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Araştırma
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In Vitro Evaluation of Selenium Against Some Plant Pathogenic Fungi

Göksel ÖZER[1], Muharrem TÜRKKAN[2], Ferit SÖNMEZ[3], Hüseyin KABAKCI[1]
Mehtap ALKAN[1], Sibel DERVİŞ[4,5*]

[1]Department of Plant Protection, Faculty of Agriculture, Bolu Abant Izzet Baysal University, Bolu, Türkiye
[2]Department of Plant Protection, Faculty of Agriculture, Ordu University, Ordu, Türkiye
[3]Department of Seed Science and Technology, Faculty of Agriculture, Bolu Abant Izzet Baysal University, Bolu, Türkiye
[4]Department of Plant and Animal Production, Vocational School of Kızıltepe, Mardin Artuklu University, Mardin, Türkiye
[5]Department of Plant Protection, Faculty of Kızıltepe Agricultural Sciences and Technologies, Mardin Artuklu University, Mardin, Türkiye

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Sorumlu yazar: Sibel DERVİŞ, e-posta: sibeldervis@gmail.com

Abstract

Objective: Selenium (Se) is garnering interest as a promising environmentally friendly element for controlling fungal pathogens in agricultural production. This study evaluated the impact of Se treatments, comprising sodium selenite (selenite) and sodium selenate (selenate) forms, on the growth of 10 plant pathogenic fungi.

Materials and Methods: The impact of Se treatments on the mycelial growth and sporulation of fungi was assessed in *in vitro* conditions. Probit analysis was used to determine the concentrations of salts that induced a 50% reduction (EC_{50}) in both mycelial growth and sporulation of fungi.

Results: At the highest concentration (120 ppm), selenite demonstrated inhibitory effects on mycelial growth across various species, with a reduction in growth ranging from 6.82% to 62.46%. In contrast, selenate exhibited a broader spectrum of inhibition, affecting mycelial growth from 0% to 87.14%. Across different concentrations, *Fusarium pseudograminearum* displayed the highest sensitivity to selenite (EC_{50}<24 ppm), followed by *Bipolaris sorokiniana* and *Verticillium dahliae*. Similarly, *Colletotrichum coccodes* exhibited the highest sensitivity to selenate treatment (EC_{50}<24 ppm), followed by *B. sorokiniana*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *V. dahliae*. Both salts effectively inhibited sporulation across fungal species, with no significant difference observed. *Colletotrichum coccodes*, *F. pseudograminearum*, *B. cinerea*, *F. culmorum*, *V. dahliae*, and *B. sorokiniana* were significantly inhibited by selenite, while *F. oxysporum* exhibited lower inhibition. Similarly, these species, along with *V. dahliae* and *F. oxysporum*, were significantly inhibited by selenate, with slight differences between their inhibition percentages. EC_{50} values below 24 ppm were observed for *C. coccodes*, *B. cinerea*, *F. culmorum*, *B. sorokiniana*, and *F. oxysporum*, indicating potent inhibition of sporulation by both salts. *Fusarium pseudograminearum* required slightly higher concentrations for 50% inhibition. *Verticillium dahliae* showed higher sensitivity to selenate than selenite, with EC_{50} values of 33.16 ppm and below 24 ppm, respectively.

Conclusion: The findings of this study contribute to our understanding of Se's antifungal potential across diverse plant pathogenic fungal species in sustainable agriculture. Further research is warranted to elucidate its mechanisms and optimize treatment protocols for disease management.

Keywords: Na_2SeO_3, Na_2SeO_4, antifungal, mycelial growth, sporulation, EC_{50}

Selenyumun Bazı Bitki Patojeni Funguslara Karşı In Vitro Değerlendirmesi

Öz

Amaç: Selenyum (Se), tarımsal alanlarda fungal patojenlerini kontrol etmek için umut vaat eden çevre dostu bir element olarak ilgi çekmektedir. Bu çalışmada, sodyum selenit (selenit) ve sodyum

selenat (selenat) formlarını içeren Se uygulamalarının 10 farklı bitki patojeni fungus türünün büyümesi üzerindeki etkisi değerlendirilmiştir.

Materyal ve Yöntem: Selenyum uygulamalarının fungusların miselyal gelişmesi ve spor oluşumu üzerindeki etkisi *in vitro* koşullarda değerlendirilmiştir. Probit analizi, fungusların hem miselyal gelişme hem de spor oluşumunda %50 azalmaya (EC_{50}) yol açan tuz konsantrasyonlarını belirlemek için kullanılmıştır.

Araştırma Bulguları: En yüksek konsantrasyonda (120 ppm), selenit tüm türlerin miselyal gelişmesini %6.82 ile %62.46 arasında engellemiştir. Buna karşın, selenat daha geniş bir engelleme spektrumu göstermiş ve miselyum büyümesini %0 ile %87.14 arasında etkilemiştir. Farklı konsantrasyonlarda, *Fusarium pseudograminearum* selenit karşısında (EC_{50}<24 ppm) en yüksek duyarlılığı gösterirken onu *Bipolaris sorokiniana* ve *Verticillium dahliae* izlemiştir. Benzer şekilde, *Colletotrichum coccodes* selenat uygulamasına karşı (EC_{50}<24 ppm) en yüksek duyarlılığı gösterirken, onu *B. sorokiniana*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* ve *V. dahliae* takip etmiştir. Her iki tuz da anlamlı bir farklılık gözlenmeksizin fungal türleri üzerinde spor oluşumunu etkili bir şekilde inhibe etmiştir. *Colletotrichum coccodes*, *F. pseudograminearum*, *B. cinerea*, *F. culmorum*, *V. dahliae* ve *B. sorokiniana* selenit tarafından anlamlı şekilde inhibe edilmiştir fakat *F. oxysporum*'a karşı daha düşük bir inhibisyon gözlenmiştir. Benzer şekilde, engelleme yüzdeleri arasında küçük farklar bulunmakla birlikte bu türler, *V. dahliae* ve *F. oxysporum* ile birlikte, selenat tarafından anlamlı şekilde inhibe edilmiştir. *Colletotrichum coccodes*, *B. cinerea*, *F. culmorum*, *B. sorokiniana* ve *F. oxysporum* için 24 ppm'nin altındaki EC_{50} değerleri, her iki tuzun da spor oluşumunu etkin bir şekilde inhibe ettiğini göstermiştir. *Fusarium pseudograminearum*'un %50 inhibisyonu için daha yüksek konsantrasyonların gerektiği anlaşılmıştır. *Verticillium dahliae*, selenit karşısında 33.16 ppm ve selenat karşısında 24 ppm'nin altında olan EC_{50} değerleri ile selenata karşı daha yüksek duyarlılık göstermiştir.

Sonuç: Bu çalışmanın bulguları, Se'nin sürdürülebilir tarımda çeşitli bitki patojen fungus türleri üzerindeki antifungal potansiyeline ilişkin anlayışımıza katkıda bulunmaktadır. Hastalık yönetimi için elementin mekanizmalarını anlamak ve uygulama protokollerini optimize etmek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Na_2SeO_3, Na_2SeO_4, antifungal, miselyal gelişme, sporulasyon, EC_{50}

Introduction

Selenium (Se), present in various forms in nature, plays a crucial role in modulating physiological and biochemical traits in plants, exerting positive effects on plant growth at low concentrations (El-Ramady et al., 2016). Spallholz (1997) highlights that Se and its compounds rank among the most toxic of nutrients. Se toxicity was initially observed in grazing animals in the western United States during the 1930s, which consumed plants known as "Se accumulators" belonging to the genera *Astragalus*, *Xylorrhiza*, *Oonopsis*, and *Stanleya*. These plants accumulate selenites and selenates from the soil, primarily as methylated Se compounds, and subsequently release dimethyldiselenide and dimethylselenide into the environment. Se hyperaccumulation in plant not only benefits ecosystems by reducing herbivory and pathogen infections but also enhances plant growth while diminishing herbivory in neighboring plants (Mehdawi and Pilon-Smits, 2012). Se treatments have been found to enhance plant resistance against fungal diseases and insect pests by fortifying defense mechanisms, impeding pathogen intrusion, and altering soil microbial communities (Li et al., 2023). Studies have shown that Se treatments positively impact potato growth, carbohydrate accumulation, and potentially yield formation, with higher Se additions possibly extending stolon and root lifespan (Turakainen et al., 2004).

Fungi in the rhizosphere of Se hyperaccumulator *Stanleya pinnata* can amplify root accumulation and reduce Se translocation in root crops, potentially enhancing Se biofortification and phytoremediation efforts (Lindblom et al., 2014). Optimal exogenous Se concentrations have been demonstrated to promote antioxidative and osmoregulatory capacities, thereby enhancing salt resistance in sorrel seedlings (Kong et al., 2005). Additionally, combining arbuscular mycorrhizal fungus inoculation with Se fertilization has been shown to boost organic Se accumulation in rice grain, potentially improving Se biofortification in rice (Chen et al., 2020). Se also acts as an antioxidant in soybean plants, inhibiting lipid peroxidation and cell membrane injury, thus positively influencing growth (Djanaguiraman et al., 2005).

Furthermore, Se treatment has been found to delay tomato fruit ripening by suppressing ethylene biosynthesis and enhancing the antioxidant defense system, thereby reducing reactive oxygen species generation and membrane damage (Zhu et al., 2017). Se treatment in salt-stressed seedlings has also been shown to heighten antioxidant defense and methylglyoxal detoxification system activities, mitigating salt-induced damage (Hasanuzzaman et al., 2011).

Under certain experimental conditions, Se displays dual effects, acting beneficially for plants while also inhibiting plant pathogens (Bhatia et al., 2013; Hasanuzzaman et al., 2011; Wu et al., 2014). Consequently, understanding the activity and role of Se in plant-pathogen interactions warrants further investigation. Several studies have explored the efficacy of Se salt treatment in controlling various plant pathogens. These studies encompass investigations into *Aspergillus funiculosus*, *Alternaria tenuis*, *Fusarium* spp., and *F. graminearum* in artificial media; *Fusarium* spp., and *Alternaria brassicicola* in Indian mustard; *F. oxysporum* f. sp. *lycopersici* in tomato; *Penicillium expansum* in artificial media; *Botrytis cinerea* in tomato; and *F. graminearum* in wheat (Razak et al., 1991; Ramadan et al., 1988; Mao et al., 2020; Hanson et al., 2003; Companioni et al., 2012). Previous studies have highlighted the inhibitory effects of Se treatments, particularly selenite, on fungal growth and spore germination (Wu et al., 2014; 2016). Se has been investigated for its role in enhancing plant defense mechanisms against fungal pathogens such as *Sclerotinia sclerotiorum* (Xu et al., 2019). Additionally, research has demonstrated Se's protective effect in plants against mycotoxins such as zearalenone and aflatoxin B1 (Filek et al., 2017; Kornaś et al., 2019; Agar et al., 2013). Furthermore, studies have investigated Se's ability to reduce deoxynivalenol production by *F. graminearum* both *in vitro* and *in vivo* (Mao et al., 2020). In soil, Se supplementation has been shown to enhance microbiome diversities and increase the relative abundance of plant growth promoting bacteria while decreasing the number of pathogenic fungi (Liu et al., 2015).

In agricultural production, Se has been explored as a potential fungicide to control *S. sclerotiorum* by damaging its membrane system, osmoregulation, and reducing cell wall degrading enzymes (Jia et al., 2018). The combination of arbuscular mycorrhizal fungi and Se has been found to enhance garlic and onion yield, biochemical characteristics, and mineral composition under different environmental conditions (Golubkina et al., 2020). The combination of melatonin and Se has shown significant improvements in resistance to postharvest gray mold disease in tomato fruits by activating antioxidant enzymes and increasing pathogenesis-related protein expression (Zang et al., 2022). Selenite has shown potential as an antifungal agent for controlling gray mold rot in tomato fruits caused by *B. cinerea* (Wu et al., 2016). Additionally, Se in soil has been implicated in enhancing plant resistance to fungal diseases like Sclerotinia stem rot of oilseed rape (Cheng et al., 2020).

Se treatments represent a multifaceted approach to fungal disease management in agriculture, with potential applications ranging from direct antifungal effects to enhancing plant defense mechanisms. Understanding the mechanisms underlying Se's antifungal activity and its interactions with plant-pathogen systems is essential for optimizing its use in sustainable agricultural practices. The study aimed to investigate the impact of sodium selenite and sodium selenate treatments on the mycelial growth and spore production of selected fungal pathogens, including *Fusarium oxysporum*, *F. culmorum*, *F. pseudograminearum*, *Bipolaris sorokiniana*, *Botrytis cinerea*, *Verticillium dahliae*, *Colletotrichum coccodes*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, and *Rhizoctonia solani* isolated from various crop plants.

Materials and Methods

Fungal cultures and inoculum preparation

Pure cultures of the selected fungal pathogens were obtained from culture collections housed in the Plant Pathology Laboratory's collection at Bolu Abant Izzet Baysal University in Bolu. The isolates had been stored and maintained on Potato Dextrose Agar (PDA) slants at 4°C in the dark prior to experimentation. Inoculum for each fungus was prepared by transferring actively growing fungal mycelium onto sterile PDA plates. These plates were then incubated until optimal growth was observed, ensuring the availability of viable and actively growing fungal inoculum for subsequent experiments (Türkkan and Erper, 2015).

Selenium treatment preparation

Sodium selenite (Na_2SeO_3) (selenite) and sodium selenate (Na_2SeO_4) (selenate) were acquired from Sigma-Aldrich (Saint Louis, MO, USA) as γ-irradiated and lyophilized powders, selected for their suitability in cell culture applications.

Prior to use, both compounds were reconstituted to the desired concentrations using sterile distilled water to ensure uniformity of the treatment solutions (Mecteau et al., 2002).

Selenium applications and probit analysis

The impact of Se treatments, including selenite and selenate, on the mycelial growth and sporulation of fungi was assessed following the methodology outlined by Türkkan and Erper (2015), with minor modifications. Mycelial growth assessments were performed in Potato Dextrose Agar (PDA) supplemented with both forms of Se at concentrations of 24, 48, 96, and 120 ppm, following the methodology outlined below. Selenite and selenate solutions were incorporated into autoclaved and cooled PDA at 50°C. Subsequently, 15 ml of the treated PDA medium for each variant was dispensed aseptically into 9-cm-diameter petri plates, with unaltered PDA plates serving as controls. Upon placing a 5-mm-diameter mycelial disc sourced from 7-day-old fungal cultures at the center of each plate, the plates were sealed with Parafilm and incubated in darkness at 25°C for 3–10 days. The colony diameter was determined by averaging the longest and shortest diameters measured after incubation. The inhibition of mycelial growth was calculated using the formula: [(control radial growth - salt-amended radial growth) / control radial growth] × 100 (Türkkan, 2013). The experimental setup followed a completely randomized block design with five replicates.

Simultaneously, the conidiation of fungi was investigated in order to determine the effects of these selenium forms (selenite and selenate) and concentrations (24, 48, 96, and 120 ppm). This assessment was conducted by adapting the method proposed by Mecteau et al. (2002). Conidia were delicately extracted using a sterile scalpel after the addition of 10 mL of distilled water to each plate, followed by filtration through two layers of sterile cheesecloth to eliminate hyphal fragments. The resulting conidial suspensions were transferred to sterile microtubes and homogenized by vortexing for 30 seconds. Subsequently, the conidia were counted using a microscope (DM1000 model, Leica Microsystems, Wetzlar, Germany) and a hemacytometer (Thoma, Marienfeld, Germany). The spore density per square centimeter of colony was calculated for each plate using the number of spores per plate and the colony diameter, following the method described by Mecteau et al. (2002). The inhibition of conidiation was expressed as a percentage: [(number of spores in control plates - number of spores in salt-amended plates) / number of spores in control plates] × 100 (Türkkan, 2013). *Sclerotinia sclerotiorum*, *S. rolfsii*, and *R. solani* were excluded from the spore count analysis as they typically do not form spores under these conditions.

Probit analysis was employed to determine the concentrations of salts that induced a 50% reduction (EC_{50}) in both mycelial growth and sporulation of fungi (Türkkan, 2013), utilizing the IBM SPSS Statistics Program (New York, USA).

Data analysis

Quantitative data, including fungal growth measurements and spore counts, underwent statistical analysis using the XLSTAT software (Version 2016.02.28451, Addinsoft, Long Island, NY, USA). The Shapiro-Wilk test was utilized to evaluate the normality of the inhibition percentages data for both mycelial growth and sporulation, in accordance with the approach proposed by Shapiro and Francia (1972). As the data exhibited a non-normal distribution of residuals, a transformation was applied to achieve normality. Specifically, the square root transformation ($\sqrt{y+1}$) was implemented within the XLSTAT program. The Levene variance homogeneity test was subsequently conducted to verify the uniformity of variance in the dataset. Due to significant differences observed in daily mycelial growth rates, separate one-way analyses of variance (ANOVA) were performed for each dataset. Post-hoc analysis using Fisher's LSD test was then conducted to identify significant differences between means, with a significance threshold set at $P<0.05$.

Additionally, for each fungal species, a two-tailed *t*-test was conducted to ascertain whether there were differences between the applications of selenite and selenate concerning mycelial development and sporulation inhibition.

Results

Effects of selenium treatments on mycelial growth

At the highest concentration tested (120 ppm), selenite exhibited inhibitory effects on the growth of fungal species ranging from 6.82% to 62.46%, whereas selenate salt showed inhibition ranging from 0% to 87.14% (Table 1). Statistical analysis revealed a significant difference in the inhibitory effects of both Se on fungal growth ($P<0.05$). Furthermore, except for the mycelial growth of *F. oxysporum* ($P = 0.240$), the *t*-test demonstrated statistically significant

inhibitory effects of both selenite and selenate salts on fungal mycelial growth (P<0.01).

Bipolaris sorokiniana emerged as the most sensitive isolate to selenite, exhibiting a notable inhibition percentage of 62.46%. Subsequently, *V. dahliae* and *F. pseudograminearum* demonstrated inhibition percentages of 57.15% and 55.60%, respectively. Following these, *S. sclerotiorum* and *S. rolfsii* displayed similar inhibition percentages of 51.31% and 51.83%, respectively. *F. oxysporum* showed an inhibition percentage of 45.91%, while *C. coccodes* and *B. cinerea* exhibited similar inhibition percentages of 29.28% and 26.01%, respectively. Selenite salt inhibited mycelial growth of the *F. culmorum* isolate, albeit at a very low rate (20.74%). Lastly, *R. solani* demonstrated the least sensitivity to selenite, with an inhibition percentage of 6.82% (Figure 1).

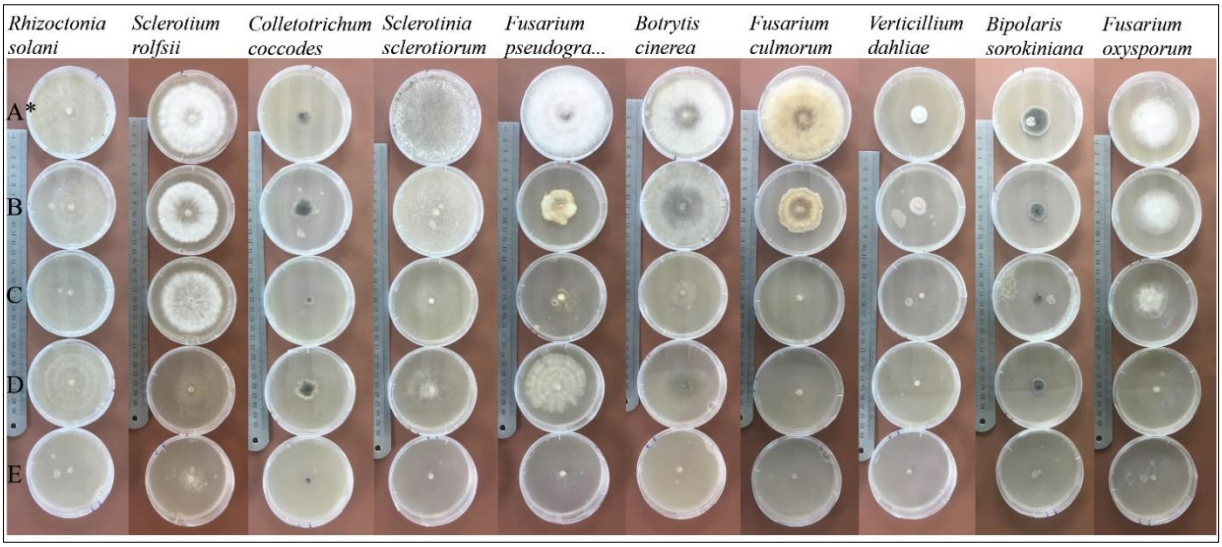

Figure 1. Effects of selenate and selenite at 24 ppm and 120 ppm on mycelial growth plant pathogenic fungi. * A: Control, B: 24 ppm selenite, C: 24 ppm selenate, D: 120 ppm selenite, E: 120 ppm selenate

The species most responsive to selenate were *S. sclerotiorum* and *B. cinerea*, both exhibiting comparable levels of sensitivity, with inhibition percentages of 87.14% and 86.28%, respectively, representing the highest inhibition rates (Table 1). Following these, *C. coccodes* and *B. sorokiniana* exhibited high sensitivity to selenate, with similar inhibition percentages of 80.08% and 79.74%, respectively. *V. dahliae* and *F. oxysporum* displayed intermediate levels of sensitivity, with inhibition percentages of 68.52% and 42.47%, respectively. *S. rolfsii* exhibited a slightly lower sensitivity, with an inhibition percentage of 38.51%. *F. culmorum* and *F. pseudograminearum* demonstrated lower sensitivity, with inhibition percentages of 31.63% and 27.24%, respectively. *R. solani* exhibited insensitivity to selenate treatment. However, its mycelial growth appeared sparse compared to the dense controls (Figure 1).

At the lowest concentration (24 ppm) of both salts against all fungal species, the percentage inhibition effects were consistent with those observed at 120 ppm (Table 1). Overall, selenate demonstrated a higher inhibitory effect compared to selenite across all tested concentrations (Figure 1).

Analyzing the EC_{50} values in mycelial growth for selenite treatment, *F. pseudograminearum* demonstrated the highest sensitivity, with an EC_{50} value below 24 ppm, indicating pronounced susceptibility to this salt (Table 2). *Bipolaris sorokiniana* exhibited high sensitivity, with an EC_{50} value of 58.21 ppm, followed by *V. dahliae* with a value of 93.34 ppm. *S. sclerotiorum* and *S. rolfsii* displayed moderate sensitivity, with EC_{50} values of 118.52 ppm and 113.96 ppm, respectively. Conversely, *C. coccodes*, *R. solani*, *B. cinerea*, *F. culmorum*, and *F. oxysporum* showed low sensitivity, with EC_{50} values exceeding 120 ppm.

Regarding selenate treatment, *C. coccodes* demonstrated the highest sensitivity, with an EC_{50} value below 24 ppm, indicating pronounced susceptibility (Table 2). *S. sclerotiorum* exhibited high sensitivity, with an EC_{50} value of 14.78 ppm, followed by *B. cinerea* with 19.54 ppm. *B. sorokiniana* and *V. dahliae* also showed considerable sensitivity, with EC_{50} values below 24 ppm.

Table 1. Mycelial growth inhibition percentages of 10 fungal species under selenite and selenate treatments

Fungal species	Host	Isolation source	Inhibition of mycelial development (%)			
			Selenite		Selenate	
			24 ppm	120 ppm	24 ppm	120 ppm
Rhizoctonia solani	Potato	Tuber	0.00±0.00 g*	6.82 ±1.05 g*	0.00±0.00 i*	0.00 ±0.00h*
Sclerotium rolfsii	Candy leaf	Crown	8.28±1.55 de	51.83 ±0.82bc	4.27±1.50 h	38.51 ±1.20e
Colletotrichum coccodes	Potato	Tuber	12.09±1.48 cd	29.28 ±1.18 e	72.94±0.47 a	80.08 ±0.45b
Sclerotinia sclerotiorum	Lettuce	Crown	0.00±0.00 g	51.31 ±1.06 c	60.35±1.50 bc	87.14 ±0.27a
Fusarium pseudograminearum	Wheat	Crown	52.05±0.66 a	55.60 ±0.75bc	15.09±1.25 f	27.24 ±1.20g
Botrytis cinerea	Sweet basil	Stem	2.11±0.95 f	26.01 ±2.73 e	54.93±0.46 cd	86.28 ±0.63a
Fusarium culmorum	Wheat	Crown	5.48±0.41 e	20.74 ±0.72 f	8.28±0.40 g	31.63 ±0.51f
Verticillium dahliae	Goji berry	Root	16.49±1.90 c	57.15 ±1.16ab	51.52±1.58 d	68.52 ±0.73c
Bipolaris sorokiniana	Wheat	Internode	34.86±0.75 b	62.46 ±0.69 a	63.84±0.66 b	79.74 ±1.38b
Fusarium oxysporum	Lavander	Root	14.70±2.62 c	45.91 ±2.05 d	26.88±2.96 e	42.47 ±1.73d

*Based on the Fisher's LSD test (P<0.05), fungal isolates denoted by the same letter within the same column do not exhibit a statistically significant difference in mycelial development inhibition caused by either selenite or selenate salts.

Table 2. Effective concentration causing 50% inhibition (EC_{50}) values of selenite and selenate treatments inhibiting the mycelial growth of 10 fungal species

Fungal species	**EC_{50} values for inhibiting mycelial growth**	
	Selenite (ppm)	**Selenate (ppm)**
Rhizoctonia solani	>120	Not determined*
Sclerotium rolfsii	113.96	>120
Colletotrichum coccodes	>120	<24
Sclerotinia sclerotiorum	118.52	<24
Fusarium pseudograminearum	<24	>120
Botrytis cinerea	>120	<24
Fusarium culmorum	>120	>120
Verticillium dahliae	93.34	<24
Bipolaris sorokiniana	58.21	<24
Fusarium oxysporum	>120	>120

*At the highest concentration tested in the study, no inhibitory effects were observed.

In contrast, *R. solani*, *S. rolfsii*, *F. pseudograminearum*, *F. culmorum*, and *F. oxysporum* exhibited low sensitivity, with EC_{50} values exceeding 120 ppm.

Effects of selenium treatments on spore production

The inhibitory effects of both selenite and selenate salts on sporulation ranged from 76.29% to 100%, indicating substantial inhibition across fungal species. Overall, the inhibitory effects of both salts on sporulation were statistically similar (P>0.05). Notably, *C. coccodes*, *F. pseudograminearum*, *B. cinerea*, *F. culmorum*, *V. dahliae*, and *B. sorokiniana* all exhibited statistically significant inhibition of sporulation with selenite treatment (P<0.05) (Table 3). Conversely, *F. oxysporum* displayed a lower inhibition percentage and showed statistical differences from the other species.

Similarly, *C. coccodes*, *F. pseudograminearum*, *B. cinerea*, *F. culmorum*, and *B. sorokiniana* all exhibited statistically significant inhibition of sporulation with selenate treatment (P<0.05) (Table 3). *V. dahliae* and *F. oxysporum* exhibited inhibition percentages slightly lower than the first group and displayed statistical differences from both the first group and each other.

At the lowest concentration (24 ppm), both selenite and selenate salts demonstrated significant inhibition or complete suppression of sporulation in *C. coccodes*, *B. cinerea*, *F. culmorum*, and *B. sorokiniana* (Table 3). Conversely, selenite exhibited moderate inhibition levels on *V. dahliae* and *F. oxysporum*, with percentages of 23.73% and 57.03%, respectively, whereas selenate showed considerably higher inhibition rates of 91.34% and 94.21%, respectively, on the same species. Moreover, both selenite and selenate salts had minimal effects on the sporulation of *F. pseudograminearum*.

Table 3. Spore production inhibition percentages of seven fungal species under selenite and selenate treatments

Fungal species	Inhibition of sporulation (%)			
	Selenite		Selenate	
	24 ppm	120 ppm	24 ppm	120 ppm
Colletotrichum coccodes	88.70±0.65 a*	97.71 ± 0.50 a*	100.00±0.00 a*	100.00 ± 0.00 a*
Fusarium pseudograminearum	29.11±9.29 c	100.00 ± 0.00 a	34.30±248 c	100.00 ± 0.00 a
Botrytis cinerea	93.26±0.89 a	98.03 ± 0.16 a	94.37±0.36 b	100.00 ± 0.00 a
Fusarium culmorum	100.00±0.00 a	100.00 ± 0.00 a	100.00±0.00 a	100.00 ± 0.00 a
Verticillium dahliae	23.73±6.73 c	99.78 ± 0.05 a	91.34±1.57 b	98.61 ± 0.11 b
Bipolaris sorokiniana	99.32±0.68 a	100.00 ± 0.00 a	100.00±0.00 a	100.00 ± 0.00 a
Fusarium oxysporum	57.03±13.67 b	76.29 ± 1.78 b	94.21±1.19 b	98.25 ± 0.08 c

*Based on the Fisher's LSD test (P<0.05), fungal isolates denoted by the same letter within the same column do not exhibit a statistically significant difference in spore production inhibition caused by either selenite or selenate salts.

For *C. coccodes*, *B. cinerea*, *F. culmorum*, *B. sorokiniana*, and *F. oxysporum,* both selenite and selenate salts exhibited EC_{50} values below 24 ppm, indicating robust potency in inhibiting sporulation for these fungal species (Table 4). *F. pseudograminearum* showed slightly higher EC_{50} values, with selenite at 28.82 ppm and selenate at 27.49 ppm, suggesting that slightly elevated concentrations of the salts are necessary to achieve 50% inhibition of sporulation in this species. *V. dahliae* demonstrated an EC_{50} value of 33.16 ppm for selenite, while the EC_{50} value for selenate was below 24 ppm, indicating that selenate is more potent in inhibiting sporulation in this species compared to selenite.

Table 4. Effective concentration causing 50% inhibition (EC_{50}) values of selenite and selenate treatments inhibiting the spore production of seven fungal species

Fungal species	EC_{50} values for inhibiting spore production	
	Selenite (ppm)	Selenate (ppm)
Colletotrichum coccodes	<24	<24
Fusarium pseudograminearum	28.82	27.49
Botrytis cinerea	<24	<24
Fusarium culmorum	<24	<24
Verticillium dahliae	33.16	<24
Bipolaris sorokiniana	<24	<24
Fusarium oxysporum	<24	<24

Discussion

This study explored the impact of Se treatments on mycelial growth and sporulation across diverse fungal species. Both selenite and selenate salts exhibited significant inhibitory effects on mycelial growth, with varying degrees of sensitivity observed among different fungi. Selenate treatments consistently exhibited stronger suppression of fungal growth compared to selenite treatments, with toxicity assessments revealing selenate salt to be generally more toxic than selenite salt, impacting both mycelial growth and sporulation across most fungal species.

The findings from multiple studies underscore the potential of Se treatments as effective means of controlling various plant pathogenic fungal diseases. Se has been shown to effectively control gray mold rot in tomato fruits by inhibiting spore germination and causing membrane integrity damage in the fungal pathogen *B. cinerea* (Wu et al., 2016). Moreover, Se treatments have shown promise in inhibiting fungal growth and substrate consumption, particularly with selenite treatment, which significantly inhibits spore germination of *B. cinerea* (Wu et al., 2016). Zang et al. (2022) examined the synergistic effects of Se and melatonin against gray mold decay caused by *B. cinerea* in tomato fruits. While melatonin alone did not display antifungal properties, Se significantly suppressed the development of gray mold. Notably, the combination of melatonin and Se exhibited

substantial inhibition of disease spread and growth, resulting in a control efficacy of 74.05%.

Selenite was part of a study evaluating its efficacy, along with zinc sulfate, oxalic acid, and sodium malonate, against *S. sclerotiorum* (Sarma et al., 2007). *In vitro* assays confirmed selenite as the only chemical with antifungal properties. Additionally, Se treatments have demonstrated efficacy against *S. sclerotiorum* by increasing plant Se concentration and altering soil microbial communities, suggesting its potential as an ecological fungicide for biological disease control (Liu et al., 2019). This inhibitory effect extends to the growth of *S. sclerotiorum*, where Se treatments damage the membrane system, affect osmoregulation, and reduce cell wall-degrading enzyme activities (Jia et al., 2018). Xu et al. (2019) highlighted Se's role as a potential eco-fungicide to protect oilseed rape leaves from *S. sclerotiorum* infection, enhancing plant defense mechanisms. Furthermore, Se effectively reduces the pathogenicity of *S. sclerotiorum* by inhibiting sclerotial formation and germination by damaging sclerotial ultrastructure, reducing acid production, and increasing hydrogen peroxide and superoxide anion content, presenting an eco-friendly approach for controlling it (Cheng et al., 2019).

Razak et al. (1991) aimed to study Se's impact on fungicide effectiveness, isolating *Aspergillus funiculosus* from decayed banana, and *Alternaria tenuis* and *Fusarium* sp. from tomato fruits. These fungi showed resilience to high Dithane levels and thrived in its presence, as well as tolerating selenite concentrations up to 2%. However, *A. funiculosus* and *Fusarium* sp. struggled to grow in the presence of Se-Dithane mixtures, whereas *A. tenuis* showed greater tolerance. Hanson et al. (2003) investigated the Se (selenate) tolerance of *Fusarium* species and *Alternaria brassicicola*, finding that Se-treated *Brassica juncea* plants demonstrated reduced lesions when exposed to *A. brassicicola*, suggesting Se's protective effect against this leaf pathogen. In line with the findings of Hanson et al. (2003), the EC_{50} values for *Alternaria* and *Fusarium* isolates were determined to be approximately 55 mg/l and 60 mg/l Se, respectively. However, our study revealed significant differences in the sensitivity of three *Fusarium* species, namely *F. pseudograminearum*, *F. culmorum*, and *F. oxysporum*, with EC_{50} values exceeding 120 ppm Se for inhibiting mycelial growth. Interestingly, the EC_{50} values for inhibiting spore production in our study for three *Fusarium* spp. were all below 24 ppm, except for *F. pseudograminearum*, which exhibited a slightly higher value of 27.49 ppm. Troni et al. (2021) investigated the *in vitro* effects of different Se concentrations from various Se forms (selenite, selenate, selenomethionine, and selenocystine) on the development of a *F. proliferatum* strain isolated from rice. Concentration-dependent inhibition of fungal growth was observed for both selenite and selenate, with selenite being effective at 20 mg kg^{-1}. The study suggests that incorporating low concentrations of selenite with conventional fungicides may offer a promising alternative for controlling *Fusarium* species. Espinosa-Ortiz et al. (2015) demonstrated variations in the inhibitory effects of Se salts on fungal growth, with selenite exhibiting stronger inhibition compared to selenate.

Our study confirms and extends previous research on selenium's inhibitory effects on fungal pathogens by quantifying the impacts of selenite and selenate on multiple fungal species. It is worth mentioning that different fungal species exhibited varying sensitivities to selenium treatments. *Fusarium pseudograminearum* and *B. sorokiniana* were most sensitive to selenite, while *C. coccodes* and *S. sclerotiorum* were sensitive to selenate. *Rhizoctonia solani*, on the other hand, remained unaffected by both salts. These results emphasize the need for species-specific strategies in disease management and suggest further research to optimize selenium treatment protocols and understand the underlying mechanisms of inhibition.

Moreover, our investigation revealed potent inhibition of sporulation by both selenite and selenate across multiple fungal species, with some species exhibiting EC_{50} values below 24 ppm, indicating strong efficacy of Se treatments in suppressing fungal reproduction. However, our study also identified variations in fungal sensitivity to Se treatments, suggesting the need for further research to optimize treatment protocols and elucidate underlying mechanisms of Se-mediated inhibition. By expanding our understanding of Se's role in fungal pathogenesis, future studies can contribute to the development of more effective and environmentally sustainable strategies for disease management in agriculture.

In conclusion, previous studies and our experiments emphasize selenium's potential against fungal pathogens in agriculture, indicating the promise of developing effective and sustainable selenium-based fungicides. Ongoing research offers hope for the

future of Se-based fungicides. The observed inhibition of fungal growth by Se treatments highlights its potential for controlling fungal pathogens in agricultural settings, albeit with variations based on fungal species, Se concentration, and form. Further investigations are essential to understand the mechanisms behind the inhibitory effects of selenite and selenate on plant pathogens. Elucidating these mechanisms is crucial for effectively utilizing Se-based treatments in disease management strategies. *In vivo* efficiency tests, along with proteomics, metabolomics, genomics, and transcriptomics analyses, are needed to comprehensively explore these underlying mechanisms**.**

Conflicts of interest

The authors declare that there are no conflicts of interest.

Authorship contribution statement

GÖ: Supervised the research project, conceived and designed the study, performed the *in vitro* experiments, analyzed the data, and wrote the initial draft of the manuscript.

MT: Performed statistical analysis, assisted in data interpretation, and contributed to the revision of the manuscript.

FS: Provided guidance and expertise on selenium applications and obtained funding for the project.

HK: Provided support with experimental setup and culture maintenance.

MA: Assisted with the *in vitro* experiments and contributed to data collection and analysis.

SD: Provided critical input on study design, data analysis, and manuscript preparation, wrote the manuscript, and finalized the manuscript for submission.

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