerea* and other postharvest pathogens in different crops. Metal-oxide nanoparticles have also been investigated for plant protection. Zinc oxide nanoparticles (ZnO NPs) may influence fungal growth and provide zinc, an element involved in plant metabolism, while copper-based nanoparticles (CuO NPs) have demonstrated antimicrobial properties (**Kanhed *et al*., 2014; Tymoszuk and Wojnarowicz, 2020; Mohamed *et al*., 2021**). Their effectiveness requires evaluation alongside biological approaches under relevant production conditions. Information on blueberry fruit rot and its management in Egypt remains limited. Therefore, this study investigated fruit rot disease incidence and severity on cv. Eureka Sunrise and cv. Eureka Dawn, identified associated fungal pathogens, assessed the pathogenicity of recovered isolates, and compared selected biological agents, commercial bio-based products and CuO and ZnO NPs for controlling *B. cinerea* in laboratory, fruit and greenhouse experiments.

2. Materials and Methods

Survey of blueberries fruit rot

The survey was conducted in plastic greenhouses in Sadat city, Menoufia governorate, and Badr city, Beheira governorate, Egypt, during the 2024 and 2025 growing seasons. Two-year-old Eureka Sunrise and Eureka Dawn trees were examined. For each cultivar, twenty-five naturally infected trees were selected randomly as five replicates, and 50 fruits were sampled per replicate. Disease incidence and disease severity were calculated as follow:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits of blueberries}}{\text{Total number of inspected blueberries fruits}} \text{ x } 100$$

Disease severity was rated on a 0–5 scale according to the percentage of fruit surface showing rot discoloration, adapted from **Liu *et al*. (1995):**

$$\text{Disease severity (\%)} = \frac{\Sigma\,(n \times v)}{5N} \times 100$$

Where; **n** = Number of infected blueberry fruits in each category (grade), **N** = Total number of the inspected blueberry fruits, and **v** = Numerical value of each category as follows:

0 = healthy fruit (no fruit rot observed).

1 = 1-10% of the blueberry fruit area infected.

2 = 11-25% of the fruit blueberry area infected.

3 = 26-50% of the blueberry fruit area infected.

4 = 51-75% of the blueberry fruit area infected.

5 = 76-100% of the blueberry fruit area infected.

Isolation and identification of the associated fungi

Symptomatic fruits collected in 2024 and 2025 seasons were rinsed, and diseased tissue was cut into approximately 0.5-cm pieces. Pieces were surface disinfected in 1% sodium hypochlorite for 0.5 min, rinsed three times with sterile distilled water, dried on sterile filter paper and plated on potato dextrose agar (PDA). Plates were incubated at 25±1°C for 5 days. Colonies were purified by hyphal-tip transfer and identified morphologically using **Barnett and Hunter (1998)**. Pure cultures were maintained on PDA slants at 4°C, and the frequency of each fungus was determined using the following formula.

$$\text{Frequency (\%)} = \frac{\text{Number of colonies of each fungal species}}{\text{Total number of all fungi}} \times 100$$

Pathogenicity test

Mature healthy fruits of both cultivars (Eureka Sunrise and Eureka Dawn) were collected during 2024 and 2025. Fruits of uniform size without visible wounds or decay were washed, surface-sterilized with 1% sodium hypochlorite for 0.5 min, rinsed in sterile water and air-dried. Three replicates of 10 fruits were used per fungus. *B. cinerea* was inoculated individually into wounded fruits as a spore suspension (1×10^6 spores ml^{-1}); *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp. suspensions of the same concentration, prepared from 5-7-day-old cultures, were sprayed onto fruits (**Lachhab *et al*., 2015**). After 7 days at room temperature, disease severity was rated using the described scale, and the causal fungi were re-isolated to confirm identity.

Use of *Trichoderma album*, *Bacillus subtilis* and metal oxide nanoparticles as control agents

Identified cultures of *Trichoderma album* and *Bacillus subtilis* were supplied by the Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. Cultures were maintained on suitable media; *T. album* was incubated at 22–25°C for 7 days and *B. subtilis* at 30°C for 48 h. The CuO and ZnO NPs were supplied by the nanotechnology laboratory and advanced materials at PPRI, ARC, Egypt.

***In vitro* antagonistic experiments of *Trichoderma album* and *Bacillus subtilis* against *Botrytis cinerea* to control fruit rot of Blueberries:**

Mycelial growth inhibition and treatment efficacy against *B. cinerea* were calculated as percentages using the common equation described for each assay.

$$\text{Inhibation or Efficacy (\%)} = \frac{\text{C} - \text{T}}{\text{C}}\ x\ 100$$

Where; C is the mean measurement in the untreated control (mycelial colony diameter in mm or disease severity %), and T is the mean measurement in the treated plates or fruits.

***In vitro* dual culture assay**

Antagonism was assessed by the dual-culture method of **Yassin *et al*. (2021)**. A 5 mm disc from a 5-day-old *B. cinerea* culture was placed 1 cm from the edge of a 9-cm PDA plate. Opposite it, either a 5-mm *T. album* disc or a loopful of *B. subtilis* was inoculated 1 cm from the edge. Three replicate plates were prepared for each antagonist, with *B. cinerea* only plates as controls. Plates were incubated

at 22-25°C for 5 days, and radial growth inhibition was calculated using the stated mentioned above (**Abd-El-Motty and Shatla, 1981**), **Where** C= average linear growth of the pathogen in control plates (pathogen inoculated alone), and T= average linear growth of the pathogen in presence of the bioagent.

Culture filtrate assay

A 5-mm *T. album* mycelial disc or a loopful of *B. subtilis* was inoculated into 100 ml potato dextrose broth (PDB; pH =7) in 250 ml flasks and incubated for 7 days at suitable temperatures (**You *et al.*, 2016**). Cultures were filtered through Whatman No. 1 paper and sterilized through 0.22-µm millipore filters. Each sterile filtrate was added at 10% (v/v) to molten PDA and poured into 9-cm plates; unamended PDA served as the control. A 5-mm *B. cinerea* disc was placed at each plate's center, and were incubated at 22-25°C for 5 d. Three replicates were used per bioagent. Growth inhibition was calculated by comparing colony diameters in treated and control plates (**Zaki *et al.*, 2021**). **Where** C and T are the mycelial growth diameter of the pathogen *B. cinerea* in the control and filtrate-amended plates, respectively.

In vitro* assessment of antifungal activities of different bio and agrochemical products against *B. cinerea

Products listed in Table 1 were incorporated into molten PDA at the recommended concentrations; PDA without additives served as the control. Each plate was inoculated centrally with a 5-mm disc from a 7-day-old *B. cinerea* culture and incubated at 22-25°C. Six plates per treatment were arranged as three replicates. When the control colonies covered the plate, colony diameters were measured and inhibition was calculated using the equation cited by **Guo *et al*. (2006)**.

***In vivo* evaluation experiments on blueberry fruits**

The Table 1 products were tested on fruits at the same concentrations used *in vitro*. Fruits of both cultivars Eureka Sunrise and Eureka Dawn were washed, treated with the relevant product and needle-wound-inoculated with *B. cinerea* as described above. Inoculated fruits were maintained at room temperature for 15 days under conditions conducive to disease. Disease severity was scored using the scale described by **Mousa *et al*. (2006)**, and treatment efficacy (%) was calculated by comparing treated fruits with control.

Extraction and Gas Chromatography–Mass Spectrometry (GC-MS) characterization of antifungal compounds from *T. album* and *B. subtilis*

For metabolite extraction, approximately 5 ml of inoculum from a 5-day-old *T. album* culture or a 48-h *B. subtilis* culture was transferred separately to 100 ml PDB in 250-ml flasks. *T. album* cultures were incubated at 22–25°C for 7 days and *B. subtilis* at 30-35°C for 48 h. Filtrates were collected through Whatman No. 1 paper, centrifuged at 9,000 rpm for 15 min, extracted with ethyl acetate and concentrated by rotary evaporation (**Zaki *et al.*, 2021**). Extracts were analysed by GC–MS following **Shahiri Tabarestani *et al*. (2016)**, using an Agilent 8890 gas chromatograph coupled to an Agilent 5977B mass spectrometer and an HP-5MS capillary column (30 m × 0.25 mm; 0.25-µm film). The oven was held at 40°C for 2 min, increased to 200°C at 5°C min^{-1}, then to 260°C at 25°C min^{-1} and held for 25 min. Helium flow was 1.1 ml min^{-1} and the injector temperature was 250°C. Mass spectra were acquired at 70 eV over m/z 39–500, and compounds were tentatively identified by comparison with the NIST mass-spectral library.

Greenhouse experiment

Greenhouse trials were conducted on two-year-old cv. Eureka Sunrise and cv. Eureka Dawn plants in Menoufia governorate during the 2024 and 2025 seasons. Five replicates comprising 25 trees were assigned to each treatment, applied at the concentrations listed in Table 1. Sprays were applied

three times: at fruit set and 14 and 21 days before harvest. Control trees received water only. At harvest, 25 fruits were randomly collected from each replicate and kept in cardboard boxes at room temperature for 15 days. Disease severity and treatment efficacy were then assessed as described above (**Hassan *et al*., 2025**).

Table (1). Tested bio and agrochemical products

Product	Active ingredients	Status	*conc./l
Bio Zeid 2.5%	*Trichoderma album*	WP	2.5g
Biocontrol T34 12%	*Trichoderma asperellum* strain T34	WP	2g
Bio Arc 6.0%	*Bacillus megaterium*	WP	2.5g
Fungisei 1%	*Bacillus subtilis* strain (IAB/BSO3 1×10^8/cfu ml)	SC	1.5ml
Zn nanoparticles	ZnO NPs	WP	0.2g
Cu nanoparticles	CuO NPs	WP	0.2g

Where; *conc.= concentration.

Statistical analysis

Analysis of variance (ANOVA) was applied to analyze the data collected over the two growth seasons in accordance with **Snedecor and Cochran (1989)**. Duncan's Multiple Range Test (**Duncan, 1955**) was used to examine the differences between the treatment means.

3. Results

Survey of blueberries fruit rot

Blueberry fruit rot symptoms were observed on cv. Eureka Sunrise and cv. Eureka Dawn in plastic greenhouses across both surveyed governorates (Figures 1 and 2). As shown in Table 2, disease incidence ranged from 6.00% to 9.60%, while severity ranged from 4.88% to 7.68%. Both measures were higher in cv. Eureka Sunrise than cv. Eureka Dawn. In 2024 and 2025 seasons, Menoufia governorate recorded greater incidence and severity than Beheira governorate. The highest incidence (9.60%) occurred on cv. Eureka Sunrise in Menoufia governorate in 2025, whereas the lowest incidence (6.00%) was recorded for cv. Eureka Dawn in both governorates in 2024 and 2025. Cv. Eureka Sunrise in Menoufia showed the highest disease severity in 2024.

Table (2). Disease incidence (D.I.%) and disease severity (D.S.%) of blueberries fruit rot in the two surveyed governorates during 2024 and 2025 seasons

Governorates	Cultivar	2024		2025	
		D.I.%	D.S.%	D.I.%	D.S.%
Menoufia	Eureka Sunrise	9.20a	7.68a	9.60a	6.80a
	Eureka Dawn	6.00c	5.20c	8.88b	4.88d
Beheira	Eureka Sunrise	8.00b	5.60b	8.00c	5.60b
	Eureka Dawn	6.00c	5.20c	6.00d	5.20c

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$).



Figure (1). Overview of the Eureka Sunrise blueberry growth cycle: seedling propagation in peat moss (A); greenhouse cultivation under controlled temperature (21–24°C) and bright, indirect light (B); established plants managed with regular fertilization and disease control (C); fruit harvest upon reaching full blue coloration (D)



Figure (2). Overview of the Eureka Dawn blueberry growth cycle: seedling propagation in peat moss (A); greenhouse cultivation under controlled temperature (21–24°C) and bright, indirect light (B); established plants managed with regular fertilization and disease control (C); fruit harvest upon reaching full blue coloration (D)

Isolation and identification of the associated fungi

Pure cultures were identified microscopically and by cultural characteristics as *Botrytis cinerea*, *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. Their isolation frequencies from symptomatic blueberry fruit are presented in Table 3. Across both study years and governorates, *B. cinerea* was isolated most frequently, whereas *Penicillium* spp. had the lowest frequency.

Table (3). Isolation frequency (%) of fungi recovered from naturally infected blueberry fruit in Menoufia and Beheira governorates during 2024 and 2025 seasons

Isolated fungus	Isolation frequency %			
	2024		2025	
	Menoufia	Beheira	Menoufia	Beheira
Botrytis cinerea	72.22a	66.66a	77.77a	61.12a
Aspergillus spp.	11.11b	16.66b	11.11b	16.66b
Rhizopus spp.	11.11b	11.11c	5.56c	16.66b
Penicillium spp.	5.56c	5.56d	5.56c	5.56c
Total	100	100	100	100

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$).

Pathogenicity test

All identified fungi showed some ability to cause blueberry fruit rot in the pathogenicity test. Table 4 and Figure 3 show that *B. cinerea* produced the greatest mean disease severity on cv. Eureka Sunrise and cv. Eureka Dawn, reaching 69.995% in 2024 and 61.66% in 2025. In comparison, fruit inoculated with *Penicillium* spp. (4.330% and 2.995%), *Rhizopus* spp. (3.665% and 2.995%), and *Aspergillus* spp. (1.995% and 1.330%) developed only small decay spots. In both years, Eureka Sunrise was more susceptible to artificial inoculation than Eureka Dawn.

Table (4). Fruit rot severity caused by fungal species isolated during the 2024 and 2025 surveys on Eureka Sunrise and Eureka Dawn blueberry fruit

Tested fungi	Disease severity (Fruit rot) %					
	2024			2025		
	Eureka Sunrise	Eureka Dawn	Mean	Eureka Sunrise	Eureka Dawn	Mean
Botrytis cinerea	73.33a	66.66a	69.995a	66.66a	56.66a	61.66a
Aspergillus spp.	2.66d	1.33c	1.995d	1.33c	1.33c	1.330c
Rhizopus spp.	4.00c	3.33b	3.665c	3.33b	2.66b	2.995b
Penicillium spp.	5.33b	3.33b	4.330b	3.33b	2.66b	2.995b
Control	00e	00d	00e	00d	00d	00d

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$).

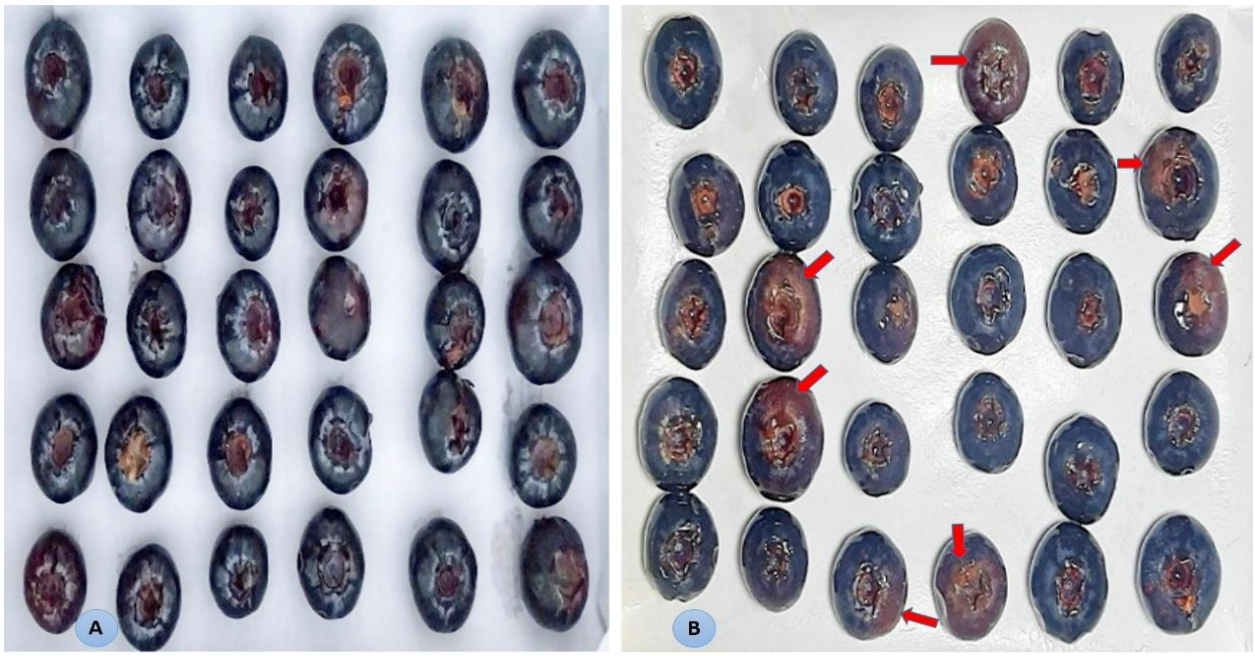


Figure (3). Blueberry fruits after artificial inoculation with *Botrytis cinerea*: control (A) and infected fruits (B)

Efficacy of *T. album* and *B. subtilis* against *B. cinerea* in the dual culture assay

Data in Table 5 and Figures 4 and 5, *T. album* and *B. subtilis* antagonized *B. cinerea*, inhibiting its mycelial growth by 73.33% and 77.77%, respectively. *B. subtilis* produced the stronger antagonistic effect. Scanning electron microscopy illustrated hyphal interactions and the antagonistic or mycoparasitic effects of both agents against *B. cinerea* (Figure 5).

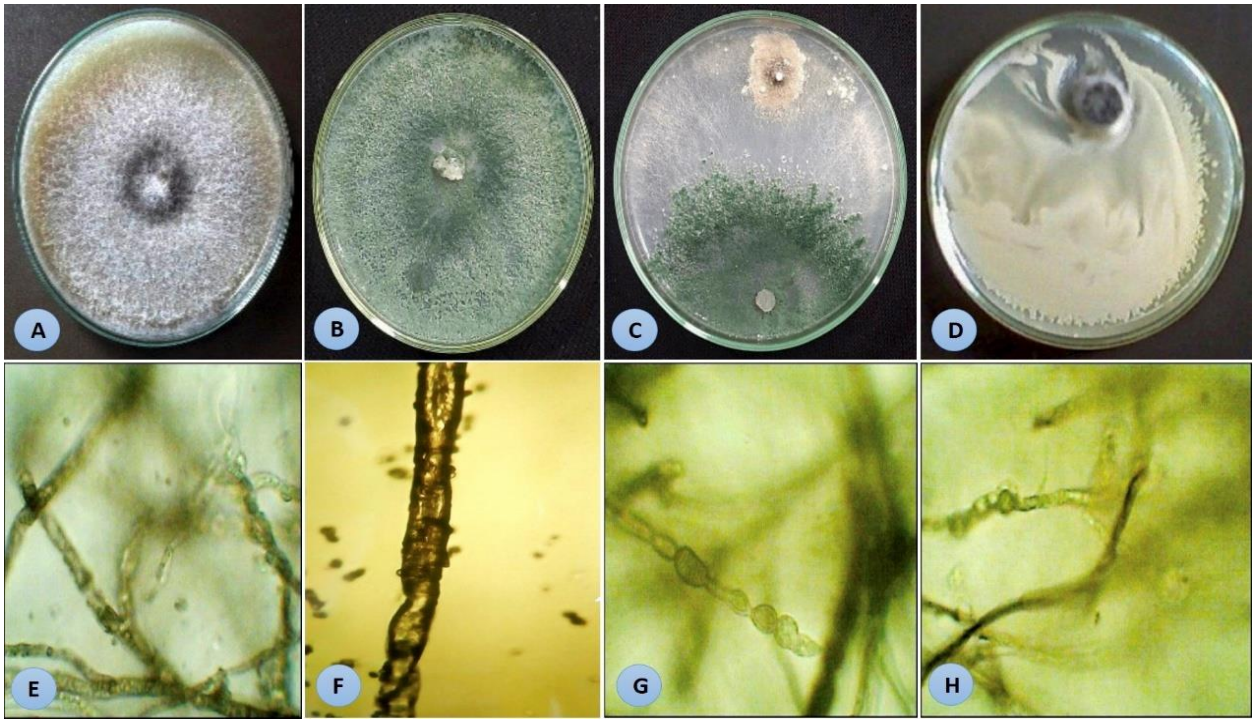


Figure (4). Colony morphology of *Botrytis cinerea* on PDA (A) and *Trichoderma album* (B); interactions of *B. cinerea* with *T. album* (C) and *Bacillus subtilis* (D); and malformed *B. cinerea* hyphae in the presence of *T. album* (E, F) and *B. subtilis* (G, H)

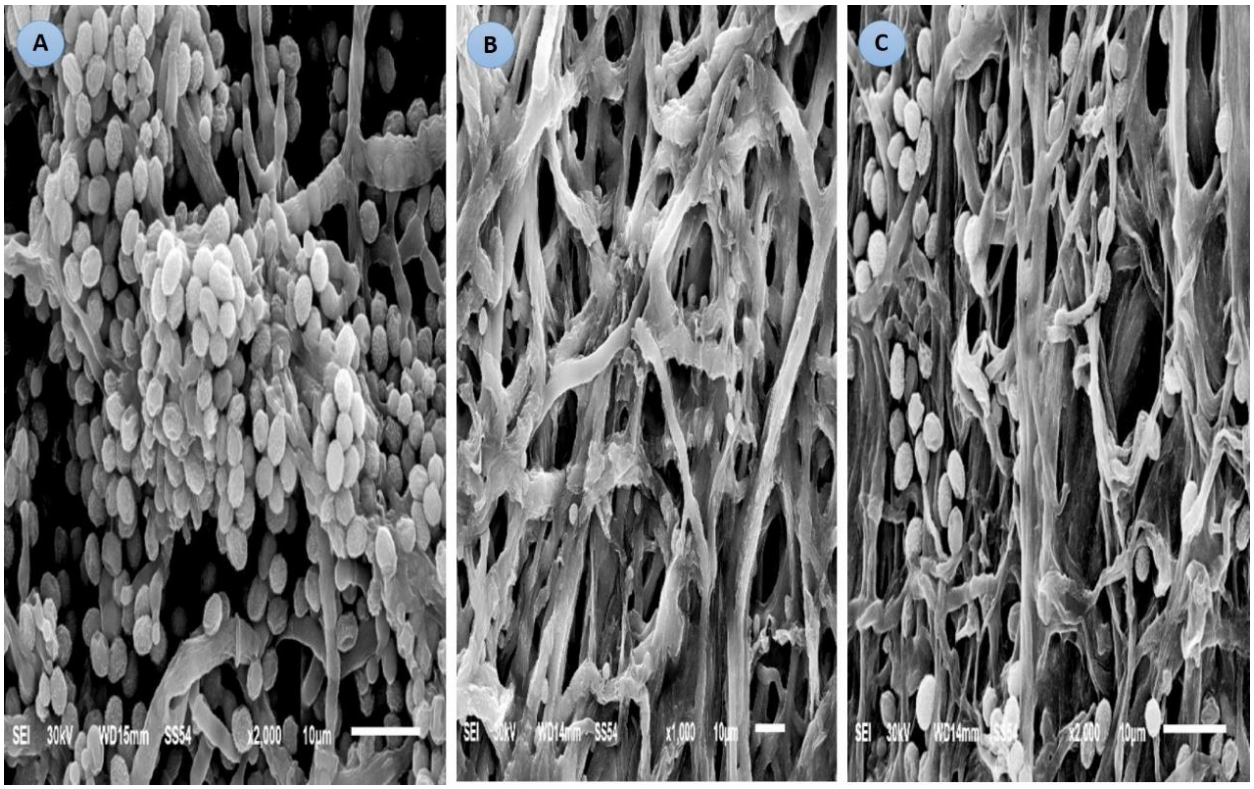


Figure (5). Scanning electron micrographs showing hyphal interactions between *Botrytis cinerea* (control) and *Trichoderma album* (A, B), and between *B. cinerea* (A) and *Bacillus subtilis* (C)

Table (5). Dual culture efficacy of *Trichoderma album* and *Bacillus subtilis* against *Botrytis cinerea*

Bioagent	Mycelial growth diameters (mm) of *B. cinerea*	Reduction (%)
Trichoderma album	24b	73.33
Bacillus subtilis	20c	77.77
Control	90a	00

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$).

Efficacy of *T. album* and *B. subtilis* against *B. cinerea* using culture filtrates

Table 6 presents the inhibitory effects of *T. album* and *B. subtilis* culture filtrates against *B. cinerea*. Both significantly suppressed mycelial growth, with inhibition rates of 80.00% and 82.22%, respectively. The *B. subtilis* filtrate had the greater inhibitory effect.

Table (6). Efficacy of *Trichoderma album* and *Bacillus subtilis* culture filtrates against *Botrytis cinerea*

Bioagent Filtrate	Mycelial growth diameters (mm) of *B. cinerea*	Reduction (%)
Trichoderma album	18c	80.00
Bacillus subtilis	16b	82.22
Control	90a	00

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$).

In vitro evaluation of bio- and agrochemical products against *B. cinerea*, the causal agent of blueberry fruit rot

Table 7 show that all evaluated bio and agrochemical products reduced the linear growth of *B. cinerea* on PDA medium as compared to the control. The most effective treatments were Bio Arc 6.0%, Fungisei 1%, Bio Zeid 2.5%, and Biocontrol T34 12%, all of which prevented the tested fungus from growing. Nevertheless, only 75.55% and 80%, respectively, of the investigated pathogens' colony growth were reduced by ZnO and CuO nanoparticles.

Table (7). *In vitro* efficacy of bio- and agrochemical products against *Botrytis cinerea*, the causal agent of blueberry fruit rot

Product	*conc./l	Colony diameter (mm) of *B. cinerea*	Reduction (%)
Bio Zeid 2.5%	2.5g	12d	86.66
Biocontrol T34 12%	2g	12d	86.66
Bio Arc 6.0%	2.5g	10e	88.88
Fungisei 1%	1.5ml	10e	88.88
ZnO nanoparticles	0.2g	22b	75.55
CuO nanoparticles	0.2g	18c	80
Control	---	90a	---

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$); *conc.= concentration.

In vivo evaluation of treatments on blueberry fruit

Mature Eureka Sunrise and Eureka Dawn fruit were sprayed separately with the test treatments and then artificially inoculated with *B. cinerea*. Disease severity was assessed after 15 days at room temperature. All treatments reduced severity relative to the control, which generally showed the highest values (Table 8). Across both seasons, Bio Arc 6.0% and Fungisei 1% were most effective, followed by Biocontrol T34 12% and Bio Zeid 2.5%; these four treatments outperformed ZnO and CuO nanoparticles.

Table (8). Efficacy (Eff.%) and disease severity (D.S.%) of bio- and agrochemical products against blueberry fruit rot on cvs. Eureka Sunrise and Eureka Dawn under *in vivo* artificial inoculation in 2024 and 2025 seasons

Product	*conc./l	2024				2025			
		Eureka Sunrise		Eureka Dawn		Eureka Sunrise		Eureka Dawn	
		D.S.(%)	Eff.(%)	D.S.(%)	Eff.(%)	D.S.(%)	Eff.(%)	D.S.(%)	Eff.(%)
Bio Zeid 2.5%	2.5g	5.33d	92.00	6.00d	91.81	4.66d	91.77	5.33e	92.38
Biocontrol T34 12%	2g	4.66e	93.00	6.00d	91.81	4.00e	92.94	6.00d	91.42
Bio Arc 6.0%	2.5g	2.66f	96.00	4.00e	94.54	3.33f	94.12	5.33e	92.38
Fungisei 1%	1.5ml	2.66f	96.00	4.00e	94.54	4.00e	92.94	6.00d	91.42
ZnO (NPs)	0.2g	14.00b	78.99	14.66b	80.90	10.00b	82.35	12.00b	82.85
CuO (NPs)	0.2g	10.00c	84.99	12.00c	83.63	6.00c	89.41	10.00c	85.71
Control	Water	66.66a	---	73.33a	---	56.66a	---	70.00a	---

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$); *conc.= concentration.

GC–MS characterization of antifungal compounds extracted from *T. album* and *B. subtilis*

Gas chromatography–mass spectrometry (GC–MS) was used to identify compounds in *T. album* and *B. subtilis* extracts associated with inhibition of *B. cinerea*. The *T. album* extract contained palmitic, oleic, and linoleic acids, ergosterol, benzeneacetaldehyde, 2-methyl-1-hexadecanol, phthalic acid di(2-propylpentyl) ester, and other compounds (Table 9). Compounds detected in the *B. subtilis* extract included propanoic acid 2-methyl-; pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-; L-5-propylthiomethylhydantoin; benzeneacetic acid; and others (Table 10).

Greenhouse experiment

Six bio- and agrochemical treatments, at the concentrations used in the *in vitro* and *in vivo* assays, were evaluated against naturally occurring fruit rot on cv. Eureka Sunrise and cv. Eureka Dawn in greenhouses during 2024 and 2025. All treatments lowered disease severity relative to the control (Table 11). Across both seasons, Bio Arc 6.0% and Fungisei 1% performed best, followed by Bio Zeid 2.5% and Biocontrol T34 12%. ZnO and CuO nanoparticles were the least effective treatments.

Table (9). Gas chromatography–mass spectrometry (GC–MS) characterization of antifungal compounds extracted from *Trichoderma album*

Peak	Retention Time (min)	Compound Name	Area %	Activity	References
1	36.800	Oleic Acid	2.690	Antioxidant	**Ertürk *et al.* (2016)**
2	35.230	Palmitic acid vinyl ester	2.490	Antimicrobial	**Ertürk *et al.* (2016)**
3	33.670	2-Methyl-1-hexadecanol	0.930	Antioxidant	**Tahara *et al.* (1979)**
4	28.900	Retinal	1.850	Antioxidant	--
5	12.660	Benzeneacetaldehyde	1.110	Antioxidant	--
6	17.800	2,5-Dimethylbenzaldehyde	0.900	Antimicrobial	--
7	18.00	Palmitic acid	2.998	Antimicrobial	**Teresa *et al.* (2014)**
8	36.600	Linoleic acid	2.830	Antimicrobial	--
9	40.250	Phthalic acid, di(2-propylpentyl) ester	2.100	Antimicrobial	--
10	32.00	Ergosterol	5.000	Antifungal	**Mulatu *et al.* (2022)**
11	4.440	Furfural	0.550	--	--
12	38.000	Ethyl iso-allocholate	1.002	--	--
13	36.765	Ethyl 13-methyltetradecanoate	0.995	Antimicrobial	--
14	35.089	Tributyl acetylcitrate	2.220	--	--
15	26.900	Octadecadienoic acid	8.900	Antimicrobial	--
16	31.789	Tetradecanoic acid	3.222	Antimicrobial	--

Table (10). Gas chromatography–mass spectrometry (GC–MS) characterization of compounds extracted from *Bacillus subtilis*

Peak	Retention Time (min)	Compound Name	Area %	Activity	References
1	3.344	Propanoic acid, 2-methyl-	1.56	Antimicrobial	**Ertürk *et al.* (2016)**
2	5.480	2-methylbutanoic acid	2.21	Antimicrobial	**Hayashida-Soiza *et al.* (2008)**
3	3.070	L-5-Propylthiomethylhydantoin	22.73	Antimicrobial	**Tahara *et al.* (1979)**
4	6.090	Phenol	0.93	Antifungal	**Teresa *et al.* (2014)**
5	13.601	Benzeneacetic acid	3.94	Antifungal, Antimicrobial	**Kim *et al.* (2004)**
6	19.886	Pyrrolo[1,2-a]pyrazine-1,4-dione hexahydro-(Isomer I)	2.14	Antifungal	**Jiang *et al.* (2014)**
7	19.890	Pyrrolo[1,2-a]pyrazine-1,4-dione hexahydro-(Isomer II)	2.56	Antimicrobial	**Susanti *et al.* (2013)**
8	21.555	9-Octadecen-1-ol, (Z)-	4.44	Antifungal and antibacterial	**Hossain *et al.* (2016)**
9	22.339	2,5-Piperazinedione 3,6-bis(2-methylpropyl)-	1.54	Antimicrobial	**Altaee *et al.* (2016)**

Table (11). Efficacy (Eff.%) and disease severity (D.S.%) of preharvest bio- and agrochemical treatments against blueberry fruit-rot on cvs. Eureka Sunrise and Eureka Dawn after 15 days of storage at room temperature

Product	*conc./l	2024				2025			
		Eureka Sunrise		Eureka Dawn		Eureka Sunrise		Eureka Dawn	
		D.S.(%)	Eff.(%)	D.S.(%)	Eff.(%)	D.S.(%)	Eff.(%)	D.S.(%)	Eff.(%)
Bio Zeid 2.5%	2.5g	7.20c	89.15	8.00d	88.88	7.20e	89.41	8.00d	89.13
Biocontrol T34 12%	2g	7.20c	89.15	8.00d	88.88	8.00d	88.23	8.00d	89.13
Bio Arc 6.0%	2.5g	3.20e	95.18	4.00f	94.44	4.00g	94.11	5.60f	92.39
Fungisei 1%	1.5ml	4.80d	92.77	5.60e	92.22	5.60f	91.76	6.40e	91.30
Zn (NPs)	0.2g	10.40b	84.33	12.00c	83.33	11.20c	83.52	12.00c	83.69
Cu (NPs)	0.2g	10.40b	84.33	14.40b	80.55	14.40b	78.82	15.20b	79.34
Control	Water	66.40a	---	72.00a	---	68.00a	---	73.60a	---

Different letters within a column indicate significant differences according to ANOVA followed by Duncan's test ($P \leq 0.05$); *conc.= concentration, NPs= nanoparticles.

4. Discussion

Blueberry (*Vaccinium corymbosum* L.), a deciduous shrub native to North America, is an economically important crop cultivated across the Americas, Europe, Asia, Africa, and Oceania (**FAOSTAT, 2024**). Its nutritional value and bioactive compounds, particularly antioxidants, have increased its importance in the food and pharmaceutical industries (**Nestby *et al*., 2011; Norberto *et al*., 2013**). Growing commercial interest and the movement of plant material across regions may also facilitate the introduction of pathogens into new production areas (**Ma *et al*., 2018**). Stem blight and

dieback are common blueberry diseases associated with fungal pathogens; they reduce plant longevity and fruit production (**Scarlett *et al*., 2019; Hilário *et al*., 2020**). Several genera, including Botryosphaeria, Diaporthe, Neofusicoccum, Neopestalotiopsis, and Phoma-like fungi, have been associated with these symptoms. The principal canker groups include Botryosphaeria, Fusicoccum, and Phomopsis; *Botryosphaeria dothidea* is commonly linked to Botryosphaeria stem blight, whereas *B. corticis* is associated with stem canker (**Polashock *et al*., 2017**).

The present study showed that blubbery fruit rot disease incidence and severity varied with cultivar, governorate and season, with cv. Eureka Sunrise generally more susceptible than cv. Eureka Dawn. The highest incidence occurred on cv. Eureka Sunrise in Menoufia during 2025. *Botrytis cinerea* was the predominant fungus recovered from symptomatic blueberry fruits in both governorates and seasons. In contrast, *Penicillium* spp. showed the lowest isolation frequency. *B. cinerea* produced markedly greater fruit rot severity than the other tested fungi on both cultivars in both seasons, confirming its strong pathogenic potential under the assay conditions. Cv. Eureka Sunrise generally showed greater disease severity than cv. Eureka Dawn, whereas the uninoculated controls remained symptomless. *T. album* and *B. subtilis* suppressed *B. cinerea* mycelial growth in dual culture, with *B. subtilis* achieving a greater reduction than *T. album*. These results demonstrate substantial antagonistic activity *in vitro*, particularly by *B. subtilis*. Culture filtrates of both biological agents strongly inhibited *B. cinerea*, reducing mycelial growth by *T. album* and *B. subtilis*. The slightly greater inhibition by the *B. subtilis* filtrate suggests that diffusible metabolites may contribute to its antagonistic effect.

B. cinerea is a major plant pathogen because of its broad host range, diverse infection strategies, genetic variability, short life cycle, and ability to persist as mycelium, conidia, or sclerotia. It causes substantial economic losses (**Dean *et al*., 2012**). Although synthetic fungicides remain widely used to manage *B. cinerea*, repeated applications can select for resistance, making chemical control less reliable (**Lu *et al*., 2016**). Fungicides may also adversely affect human and animal health and environmental quality, including air, soil, and water (**Kim *et al*., 2017**). In response to health concerns and environmental regulations, the use of chemical agents against plant pathogens has declined in some regions, particularly the United States (**Carbú *et al*., 2016**). Consequently, microorganisms and their metabolites have attracted interest as potentially more environmentally compatible disease control alternatives that can preserve beneficial microorganisms (**Parnell *et al*., 2016**).

The findings of this study demonstrate that all tested products reduced *B. cinerea* colony growth relative to the untreated control, with Bio Arc 6.0% and Fungisei 1% producing the greatest reductions. CuO and ZnO nanoparticles were less effective under the tested *in vitro* conditions. All treatments substantially reduced fruit rot severity compared with the control on both cultivars in both seasons. Bio Arc 6.0% and Fungisei 1% were consistently among the most effective treatments, whereas the nanoparticles provided comparatively lower control, particularly ZnO. Under greenhouse conditions, all treatments reduced fruit-rot severity relative to the control, with Bio Arc 6.0% generally providing the greatest protection across cultivars and seasons. Fungisei 1%, Bio Zeid 2.5%, and Biocontrol T34 12% also performed well, while nanoparticle treatments were less effective, particularly CuO in 2025.

Among microbial antagonists, *B. subtilis* and *T. harzianum* have demonstrated antifungal activity. *Trichoderma* spp. can act as hyperparasites and secrete chitinases and β-1,3-glucanases that degrade *B. cinerea* cell walls (**Ting and Chai, 2015**). *Bacillus* spp. can form biofilms and produce cyclic lipopeptides, including surfactin and iturin, which inhibit spore germination (**Saiyam *et al*., 2024**). *B. subtilis*, combined with *Metschnikowia pulcherrima*, has controlled *B. cinerea* on grapes (**Mondino *et al*., 2012**). Other studies reported antagonism against *Colletotrichum gloeosporioides* from papaya (**Hasan *et al*., 2013**), suppression of *Rhizopus stolonifer* on peach *in vitro* and *in vivo* (**Wang *et al*., 2013**), and inhibition of tomato postharvest pathogens, including Rhizopus, Fusarium, and *Penicillium steckii*, by *T. harzianum* (**El-Katatny *et al*., 2011**). Commercial products containing Bacillus and Trichoderma species have also shown inhibitory effects against pathogens affecting diverse crops. *Trichoderma* spp. are widely investigated as biological control agents, with *T. harzianum* among the most studied for managing *B. cinerea* and other fungal pathogens (**Herrera-Parra *et al*., 2017; Zhang *et al*., 2018**). Their secondary metabolites have antifungal, antibacterial, and other bioactive properties and can act against pathogens such as *Fusarium* spp., *B. cinerea*, *Pythium* spp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Ustilago maydis* (**Hermosa *et al*., 2014**).

Metal oxide nanoparticles, particularly copper oxide (CuO-NPs) and zinc oxide (ZnO-NPs), may affect plant cells and influence physiological and biochemical processes (**Gowtham *et al*., 2024**). ZnO-NPs are also used as a zinc source to support crop growth and productivity. Compared with ionic zinc (Zn^{2+}) and larger particles, foliar application of ZnO-NPs may improve plant growth and yield (**Awan *et al*., 2021**), while potentially interfering with fungal nutrient uptake (**Ogwu and Izah, 2025**). Their nanoscale properties may facilitate passage through plant cell membranes and support intracellular delivery and metabolic integration (**Miliauskienė *et al*., 2022**). Zinc contributes to plant defense, membrane stability, protein translation, cell elongation, and responses to environmental stress; accordingly, ZnO-NPs have been investigated for improving productivity and yield across plant species (**Adil *et al*., 2022**).

GC–MS profiling of *T. album* and *B. subtilis* extract identified a diverse range of compounds reported to have antimicrobial or antifungal activities. These constituents provide candidate explanations for the observed antagonism against *B. cinerea*, although their individual contributions require targeted confirmation. These results are in the same trend with those recorded by (**Hayashida-Soiza *et al*., 2008; Susanti *et al*., 2013; Teresa *et al*., 2014; Ertürk *et al*., 2016; Mulatu *et al*., 2022**).

5. Conclusions

Fruit rot was observed on cv. Eureka Sunrise and cv. Eureka Dawn in Egyptian blueberry greenhouses, and pathogenicity tests identified *Botrytis cinerea* as the most aggressive associated fungus. The study provides the first documented report of *B. cinerea*-associated blueberry fruit rot in Egypt. Among the tested approaches, Bio Arc 6.0% and Fungisei 1% gave the strongest control, followed by Bio Zeid 2.5% and Biocontrol T34 12%; CuO and ZnO nanoparticles were less effective under greenhouse conditions. *Trichoderma album* and *Bacillus subtilis* also inhibited *B. cinerea*, and GC–MS detected potentially antifungal metabolites. Further multisite trials should optimize application timing and formulations and assess integrated use of bioagents and nanoparticles to support sustainable blueberry production.

Acknowledgement

The authors gratefully acknowledge the management and workers of the commercial blueberry farms in Sadat city (Menoufia governorate) and Badr city (Beheira governorate, Egypt) for their invaluable support and cooperation throughout this study. Sincere appreciation is also extended to the researchers and staff at the Plant Pathology Research Institute, El-Qanater El-Khayria Horticultural Research Station, and the Central Lab of Organic Agriculture for their kind technical assistance and support.

Novelty Statement

This study provides the first documented report of *Botrytis cinerea*-associated fruit rot on greenhouse-grown blueberries, specifically cv. Eureka Sunrise and cv. Eureka Dawn in Egypt. Beyond identifying *B. cinerea* as the pathogen, this work establishes a comprehensive framework for sustainable management by demonstrating the efficacy of indigenous bioagents, *Trichoderma album*, *Bacillus subtilis* and their bioactive metabolites, alongside commercial biopesticides and metal oxide nanoparticles, under *in vitro*, *in vivo* and greenhouse conditions.

Funding source

This study did not receive any fund.

Conflict of interests

The authors have declared no conflicts of interest.

References

Abd-El-Motty, T. H. and Shatla, M. N. (1981). Biological control of white rot disease of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. Journal of Phytopathology, 100(1): 29-23. https://doi.org/10.1111/j.1439-0434.1981.tb03287.x

Adil, M.; Bashir, S.; Bashir, S.; Aslam, Z.; Ahmad, N.; Younas, T.; Asghar, R. M. A.; Alkahtani, J.; Dwiningsih, Y. and Elshikh M. S. (2022). Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.932861

Altaee, N.; Kadhim, M. J. and Hameed, I. H. (2016). Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. International Journal of Toxicological and Pharmacological Research, 8(6): 462-470. https://doi.org/10.5281/zenodo.12805616

Awan, S.; Shahzadi, K.; Javad, S.; Tariq, A.; Ahmad, A. and Ilyas, S. (2021). A preliminary study of influence of zinc oxide nanoparticles on growth parameters of *Brassica oleracea* var *italic*. Journal of the Saudi Society of Agricultural Sciences, 20(1): 18–24. https://doi.org/10.1016/j.jssas.2020.10.003

Barnett, H. L. and Hunter, B. B. (1998). Illustrated Genera of Imperfect Fungi. 4th Ed., The American Phytopathological Society Press, St. Paul, MN, USA, 218 p.

Bell, S. R.; Hernández Montiel, L. G.; González Estrada, R. R. and Gutiérrez Martínez, P. (2021). Main diseases in postharvest blueberries, conventional and eco-friendly control methods: A review. LWT-Food Science and Technology, 149: 112046. https://doi.org/10.1016/j.lwt.2021.112046

Carbú, M.; González-Rodriguez, V. E.; Garrido, C.; Husaini, A. M. and Cantoral, J. M. (2016). New biocontrol strategies for strawberry fungal pathogens. *In*: Strawberry: Growth, Development and Diseases; Husaini, A. M. and Neri, D., Eds.; CABI international, Boston, MA, USA; pp. 196-211. https://doi.org/10.1079/9781780646633.0196

Cassidy, A.; Mukamal, K. J.; Liu, L.; Franz, M.; Eliassen, A. H. and Rimm, E. B. (2013). High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. Circulation, 127(2): 188–196. https://doi.org/10.1161/CIRCULATIONAHA.112.122408

Dean, R.; Van Kan, J. A. L.; Pretorius, Z. A.; Hammond-Kosack, K. E.; Di Pietro, A.; Spanu, P. D.; Rudd, J. J.; Dickman, M.; Kahmann, R. and Ellis, J. and Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology, 13(4): 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11(1): 1-42. http://dx.doi.org/10.2307/3001478

El-Katatny, M. H.; El-Katatny, M. S.; Fadl-Allah, E. M. and Emam, A. S. (2011). Antagonistic effect of two isolates of *Trichoderma harzianum* against postharvest pathogens of tomato (*Lycopersicon esculentum*). Archives of Phytopathology and Plant Protection, 44(7): 637-654. https://doi.org/10.1080/03235400903266438

Ertürk , Ö; Çil, E.; Yoloğlu, N. and Yavuz, C. (2016). An *in vitro* study on antimicrobial and antioxidant activity of propolis from rize province of Turkey. Mellifera, 16(1): 4-18. https://izlik.org/JA98ZC35SS

Evans, E. A. and Ballen, F. H. (2014). An overview of US blueberry production, trade, and consumption, with special reference to Florida: FE952/FE952. EDIS, 2014(8), UF/IFAS Extension, Institute of Food and Agricultural Sciences, University of Florida, Gainesville (FL). https://doi.org/10.32473/edis-fe952-2014.

FAOSTAT, (2024). Statistics of Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/#home.

Fira, D.; Dimkić, I.; Berić, T.; Lozo, J. and Stanković, S. (2018). Biological control of plant pathogens by *Bacillus species*. Journal of Biotechnology, 285: 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Gowtham, H. G.; Shilpa, N.; Singh, S. B.; Aiyaz, M.; Abhilash, M. R.; Nataraj, K.; Amruthesh, K. N.; Ansari, M. A.; Alomary, M. N.; and Murali, M. (2024). Toxicological effects of nanoparticles in plants: Mechanisms involved at morphological, physiological, biochemical and molecular levels. Plant Physiology and Biochemistry, 210: 108604. https://doi.org/10.1016/j.plaphy.2024.108604

Guo, Z.; Chen, R.; Xing, R.; Liu, S.; Yu, H.; Wang, P.; Li, C. and Li, P. (2006). Novel derivatives of chitosan and their antifungal activities in vitro. Carbohydrate Research, 341(3): 351–354. https://doi.org/10.1016/j.carres.2005.11.002

Hasan, M. F.; Mahmud, T. M. M.; Ding, P. and Kadir, J. (2013). Control of postharvest anthracnose disease and quality of papaya using *Bacillus subtilis* strain B34 enhanced with sodium bicarbonate and *Aloe vera* gel. Acta Horticulturae, 1012: 653-660. https://doi.org/10.17660/ActaHortic.2013.1012.88

Hassan, M. S. S.; Shehata, A. S. F. and Elmaghraby, I. M. K. (2025). Efficacy of pre-harvest applications of various bio and agrochemical products on anthracnose disease of mango fruits and keeping quality during storage conditions. Egyptian Journal of Phytopathology, 53(1): 55-76. https://doi.org/10.21608/EJP.2025.417823

Hayashida-Soiza, G.; Uchida, A.; Mori, N.; Kuwahara, Y. and Ishida, Y. (2008). Purification and characterization of antibacterial substances produced by a marine bacterium *Pseudoalteromonas haloplanktis* strain. Journal of Applied Microbiology, 105(5): 1672-1677. https://doi.org/10.1111/j.1365-2672.2008.03878.x

Hermosa, R.; Cardoza, R. E.; Rubio, M. B.; Gutiérrez, S. and Monte, E. (2014). Secondary Metabolism and Antimicrobial Metabolites of Trichoderma. *In*: Gupta, V. G.; Schmoll, M.; Herrera-Estrella, A.; Upadhyay, Dr. R. S.; Druzhinina, I. and Tuohy, M., Eds., Biotechnology and Biology of Trichoderma, Elsevier, Amsterdam, Chapter 10, 125-137. https://doi.org/10.1016/B978-0-444-59576-8.00010-2

Herrera-Parra, E.; Cristóbal-Alejo, J. and Ramos-Zapata, J. A. (2017). *Trichoderma* strains as growth promoters in *Capsicum annuum* and as biocontrol agents in *Meloidogyne incognita*. Chilean Journal of Agricultural Research, 77(4): 318-324. http://dx.doi.org/10.4067/S0718-58392017000400318

Hilário, S.; Lopes, A.; Santos, L. and Alves, A. (2020). *Botryosphaeriaceae* species associated with blueberry stem blight and dieback in the Centre Region of Portugal. European Journal of Plant Pathology, 156: 31–44. https://doi.org/10.1007/s10658-019-01860-6

Hossain, M. T.; Khan, A.; Chung, E. J.; Rashid, M. H. and Chung, Y. R. (2016). Biological control of rice Bakanae by an endophytic *Bacillus oryzicola* YC7007. The Plant Pathology Journal, 32(3): 228-241. http://dx.doi.org/10.5423/PPJ.OA.10.2015.0218

Ingram, R. J.; Ludwig, H. D. and Scherm, H. (2019). Epidemiology of exobasidium leaf and fruit spot of rabbiteye blueberry: Pathogen overwintering, primary infection, and disease progression on leaves and fruit. Plant Disease, 103(6): 1293-1301. https://doi.org/10.1094/PDIS-09-18-1534-RE

Jiang, Y.; Han, Q.; Shen, R.; Zang, X. and Wang B. (2014). Synthesis and antimicrobial activity of some new 4*H*-pyrrolo[1,2-a] benzimidazoles. Chemical Research in Chinese Universities, 30(5): 755-758. https://doi.org/10.1007/s40242-014-4147-2

Kanhed, P.; Birla, S.; Gaikwad, S.; Gade, A.; Seabra, A. B., Rubilar, O.; Duran, N. and Rai, M. (2014). *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. Materials Letters, 115: 13-17. https://doi.org/10.1016/j.matlet.2013.10.011

Kim, K. H.; Kabir, E. and Jahan, S. A. (2017). Exposure to pesticides and the associated human health effects. Science of The Total Environment, 575: 525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009

Kim, Y-H.; Bang, C-Y.; Won, E-K.; Kim, J-P. and Choung, S-Y. (2009). Antioxidant activities of *Vaccinium uliginosum* L. extract and its active components. Journal of Medicinal Food, 12(4): 885-892. https://doi.org/10.1089/jmf.2008.1127

Kim, Y.; Cho, J-Y.; Kuk, J-H.; Moon, J-H.; Cho, J-I.; Kim, Y-C. and Park, K-H. (2004). Identification and antimicrobial activity of phenylacetic acid produced by *Bacillus licheniformis* isolated from fermented

soybean, Chungkook-Jang. Current Microbiology, 48(4): 312-317. https://doi.org/10.1007/s00284-003-4193-3

Lachhab, N.; Sanzani, S. M.; Fallanaj, F.; Youssef, K.; Nigro, F.; Boselli, M. and Ippolito, A. (2015). Protein hydrolysates as resistance inducers for controlling green mould of citrus fruit. Acta Horticulturae, 1065: 1593-1598. https://doi.org/10.17660/ActaHortic.2015.1065.203

Liu, L.; Kloepper, J. W. and Tuzun, S. (1995). Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology, 85(6): 695-698. https://doi.org/10.1094/Phyto-85-695

Lu, X. H.; Jiao, X. L.; Hao, J. J.; Chen, A. J. and Gao, W. W. (2016). Characterization of resistance to multiple fungicides in *Botrytis cinerea* populations from Asian ginseng in northeastern China. European Journal of Plant Pathology, 144: 467–476. https://doi.org/10.1007/s10658-015-0786-5

Ma, L.; Sun, Z.; Zeng, Y.; Luo, M. and Yang, J. (2018). Molecular mechanism and health role of functional ingredients in blueberry for chronic disease in human beings. International Journal of Molecular Sciences, 19(9): 2785. https://doi.org/10.3390/ijms19092785

Miliauskienė, J.; Brazaitytė, A.; Sutulienė, R.; Urbutis, M. and Tučkutė, S. (2022). ZnO nanoparticle size-dependent effects on Swiss chard growth and nutritional quality. Agriculture, 12(11): 1905. https://doi.org/10.3390/agriculture12111905

Mohamed, A. A.; Abu-Elghait, M.; Ahmed, N. E. and Salem, S. S. (2021). Eco-friendly Mycogenic Synthesis of ZnO and CuO Nanoparticles for in Vitro Antibacterial, Antibiofilm, and Antifungal Applications. Biological Trace Element Research, 199: 2788–2799. https://doi.org/10.1007/s12011-020-02369-4

Mondino, P.; Casanova, L.; Calero, G.; Bentancur, O. and Alaniz, S. (2012). Zimevit: a biofungicide that combines the action of one bacteria and one yeast for the control of gray mold of grape caused by *Botrytis cinerea*. Revista Brasileira de Agroecologia (Brazilian Journal of Agroecology), 7(3): 165-172.

Mousa, M. S.; Ali, M. K.; Mosa, A. A. and Elewa, I. S. (2006). Root rot disease of olive transplants and its biological control. Arab Universities Journal of Agricultural Sciences, 14(1): 395-409. https://doi.org/10.21608/ajs.2006.15576

Moyer, R. A.; Hummer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*. Journal of Agricultural and Food Chemistry, 50(3): 519–525. https://doi.org/10.1021/jf011062r

Mulatu, A.; Megersa, N.; Tolcha, T.; Alemu, T. and Vetukuri, R. R. (2022). Antifungal compounds, GC-MS analysis and toxicity assessment of methanolic extracts of *Trichoderma* species in an animal model. PLoS ONE, 17(9): e0274062. https://doi. org/10.1371/journal.pone.0274062

Nestby, R.; Percival, D.; Martinussen, I.; Opstad, N. and Rohloff, J. (2011). The European blueberry (*Vaccinium myrtillus* L.) and the potential for cultivation. A review. The European Journal of Plant Science and Biotechnology, 5(1): 5–16.

Norberto, S.; Silva, S.; Meireles, M.; Faria, A.; Pintado, M. and Calhau C. (2013). Blueberry anthocyanins in health promotion: a metabolic overview. Journal of Functional Foods, 5(4): 1518-1528. https://doi.org/10.1016/j.jff.2013.08.015

Ogwu, M. C. and Izah S. C. (2025). Nanotechnology for fungal pathogen control in crops: innovations, public health impacts, and disease prevention. Frontiers in Fungal Biology, 6: 1653214. https://doi.org/10.3389/ffunb.2025.1653214

Parnell, J. J.; Berka, R.; Young, H. A.; Sturino, J. M.; Kang, Y.; Barnhart, D. M. and DiLeo, M. V. (2016). From the lab to the farm: An industrial perspective of plant beneficial microorganisms. Frontiers in Plant Science, 7: 1110. https://doi.org/10.3389/fpls.2016.01110

Polashock, J. J.; Caruso, F. L.; Averill, A. L. and Schilder, A. C. (2017). Compendium of blueberry, cranberry, and lingonberry diseases and pests, 2nd ed., Part I: Infectious Diseases, APS Press, St. Paul, MN, USA, pp. 9-134. https://doi.org/10.1094/9780890545386.002

Rivera, S. A.; Zoffoli, J. P. and Latorre, B. A. (2013). Infection risk and critical period for the postharvest control of gray mold (*Botrytis cinerea*) on blueberry in Chile. Plant Disease, 97(8): 1069–1074. https://doi.org/10.1094/PDIS-12-12-1112-RE

Saiyam, D.; Dubey, A.; Malla, M. A. and Kumar, A. (2024). Lipopeptides from Bacillus: unveiling biotechnological prospects-sources, properties, and diverse applications. Brazilian Journal of Microbiology, 55(1): 281-295. https://doi.org/10.1007/s42770-023-01228-3

Scarlett, K. A.; Shuttleworth, L. A.; Collins, D.; Rothwell, C. T.; Guest, D. I. and Daniel, R. (2019). Botryosphaeriales associated with stem blight and dieback of blueberry (*Vaccinium* spp.) in New South Wales and Western Australia. Australasian Plant Pathology, 48: 45-57. https://doi.org/10.1007/s13313-018-0584-6

Scherm, H.; Nesmith, D. S.; Horton, D. L. and Krewer, G. (2001). A survey of horticultural and pest management practices of the Georgia blueberry industry. Small Fruits Review, 1(4): 17-28. https://doi.org/10.1300/J301v01n04_03

Shahiri Tabarestani, M.; Rahnama, K.; Jahanshahi, M.; Nasrollahnejad, S. and Fatemi, M. H. (2016). Identification of volatile organic compounds of *Trichoderma* spp. using static headspace gas chromatography-mass spectrometry. Mycologia Iranica, 3(1): 47 – 55. https://doi.org/10.22043/mi.2017.41532.1072

Snedecor, G. W. and Cochran, W. G. (1989). Statistical Methods. 8th Ed. The Iowa State Univ. Press, Ames, Iowa, USA, 503 pp.

Susanti, D.; Awang, N. A.; Qaralleh, H.; Sheikh Mohamed, H. I. and Attoumani, N. (2013). Antimicrobial activity and chemical composition of essential oil of Malaysian *Etlingera elatior* (Jack) R.M. smith flowers. Journal of Essential Oil-Bearing Plants, 16(2): 294-299. https://doi.org/10.1080/0972060X.2013.793968

Tahara, S.; Miura, Y. and Mizutani, J. (1979). Antimicrobial L-5-Alk(en)ylthiomethylhydantoin-(±)-S-oxides: mode of action. Agricultural and Biological Chemistry, 43(5): 919-924. https://doi.org/10.1271/bbb1961.43.919

Teresa, R.-C. M.; Rosaura, V.-G.; Elda, C.-M. and Ernesto, G.-P. (2014). The avocado defense compound phenol-2,4-bis (1, 1-dimethylethyl) is induced by arachidonic acid and acts via the inhibition of hydrogen peroxide production by pathogens. Physiological and Molecular Plant Pathology, 87: 32-41. https://doi.org/10.1016/j.pmpp.2014.05.003

Ting, A. S. Y. and Chai, J. Y. (2015). Chitinase and β-1,3-glucanase activities of *Trichoderma harzianum* in response towards pathogenic and non-pathogenic isolates: Early indications of compatibility in consortium. Biocatalysis and Agricultural Biotechnology, 4(1): 109-113, https://doi.org/10.1016/j.bcab.2014.10.003.

Tymoszuk, A. and Wojnarowicz, J. (2020). Zinc oxide and zinc oxide nanoparticles impact on *in vitro* germination and seedling growth in *Allium cepa* L. Materials, 13(12): 2784. https://doi.org/10.3390/ma13122784

Wang, X.; Wang, J.; Jin P. and Zheng Y. (2013). Investigating the efficacy of *Bacillus subtilis* SM21 on controlling *Rhizopus* rot in peach fruit. International Journal of Food Microbiology, 164 (2-3): 141-147. https://doi.org/10.1016/j.ijfoodmicro.2013.04.010

Wharton, P. S. and Schilder, A. C. (2008). Novel infection strategies of *Colletotrichum acutatum* on ripe blueberry fruit. Plant Pathology, 57(1): 122–134. https://doi.org/10.1111/j.1365-3059.2007.01698.x

Yassin, M. T.; Mostafa, A. A.; Al-Askar, A. A.; Sayed, S. R. M and Rady, A. M. (2021). Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against some fusarial pathogens causing stalk rot disease of maize, *in vitro*. Journal of King Saud University – Science, 33(3): 101363. https://doi.org/10.1016/j.jksus.2021.101363

You. J; Zhang, J., Wu, M.; Yang L.; Chen, W. and Li, G. (2016). Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato. Biological Control, 101: 31-38. https://doi.org/10.1016/j.biocontrol.2016.06.006

Zaki, S. A.; Ouf, S. A.; Albarakaty, F. M.; Habeb, M. M.; Aly, A. A. and Abd-Elsalam K. A. (2021). *Trichoderma harzianum*-mediated ZnO nanoparticles: a green tool for controlling soil-borne pathogens in cotton. Journal of Fungi, 7(11): 952. https://doi.org/10.3390/jof7110952

Zhang, S.; Xu, B.; Zhang, J. and Gan, Y. (2018). Identification of the antifungal activity of *Trichoderma longibrachiatum* T6 and assessment of bioactive substances in controlling phytopathgens. Pesticide Biochemistry and Physiology, 147: 59–66. https://doi.org/10.1016/j.pestbp.2018.02.006

THE FUTURE Journals

© The Author(s). 2022 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise