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Araştırma
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In vitro evaluation of salt-based antifungal compounds for sustainable control of *Neoscytalidium dimidiatum*

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Abstract

Objective: This study evaluated the antifungal potential of various salts—specifically ammonium, borate, calcium, magnesium, potassium, and sodium compounds—against two isolates (Ol_Dr04 and Ciar 64) of *Neoscytalidium dimidiatum* under *in vitro* conditions. The goal was to assess the efficacy of these salts in inhibiting mycelial growth, arthrospore germination, and germ tube elongation under both fixed and adjusted pH conditions.

Materials and Methods: In this study, the mycelial growth of *N. dimidiatum* isolates was first observed across a pH range of 2 to 12 to determine the optimal pH levels. Subsequently, the antifungal efficacy of 1% concentrations of ammonium, borate, calcium, magnesium, potassium, and sodium salts was assessed under both fixed and adjusted pH (5) conditions for both isolates. Effective salt concentrations (EC_{50}) needed to achieve a 50% reduction in mycelial growth, arthrospore germination, and germ tube elongation were calculated using probit analysis. Additionally, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were determined for each salt under the tested conditions.

Results: Under fixed pH conditions, 1% concentrations of ammonium (bicarbonate and carbonate), borate (anhydrous borax, Etidot-67, and hydrated borax), and sodium (benzoate, citrate tetrahydrate, and metabisulfite) salts completely inhibited mycelial growth in both fungal isolates. However, under adjusted pH (5) conditions, only sodium benzoate and metabisulfite maintained the same inhibitory effect. At adjusted pH, calcium oxide and propionate also fully suppressed mycelial growth. Sodium metabisulfite emerged as the most effective antifungal compound, with remarkably low EC_{50} values (0.016 and 0.017%; w/v), MIC (0.0625 and 0.0625%; w/v), and MFC (0.0625% and 0.0625%; w/v) concentrations. Furthermore, with EC_{50} below 0.03125%, sodium metabisulfite remained the strongest inhibitor in both arthrospore germination and germ tube elongation assays.

Conclusion: These results highlight the potential of sodium metabisulfite, ammonium bicarbonate, and ammonium carbonate salts as environmentally friendly alternatives to conventional fungicides. Further *in vivo* studies are recommended to validate these findings and explore practical applications in sustainable plant disease management.

Keywords: *Neoscytalidium dimidiatum*, antifungal salts, EC_{50}, MIC, MFC, mycelial growth, arthrospore germination, germ tube elongation

***Neoscytalidium dimidiatum'*un sürdürülebilir kontrolünde tuz bazlı antifungal bileşiklerin *in vitro* değerlendirilmesi**

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Öz

Amaç: Bu çalışmada, amonyum, borat, kalsiyum, magnezyum, potasyum ve sodyum bileşiklerini içeren çeşitli tuz gruplarının, *Neoscytalidium dimidiatum*'un iki izolatı (Ol_Dr04 ve Ciar 64) üzerindeki antifungal potansiyeli *in vitro* koşullarda değerlendirilmiştir. Farklı tuzların sabit ve ayarlanmış pH koşullarında miselyal gelişimi, arthrospor çimlenmesi ve çim tüpü uzamasını engelleyici etkileri analiz edilmiştir.

Materyal ve Yöntem: Çalışmada, öncelikle *N. dimidiatum* izolatlarının pH 2-12 aralığında miselyal gelişimi gözlemlenmiş ve optimum pH seviyeleri belirlenmiştir. Ardından, her iki izolat üzerinde amonyum, borat, kalsiyum, magnezyum, potasyum ve sodyum tuzlarının %1'lik konsantrasyonlarının sabit ve ayarlanmış pH (5) koşullarındaki antifungal etkinliği değerlendirilmiştir. Misel gelişiminde, arthrospor çimlenmesinde ve çim tüpü uzamasında %50 azalma sağlamak için gereken etkili tuz konsantrasyonları (EC_{50}) probit analizi kullanılarak hesaplanmıştır. Ayrıca, minimum inhibisyon konsantrasyonu (MIC) ve minimum fungisidal konsantrasyon (MFC) değerleri de tespit edilmiştir.

Araştırma Bulguları: Sabit pH koşullarında, amonyum (bikarbonat ve karbonat), borat (susuz boraks, Etidot-67 ve sulu boraks) ve sodyum (benzoat, sitrat tetrahidrat ve metabisülfit) tuzlarının %1'lik konsantrasyonları, her iki fungus izolatının miselyal gelişimini tamamen engellemiştir. Ancak, ayarlanmış pH (5) koşullarında, bu etkili tuzlardan yalnızca sodyum benzoat ve sodyum metabisülfit aynı engelleyici etkiyi göstermiştir. Ayarlanmış pH koşullarında ayrıca kalsiyum oksit ve kalsiyum propiyonat da miselyal gelişimi tamamen durdurmuştur. Sodyum metabisülfit, son derece düşük EC_{50} (%0.016 ve 0.017; w/v), MIC (%0.0625 ve 0.0625; w/v) ve MFC (%0.0625 ve %0.0625; w/v) değerleri ile en etkili antifungal bileşik olarak öne çıkmıştır. Ayrıca, %0.03125'in altındaki EC_{50} değeriyle sodyum metabisülfit, arthrospor çimlenmesi ve çim tüpü uzama testlerinde de en güçlü inhibitör olmuştur.

Sonuç: Bu sonuçlar, sodyum metabisülfit, amonyum bikarbonat ve amonyum karbonat tuzlarının geleneksel fungisitlere çevre dostu alternatif olarak potansiyelini ortaya koymaktadır. Bu bulguları doğrulamak ve bitki hastalıklarının sürdürülebilir mücadelesinde pratik uygulamaların araştırılması için daha fazla *in vivo* çalışma yapılması önerilmektedir.

Anahtar kelimeler: *Neoscytalidium dimidiatum*, antifungal tuzlar, EC_{50}, MIC, MFC, miselyal gelişim, arthrospor çimlenmesi, çim tüpü gelişimi

Introduction

Neoscytalidium dimidiatum is a significant fungal pathogen responsible for a wide array of plant diseases, including canker, dieback, leaf spots, and fruit rot, affecting over 126 plant species globally (Bragard et al. 2023; Derviş and Özer, 2023). Its devastating impact on economically important crops such as citrus, mango, pistachio, and dragon fruit poses serious threats to agricultural productivity, leading to considerable yield losses and, in severe cases, plant death. Beyond agriculture, *N. dimidiatum* also presents risks to human health, underscoring its status as a dual-threat pathogen (Silva et al., 2016).

The widespread presence of *N. dimidiatum* globally is largely due to its remarkable adaptability to diverse environments, particularly thriving in tropical and arid regions where elevated temperatures and water stress prevail. Climate change, with its intensifying droughts and extreme temperature fluctuations, is expected to further exacerbate the prevalence and virulence of this pathogen, especially in arid areas across Asia, North America, and the Middle East (Derviş and Özer, 2023). In Türkiye, *N. dimidiatum* poses significant threats to both agriculture and forestry, affecting a wide range of plants, from blue spruce (*Picea pungens*) to olives (*Olea europaea*) and chickpeas (*Cicer arietinum*) (Derviş et al., 2023; Güney et al., 2022, 2023). The pathogen's impact extends beyond key crops, as it also targets herbs like lemon balm (*Melissa officinalis*) (Özer et al., 2022). These diverse incidences underscore the pathogen's broad host range and adaptability across Türkiye's varied agricultural landscapes. Its recent identification as a postharvest pathogen on pears (*Pyrus communis*) (Derviş et al., 2024) further highlights its evolving threat to pome fruits. The pathogen's impact on forestry, where it affects European black pine (*Pinus nigra*) and other pine species (Türkölmez et al., 2019), underscores its potential for broader ecological and environmental consequences. These factors collectively emphasize the need for integrated and region-specific management strategies to mitigate the effects of *N. dimidiatum* across Türkiye's diverse agricultural and forestry sectors. The urgency of developing effective and sustainable control measures is underscored by the absence of fungicides specifically registered for *N.*

dimidiatum in Türkiye, highlighting a critical gap in current management options.

Inorganic and organic salts have emerged as promising alternatives to synthetic fungicides, demonstrating notable efficacy in managing various plant pathogens. Several studies have highlighted the potential of salts in disease control. For instance, bicarbonates and carbonates have been shown to be effective antifungal compounds. Youssef et al. (2012) demonstrated that sodium carbonate and potassium bicarbonate successfully suppressed postharvest decay in citrus, while Alvindia and Natsuaki (2007) found that sodium bicarbonate, when used with a surfactant, controlled conidial germination of *Lasiodiplodia theobromae* and other banana crown rot pathogens. Similarly, Türkkan (2015) and Türkkan et al. (2017) demonstrated the efficacy of bicarbonates (ammonium, potassium, and sodium) against *Ilyonectria liriodendri* and *Botrytis cinerea*, respectively, in kiwifruit, supporting their broader use as fungistatic agents in various crops (Deliopoulos et al., 2010). Research has demonstrated the broad-spectrum antifungal activity of calcium, magnesium, and borate salts. Ippolito et al. (2005) demonstrated the efficacy of calcium chloride against *Botrytis cinerea* in wounded sweet cherries. Türkkan (2019) and Montecalvo et al. (2023) further highlighted the potential of calcium salts in controlling postharvest diseases in carrots and mangoes, respectively. Ünsal et al. (2019) showed that calcium propionate can effectively prevent *Fusarium* basal rot in onions. While magnesium sulfate has shown efficacy against *Fusarium solani* (Elsherbiny and El-Khateeb, 2012), magnesium chloride was less effective against *Monilinia fructigena* in apple fruits (Lyousfi et al., 2023). Borates, such as boric acid and borax, have gained significant attention. Shi et al. (2011) reported that potassium tetraborate application to mango trees increased fruit set and reduced anthracnose. Recent studies by Erper et al. (2019) and Yıldırım et al. (2020) have further supported the potential of borates as sustainable alternatives to synthetic fungicides for controlling postharvest diseases in apples. Additionally, Kara et al. (2023) demonstrated the efficacy of Etidot-67, borax, and boric acid in managing fruit and crown rot caused by *Coniella granati* in pomegranates.

Recent studies have underscored the potential of salts in controlling *N. dimidiatum*. Sodium salts, particularly at concentrations above 3%, have been shown to significantly inhibit the mycelial growth of *Neoscytalidium* isolates. Field trials incorporating sodium salt treatments along with rotating fungicides demonstrated a significant reduction in stem canker severity on dragon fruit (Riska et al., 2023), further supporting the integration of salts into disease management programs. This supports earlier work by Youssef et al. (2012), which found that pre- and postharvest applications of salts could substantially suppress postharvest decay in citrus. Understanding the environmental factors influencing *N. dimidiatum* growth is crucial for developing effective management strategies. Studies have shown that this pathogen thrives at temperatures between 20°C and 35°C, with optimal growth in slightly acidic environments (Gusella et al., 2023; Kuan et al., 2023). These conditions suggest that pH modulation, in conjunction with salt treatments, could offer a synergistic strategy for managing *N. dimidiatum* infections in diverse settings.

Building on this foundation, the present study evaluates the antifungal efficacy of various salts against two isolates of *N. dimidiatum*. The study investigates the effects of these salts on key fungal growth parameters—mycelial growth, arthrospore germination, and germ tube elongation—under both fixed and adjusted pH conditions to simulate diverse environmental scenarios. This comprehensive approach aims to provide insights into developing sustainable and effective strategies for managing *N. dimidiatum*, mitigating its impact on global agriculture and food security.

Materials and Methods

Fungal isolates

Two *Neoscytalidium dimidiatum* isolates, Ol_Dr04 and Ciar 64, were used in this study. The Ol_Dr04 isolate (GenBank Acc. Nos.: OK416080, OK428813, and OK428827 for ITS, *tef1*, and *tub2*) was sourced from olive (*Olea europaea*) (Güney et al., 2022), while the Ciar 64 isolate (OQ160342, OQ161650, and OQ161662 for ITS, *tef1*, and *tub2*) was obtained from chickpea (*Cicer arietinum*) and was previously classified as *N. novaehollandiae* (Güney et al., 2023). These isolates were chosen based on their molecular clustering; however, recent DNA-based studies have consolidated both isolates under *N. dimidiatum* (Zhang et al., 2021). Stock cultures were maintained on potato dextrose agar (PDA; BD Difco, Sparks, USA) at 4°C. Fresh fungal inoculums were prepared from 7-day-old cultures on PDA before use in the experiments.

pH range experiments for mycelial growth

To assess the impact of pH on fungal growth, PDA media were adjusted to pH levels ranging from 2.0 to 12.0 in 1.0-unit increments using 1N NaOH (Riedel-de Haen AG, Buchs SG, Switzerland) or HCl (Merck, Darmstadt, Germany) following the method described by Türkkan (2013). After the medium was sterilized by autoclaving, it was cooled to 50°C, and the pH was verified and adjusted if necessary. The medium was then aseptically dispensed into 9 cm diameter Petri plates. A 5-mm mycelial plug from a 7-day-old culture of either the Ol_Dr04 or Ciar 64 isolate was placed in the center of each pH-adjusted PDA plate. Plates were incubated at 25°C for 7 days in the dark. Mycelial growth was measured daily by recording the colony diameter in two perpendicular directions, and the average colony diameter was calculated. Five replicates were conducted for each pH level and fungal isolate. The effect of pH on mycelial growth was determined by comparing colony growth at different pH levels, with both inhibitory and stimulatory effects noted.

Salt treatments and pH adjustment

A total of nineteen different salts were tested for their antifungal activity against *N. dimidiatum*, including ammonium bicarbonate, ammonium carbonate, various borates compounds (anhydrous borax, boric acid, Etidot-67, and hydrated borax), calcium salts (acetate, citrate tetrahydrate, oxide, and propionate), magnesium sulfate, potassium bicarbonate, potassium carbonate, and various sodium salts (acetate, benzoate, bicarbonate, carbonate, citrate tetrahydrate, and metabisulfite). Stock solutions for each salt were prepared at a concentration of 10% (w/v) in distilled water and stored at 4°C. Before each experiment, these solutions were sterilized using a 0.22 µm membrane filter to ensure sterility. The inhibitory effects of the salts on mycelial growth were tested under both fixed and adjusted pH conditions. In the fixed pH condition, the salt-amended media retained the intrinsic pH of each salt solution. For the adjusted pH condition, the salt-amended media was set to pH 5 using HCl or NaOH, following the method of Türkkan and Erper (2014). The pH of the media used in control experiments was either kept fixed or set to 5, depending on the experimental conditions. For experiments requiring higher concentrations, stock solutions were diluted in distilled water (10 ml of stock solution in 100 ml of water) to achieve consistent application.

Antifungal activity assays

The antifungal efficacy of salts was tested by assessing mycelial growth inhibition. PDA plates were prepared with the addition of 1% (w/v) of each salt solution. Mycelial discs (5 mm in diameter) were taken from 7-day-old fungal cultures of both isolates and placed in the center of each plate. Plates were incubated at 25°C for 7 days in the dark (Türkkan and Erper, 2014). Colony diameters were measured daily, and the percentage of mycelial growth inhibition (MGI) was calculated using the formula: MGI (%) = [(dc−dt) / dc] × 100, where "dc" represents the colony diameter in the control plates, and "dt" represents the colony diameter in the salt-treated plates. Five replicates were conducted for each treatment.

Determination of EC_{50}, MIC, and MFC

The effective concentration of salts required to achieve a 50% reduction (EC_{50}) in mycelial growth, arthrospore germination, and germ tube elongation was calculated using probit analysis. Mycelial growth and arthrospore germination were assessed as previously described, with PDA amended to contain salt concentrations of 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, and 1% (w/v). EC_{50} values were calculated based on the reduction in growth relative to untreated controls.

In parallel experiments, the minimum inhibitory concentration (MIC), defined as the lowest concentration required to completely inhibit mycelial growth, was identified. The fungistatic or fungicidal effects of salts were determined using the method described by Türkkan (2013). Discs from salt-amended PDA plates that showed no fungal growth were transferred to fresh, unamended PDA medium and incubated for 9 days at 25°C in the dark to monitor for regrowth. The lowest concentration at which no regrowth was observed, indicating complete and irreversible inhibition, was recorded as the minimum fungicidal concentration (MFC).

Arthrospore germination and germ tube elongation inhibition assay

The effect of salts on arthrospore germination and germ tube elongation was evaluated following the method described by Türkkan (2019). After 7 days of incubation, arthrospores were harvested from each plate by adding 5 ml of distilled water and gently stirring the fungal colony with a sterile scalpel. The resulting spore suspensions were filtered through two layers of sterile cheesecloth to remove hyphal fragments, then transferred to sterile microtubes and

homogenized by vortexing for 30 seconds. To assess the effects of salts on both arthrospore germination and germ tube elongation, aliquots (100 µl) of the homogenized arthrospore suspensions (1×10^4 spores/ml) were placed onto PDA plates amended with salt concentrations of 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, and 1% (w/v). The spore suspensions were evenly spread using a sterile glass rod, and the plates were incubated at 25°C for 24 hours.

Arthrospore germination was assessed using a DM 750 microscope (Leica Microsystems GmbH, Wetzlar, Germany) at 150× magnification, equipped with a micrometer. A minimum of 100 arthrospores were evaluated per replicate. An arthrospore was considered germinated if the length of the germ tube was equal to or greater than the length of the spore body. The inhibition of arthrospore germination was calculated using the formula: Inhibition of Arthrospore Germination (%) = (%) = [(agc−agt) / agc] × 100, where "agc" represents the number of germinated arthrospores in the control plates, and "agt" represents the number of germinated arthrospores in the salt-treated plates. Five replicates were conducted for each treatment. For germ tube elongation, the length of the germ tubes was measured under a microscope using a micrometer. Inhibition of germ tube elongation was calculated by comparing the average germ tube length in the salt-treated plates to that in the control plates, using the formula: Inhibition of Germ Tube Elongation (%) = [(glc−glt) / glc] × 100, where "glc" represents the average germ tube length in the control plates, and "glt" represents the average germ tube length in the salt-treated plates.

Statistical analysis

All data (mycelial growth, arthrospore germination, and germ tube elongation), were square root transformed to normalize distribution before analysis. A one-way Analysis of Variance (ANOVA) was then performed on the transformed data. Fisher's post-hoc test was employed to identify significant differences between treatments, with a significance threshold of $P < 0.05$. All statistical analyses were conducted using XLSTAT software (version 2016.02.28451; Addinsoft, USA).

Results

pH range experiments for mycelial growth

For Ol_Dr04, the highest mycelial growth was observed at pH 5 (20.5 mm), followed closely by pH 6 (20.4 mm), with no statistically significant difference between these two pH levels ($P > 0.05$) (Table 1). Mycelial growth at pH 4 (19.2 mm) was still high but significantly lower than at pH 5 and 6 ($P < 0.05$). Mycelial growth continued to decline significantly as pH deviated from this optimal range. At pH 7, mycelial growth was reduced to 18.3 mm ($P < 0.05$), followed by a further drop at pH 8 (16.7 mm) and a pronounced decline at pH 9 (7.6 mm), with each reduction being statistically significant compared to pH 5 and 6 ($P < 0.05$). Minimal mycelial growth was observed at pH 10 (5.4 mm) and pH 11 (1.9 mm), and there was complete inhibition at pH 2 and 12.

Similarly, for Ciar 64, the highest mycelial growth occurred at pH 5 (19.8 mm), with pH 6 (19.4 mm) showing comparable mycelial growth and no statistically significant difference between the two ($P > 0.05$) (Table 1). At pH 4, mycelial growth measured at 18.7 mm was significantly lower than at pH 5 and 6 ($P < 0.05$) but was still among the higher values observed. Mycelial growth decreased to 16.3 mm at pH 7, followed by significant reductions at pH 8 (14.9 mm) and pH 9 (7.7 mm) ($P < 0.05$). Mycelial growth was minimal at pH 10 (5.4 mm) and pH 11 (2.0 mm), and no growth was observed at the extreme pH values of 2 and 12.

Table 1. Impact of varying pH on mycelial growth of *Neoscytalidium dimidiatum* isolates Ol_Dr04 and Ciar 64

pH	Mycelial Growth							
	Ol_Dr04				Ciar 64			
2	0.00	±	0.00*	h**	0.00	±	0.00	i
3	12.00	±	0.35	d	11.60	±	0.37	e
4	19.20	±	0.34	b	18.70	±	0.20	b
5	20.50	±	0.22	a	19.80	±	0.20	a
6	20.40	±	0.24	a	19.40	±	0.37	ab
7	18.30	±	0.46	b	16.30	±	0.20	c
8	16.70	±	0.30	c	14.90	±	0.24	d
9	7.60	±	0.24	e	7.70	±	0.37	f
10	5.40	±	0.24	f	5.40	±	0.24	g
11	1.90	±	0.10	g	2.00	±	0.00	h
12	0.00	±	0.00	h	0.00	±	0.00	i

*Data are presented as means ± standard errors.
**Different letters indicate statistical significance at $P < 0.05$.

Antifungal activity assays

Under fixed pH conditions, several salts demonstrated complete inhibition of mycelial growth for both *N. dimidiatum* isolates, Ol_Dr04 and Ciar 64. Specifically, ammonium carbonate, ammonium bicarbonate, sodium carbonate, sodium benzoate, sodium metabisulfite, sodium citrate tetrahydrate, hydrated borax, anhydrous borax, and Etidot-67 achieved full mycelial suppression (100% inhibition), indicating strong antifungal properties regardless of

pH variation (Table 2, Figure 1). The remaining salts showed inhibition rates ranging from 25.6% to 95.6%, with statistically significant differences between the highly effective salts and others (P < 0.05). Under adjusted pH conditions (pH 5), sodium benzoate and sodium metabisulfite maintained their high efficacy, with complete inhibition observed for both isolates, illustrating their robust antifungal activity across different pH conditions. Conversely, ammonium carbonate, ammonium bicarbonate, and borax salts exhibited reduced inhibition under this pH adjustment. Ammonium carbonate showed slightly lower efficacy, reducing mycelial growth by 93.19% for Ol_Dr04 and 89.40% for Ciar 64 (P < 0.05).

Table 2. Impact of various salts on the mycelial growth of *Neoscytalidium dimidiatum* isolates Ol_Dr04 and Ciar 64 under fixed and adjusted pH

No	Salts	Mycelial Growth Inhibition (%)				
		Fixed pH			Adjusted pH = 5	
		pH	Ol_Dr04	Ciar 64	Ol_Dr04	Ciar 64
1	Ammonium bicarbonate	7.63	100.00±0.00* a**	100.00±0.00 a	83.70±1.36 d	84.79±0.91 c
2	Ammonium carbonate	7.91	100.00±0.00 a	100.00±0.00 a	93.19±1.05 bc	89.40±0.90 bc
3	Anhydrous borax	7.53	100.00±0.00 a	100.00±0.00 a	67.40±1.22 f	61.74±0.92 d
4	Boric acid	4.97	79.36±0.66 c	94.26±0.88 b	71.92±1.26 ef	84.81±0.80 c
5	Calcium acetate	5.96	25.60±0.25 h	36.62±1.63 h	95.47±0.06 ab	95.39±0.05 ab
6	Calcium citrate tetrahydrate	5.33	26.47±0.91 h	39.81±1.39 g	8.52±1.82 j	10.11±1.10 g
7	Calcium oxide	10.86	32.71±0.74 g	66.00±1.42 f	100.00±0.00 a	100.00±0.00 a
8	Calcium propionate	5.92	68.52±0.92 d	73.08±0.72 cd	100.00±0.00 a	100.00±0.00 a
9	Etidot-67	7.20	100.00±0.00 a	100.00±0.00 a	75.12±0.95 ef	82.48±2.37 c
10	Hydrated borax	7.90	100.00±0.00 a	100.00±0.00 a	55.14±1.83 g	59.90±1.71 d
11	Magnesium sulfate	5.07	35.60±1.54 f	69.74±1.09 e	10.39±0.83 i	6.37±2.18 h
12	Potassium bicarbonate	7.45	95.55±0.04 b	75.68±1.17 c	29.84±1.07 h	49.73±1.73 e
13	Potassium carbonate	10.17	95.55±0.04 b	95.58±0.04 b	12.10±2.37 i	19.76±2.30 f
14	Sodium acetate	6.14	57.75±1.08 e	70.62±0.95 de	93.68±0.81 abc	95.39±0.05 ab
15	Sodium benzoate	5.89	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
16	Sodium bicarbonate	7.28	95.55±0.04 b	92.93±0.83 b	88.68±1.02 cd	84.36±0.70 c
17	Sodium carbonate	10.32	100.00±0.00 a	100.00±0.00 a	85.09±0.76 d	89.41±0.86 bc
18	Sodium citrate tetrahydrate	6.73	100.00±0.00 a	100.00±0.00 a	95.47±0.06 ab	95.39±0.05 ab
19	Sodium metabisulfite	4.94	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a

*Data are presented as means ± standard errors.
**Different letters indicate statistical significance at P < 0.05.

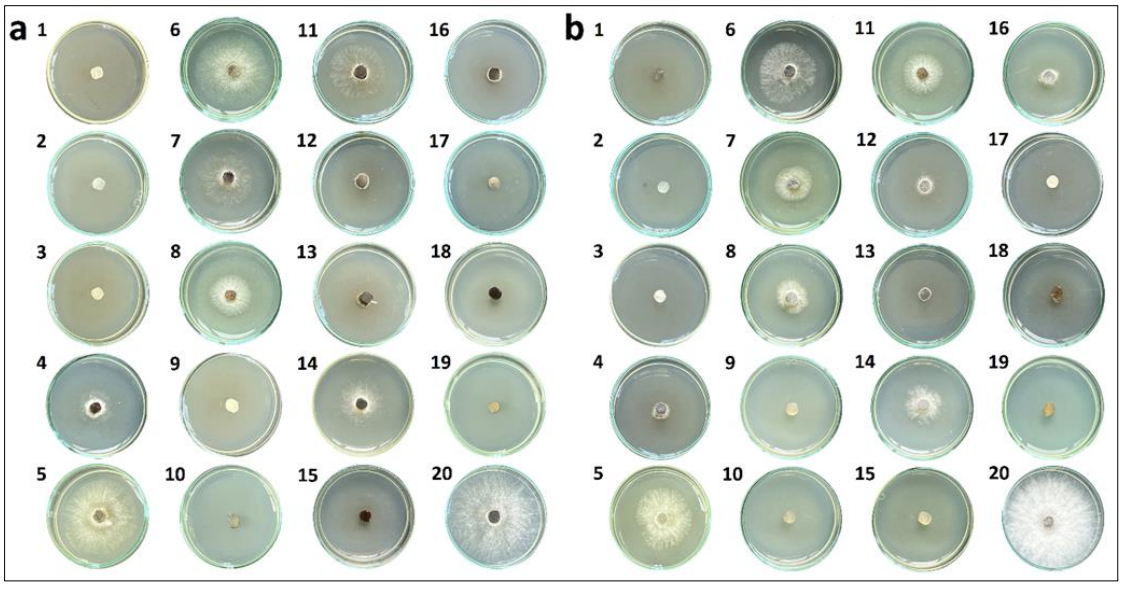

Figure 1. Mycelial growth of *Neoscytalidium dimidiatum* isolates Ol_Dr04 (a) and Ciar 64 (b) treated with 1% concentrations of various salts. The salts are numbered as follows: (1) Ammonium bicarbonate, (2) Ammonium carbonate, (3) Anhydrous borax, (4) Boric acid, (5) Calcium acetate, (6) Calcium citrate tetrahydrate, (7) Calcium oxide, (8) Calcium propionate, (9) Etidot-67, (10) Hydrated borax, (11) Magnesium sulfate, (12) Potassium bicarbonate, (13) Potassium carbonate, (14) Sodium acetate, (15)

Sodium benzoate, (16) Sodium bicarbonate, (17) Sodium carbonate, (18) Sodium citrate tetrahydrate, (19) Sodium metabisulfite, and (20) Control (no salt added).

The efficacy of sodium bicarbonate also decreased at constant pH 5, showing 88.68% inhibition for Ol_Dr04 and 84.36% for Ciar 64, a significant reduction from its effectiveness under variable pH conditions ($P < 0.05$). Potassium bicarbonate, which demonstrated high inhibition at variable pH, was notably less effective under constant pH 5, achieving only 29.84% inhibition for Ol_Dr04 and 49.73% for Ciar 64. This difference was statistically significant ($P < 0.05$), indicating the influence of pH adjustments on its antifungal properties.

Among the calcium salts, calcium acetate showed relatively low inhibition under variable pH conditions but significantly increased inhibition under constant pH 5, achieving 95.47% inhibition for both isolates ($P < 0.05$). Calcium citrate tetrahydrate, however, remained ineffective, with minimal inhibition observed across both conditions. Boric acid displayed moderate efficacy, with a decrease in inhibition at constant pH ($P < 0.05$), while hydrated borax and anhydrous borax exhibited complete inhibition under variable pH conditions, though their effectiveness decreased under constant pH 5 (Table 2, Figure 1).

Determination of EC_{50}, MIC, and MFC

The antifungal activities of the 19 salts were assessed through their EC_{50}, MIC, and MFC values (Tables 3 and 4). Sodium metabisulfite exhibited the highest antifungal activity, with EC_{50} values of 0.016% for both isolates and the lowest MIC and MFC values at 0.0625%, making it the most potent fungicidal compound tested. Ammonium salts, including ammonium carbonate and ammonium bicarbonate, also displayed strong antifungal effects, with EC_{50} values of 0.066% and 0.053% for Ol_Dr04, and 0.069% and 0.071% for Ciar 64, respectively. The MIC values for these ammonium salts were relatively low (0.250%), and they demonstrated fungicidal activity with MFC values between 0.500% and 1.000%. Sodium carbonate and sodium benzoate exhibited notable antifungal activity, though they required higher MIC and MFC values, indicating that while effective at inhibiting growth, they were less potent in suppressing the fungus. Bicarbonate salts, such as sodium bicarbonate and potassium bicarbonate, showed moderate antifungal activity with high MIC and MFC values, suggesting limited fungicidal properties. Calcium salts, including calcium acetate and calcium citrate tetrahydrate, were among the least effective, with high EC_{50} values for mycelial growth and MIC and MFC values exceeding 1.000%. Magnesium sulfate also exhibited limited antifungal activity, with EC_{50} values above 1.000% for both isolates.

Table 3. EC_{50}, MIC, and MFC values (% w/v) for *Neoscytalidium dimidiatum* isolate Ol_Dr04 under different salt treatments

No	Salts	Mycelial Growth Inhibition			Arthrospore germination	Germ tube elongation
		EC_{50}*	MIC**	MFC***	EC_{50}	EC_{50}
1	Ammonium bicarbonate	0.053 (0.012-0.075)	0.250	1.000	0.090 (0.077-0.099)	0.074 (0.058-0.085)
2	Ammonium carbonate	0.066 (0.037-0.082)	0.250	0.500	0.095 (0.083-0.103)	0.064 (0.054-0.072)
3	Anhydrous borax	0.252 (0.229-0.277)	1.000	>1.000	0.289 (0.247-0.334)	0.257 (0.248-0.267)
4	Boric acid	0.352 (0.287-0.424)	>1.000	>1.000	0.412 (0.342-0.492)	0.281 (0.263-0.300)
5	Calcium acetate	>1.000	>1.000	>1.000	>1.000	0.662 (0.621-0.708)
6	Calcium citrate tetrahydrate	>1.000	>1.000	>1.000	>1.000	0.896 (0.851-0.946)
7	Calcium oxide	>1.000	>1.000	>1.000	>1.000	0.835 (0.794-0.881)
8	Calcium propionate	0.465 (0.416-0.518)	>1.000	>1.000	>1.000	0.522 (0.498-0.547)
9	Etidot-67	0.165 (0.156-0.173)	0.500	>1.000	0.183 (0.168-0.198)	0.209 (0.201-0.219)
10	Hydrated borax	0.126 (0.103-0.147)	0.500	>1.000	0.220 (0.191-0.252)	0.195 (0.188-0.202)
11	Magnesium sulfate	>1.000	>1.000	>1.000	>1.000	0.405 (0.384-0.427)
12	Potassium bicarbonate	0.380 (0.331-0.436)	>1.000	>1.000	0.524 (0.477-0.575)	0.335 (0.313-0.357)
13	Potassium carbonate	0.206 (0.184-0.229)	>1.000	>1.000	0.157 (0.133-0.180)	0.364 (0.337-0.392)
14	Sodium acetate	0.899 (0.839-0.966)	>1.000	>1.000	0.699 (0.591-0.842)	0.155 (0.135-0.175)
15	Sodium benzoate	0.058 (0.043-0.072)	1.000	>1.000	0.122 (0.115-0.127)	0.085 (0.080-0.090)
16	Sodium bicarbonate	0.057 (0.041-0.072)	>1.000	>1.000	0.312 (0.261-0.367)	0.226 (0.214-0.237)
17	Sodium carbonate	0.027 (0.015-0.041)	1.000	>1.000	0.175 (0.160-0.191)	0.284 (0.260-0.308)
18	Sodium citrate tetrahydrate	0.073 (0.058-0.085)	0.500	>1.000	0.148 (0.133-0.163)	0.206 (0.196-0.216)
19	Sodium metabisulfite	0.016 (0.011-0.020)	0.0625	0.0625	<0.03125	<0.03125

*EC_{50} values represent the concentration causing a 50% reduction in mycelial growth, arthrospore germination, or germ tube elongation.

**MIC indicates the minimum inhibitory concentration.
***MFC indicates the minimum fungicidal concentration.

Table 4. EC_{50}, MIC, and MFC values (% w/v) for *Neoscytalidium dimidiatum* isolate Ciar 64 under different salt treatments

No	Salts	Mycelial Growth Inhibition (%)			Arthrospore germination	Germ tube elongation
		EC_{50}*	MIC**	MFC***	EC_{50}	EC_{50}
1	Ammonium bicarbonate	0.071 (0.047-0.085)	0.250	1.000	0.090 (0.077-0.099)	0.030 (0.013-0.044)
2	Ammonium carbonate	0.069 (0.043-0.084)	0.250	0.250	0.088 (0.075-0.098)	0.053 (0.039-0.063)
3	Anhydrous borax	0.202 (0.175-0.231)	1.000	>1.000	0.226 (0.191-0.261)	0.229 (0.220-0.239)
4	Boric acid	0.212 (0.195-0.229)	>1.000	>1.000	0.386 (0.316-0.466)	0.266 (0.248-0.285)
5	Calcium acetate	>1.000	>1.000	>1.000	>1.000	0.763 (0.700-0.834)
6	Calcium citrate tetrahydrate	>1.000	>1.000	>1.000	>1.000	>1.000
7	Calcium oxide	0.628 (0.573-0.691)	>1.000	>1.000	>1.000	0.176 (0.161-0.192)
8	Calcium propionate	0.249 (0.195-0.302)	>1.000	>1.000	>1.000	0.419 (0.379-0.461)
9	Etidot-67	0.158 (0.136-0.179)	0.500	>1.000	0.159 (0.151-0.167)	0.179 (0.169-0.188)
10	Hydrated borax	0.118 (0.107-0.128)	0.500	>1.000	0.193 (0.174-0.214)	0.193 (0.183-0.203)
11	Magnesium sulfate	0.561 (0.512-0.616)	>1.000	>1.000	>1.000	0.484 (0.443-0.526)
12	Potassium bicarbonate	0.384 (0.328-0.445)	>1.000	>1.000	0.631 (0.579-0.690)	0.168 (0.156-0.180)
13	Potassium carbonate	0.229 (0.210-0.249)	>1.000	>1.000	0.104 (0.072-0.136)	0.257 (0.240-0.274)
14	Sodium acetate	0.423 (0.368-0.483)	>1.000	>1.000	0.472 (0.421-0.528)	0.226 (0.210-0.243)
15	Sodium benzoate	0.073 (0.059-0.087)	1.000	>1.000	0.117 (0.111-0.123)	0.096 (0.092-0.100)
16	Sodium bicarbonate	0.038 (0.023-0.055)	>1.000	>1.000	0.717 (0.626-0.830)	0.329 (0.310-0.349)
17	Sodium carbonate	0.042 (0.028-0.056)	1.000	>1.000	0.228 (0.188-0.268)	0.211 (0.202-0.219)
18	Sodium citrate tetrahydrate	0.035 (0.018-0.051)	0.500	>1.000	0.119 (0.108-0.130)	0.199 (0.186-0.213)
19	Sodium metabisulfite	0.017 (0.012-0.020)	0.0625	0.0625	<0.03125	<0.03125

*EC_{50} values represent the concentration causing a 50% reduction in mycelial growth, arthrospore germination, or germ tube elongation.
**MIC indicates the minimum inhibitory concentration.
***MFC indicates the minimum fungicidal concentration

In terms of arthrospore germination, sodium metabisulfite was highly effective, achieving complete inhibition at concentrations below 0.03125%. Ammonium carbonate and ammonium bicarbonate also showed strong inhibition, with EC_{50} values from 0.085% to 0.090%. Sodium and potassium salts showed moderate inhibition, with EC_{50} values between 0.104% and 0.700%, while calcium and magnesium salts were the least effective, with EC_{50} values above 1.000%. Sodium metabisulfite continued to be the most potent inhibitor in germ tube elongation assays, with an EC_{50} below 0.03125%. Ammonium carbonate, ammonium bicarbonate, and sodium benzoate exhibited moderate inhibition, with EC_{50} values between 0.030% and 0.096%, while the remaining salts required higher concentrations to achieve significant inhibition.

Discussion

This study provides a comprehensive evaluation of the antifungal efficacy of various salts against *Neoscytalidium dimidiatum* isolates Ol_Dr04 and Ciar 64, focusing on their impact on mycelial growth, arthrospore germination, and germ tube elongation. The results demonstrate that ammonium salts (ammonium carbonate and ammonium bicarbonate), sodium metabisulfite, and sodium benzoate consistently exhibited the strongest antifungal activity, while calcium salts and magnesium sulfate were among the least effective salts across all assays.

The pH sensitivity of both isolates significantly influenced their growth patterns. Optimal mycelial growth occurred in slightly acidic to neutral pH conditions (pH 4 to 6), while growth was notably inhibited under acidic (pH 2) and alkaline (pH 12) extremes, indicating a high susceptibility to pH variations. This observation aligns with previous research on fungal pathogens, including *Neoscytalidium*, which often prefer slightly acidic environments for growth (Türkkan, 2013; Türkkan and Erper 2015; Deliopoulos et al., 2010). Consequently, pH modulation may be an effective component of integrated disease management strategies for *N. dimidiatum*.

Among the tested salts, ammonium carbonate and ammonium bicarbonate demonstrated potent antifungal activity with low EC_{50}, MIC, and MFC values, consistent with findings by Türkkan (2013) on ammonium salts' efficacy against *F. oxysporum* f. sp. *cepae*. This suggests that ammonium salts likely exert their antifungal effects by disrupting fungal cell walls and membranes, which contributes to their superior efficacy (Türkkan et al., 2017). Given their consistent performance across pH variations, ammonium salts

represent promising alternatives to conventional fungicides for managing *Neoscytalidium* infections.

Sodium metabisulfite was particularly notable, exhibiting the lowest EC_{50}, MIC, and MFC values. Its ability to completely inhibit mycelial growth, arthrospore germination, and germ tube elongation, even at low concentrations, underscores its potential as a highly effective fungicide. These findings corroborate previous research by Türkkan and Erper (2015), which demonstrated comparable efficacy against various *Fusarium* species (*Fusarium equiseti*, *F. proliferatum*, *F. semitectum*, *F. solani* f. sp. *phaseoli*, *F. verticillioides*), *Rhizoctonia solani* AG4–HG I, and *Macrophomina phaseolina*, likely attributed to the release of sulfur dioxide, which disrupts fungal cellular respiration (Palou et al., 2016). Given its broad-spectrum activity and low EC_{50} values, sodium metabisulfite is a strong candidate for both pre- and postharvest fungal management applications.

The high efficacy of these salts is comparable to or exceeds that of certain conventional fungicides. Xu et al. (2018) and Xian et al. (2018) reported control rates up to 85% with fungicides like pyraclostrobin, while sodium metabisulfite here achieved complete inhibition with very low EC_{50} values. Furthermore, sodium metabisulfite and ammonium salts' adaptability across pH variations resemble the versatility observed in fungicides like fluopyram combined with tebuconazole, which exhibit efficacy across diverse conditions (Eraslan Sür and Oksal, 2021). This adaptability indicates that these salts could be integrated into rotation programs with conventional fungicides to mitigate resistance, as demonstrated by Riska et al. (2023) for sodium salts in managing dragon fruit diseases.

In the context of previous research, the antifungal activity of salts like calcium oxide and aluminum sulfate (Najarpour et al., 2018; Kolaei et al., 2013) underscores the potential of metal-based salts in agricultural disease management. Calcium oxide has shown efficacy in controlling *Phytophthora pistaciae*, and aluminum sulfate has completely inhibited *Pythium sulcatum* in carrots. Similarly, copper and potassium phosphides have reduced the disease severity of Verticillium wilt in olives (López-Moral et al., 2021). While not as potent as ammonium and sodium salts in this study, these findings support the utility of metal-based salts in specific contexts. Furthermore, electrolyzed water solutions containing ammonium molybdate, sodium metabisulfite, and potassium sorbate have been effective in postharvest disease control, particularly for citrus fruits (Hussien et al., 2018). This highlights the potential of salts, not only as standalone antifungal agents but also as part of combined treatments, for managing plant diseases across diverse environments and crop systems.

Although Baker et al. (2006) reported moderate antifungal properties of boron-based salts, subsequent research, including studies by Erper et al. (2019) and Yıldırım et al. (2020), has strengthened the case for borates as viable and environmentally friendly substitutes for synthetic fungicides, especially in managing postharvest diseases like apple blue mold caused by *Penicillium expansum*. The varying efficacy observed across different salts can be attributed to distinct modes of action. Ammonium, potassium and sodium salts likely disrupt fungal cell structure, whereas borates and bicarbonates alter osmotic balance and intracellular pH, providing fungistatic effects (Deliopoulos et al., 2010; Shi et al. 2011; Türkkan et al., 2018).

In summary, this study confirms that ammonium salts, sodium metabisulfite, and sodium benzoate are among the most effective antifungal agents tested, with sodium metabisulfite demonstrating particularly low EC_{50}, MIC, and MFC values. These results highlight the potential for integrating salts into sustainable disease management frameworks. Further research, particularly *in vivo* trials, is necessary to validate these results, explore their practical applications within agricultural systems, and support a shift towards more sustainable practices. Incorporating salts with fungicides or other biocontrol methods could enhance disease control efficacy, reduce environmental impact, and delay fungicide resistance.

Conclusion

The findings underscore the strong potential of ammonium salts, particularly ammonium carbonate and ammonium bicarbonate, alongside sodium metabisulfite, as highly effective fungicidal agents against *N*. *dimidiatum*. These salts consistently inhibited mycelial growth, arthrospore germination, and germ tube elongation, suggesting that they may serve as viable alternatives to conventional fungicides. In contrast, calcium and boron-based salts demonstrated limited efficacy, requiring substantially higher concentrations to achieve comparable fungicidal effects. These results, supported by existing literature, indicate that salt-based treatments could become integral to sustainable and environmentally friendly plant

pathogen control strategies. However, further research, including *in vivo* studies and field trials, will be essential to validate these *in vitro* findings and assess the feasibility of incorporating salts into broader disease management programs. This approach holds promise for reducing the environmental impact of chemical fungicides and advancing the objectives of sustainable agriculture.

Conflict of Interest

The authors declare no conflicts of interest.

Authorship contribution statement

E.Y: Actively participated in conducting *in vitro* experiments and contributed to the collection and analysis of experimental data. M.T: Supervised the research project, conceived and designed the study, analyzed the data, and contributed both the original draft and the final submission of the manuscript. S.D: Provided critical input on study design and manuscript preparation, wrote the manuscript, and finalized the manuscript for submission. N.B: Assisted in conducting the *in vitro* experiments and contributed to data collection. GÖ and IE: Actively participated in reviewing and revising the manuscript.

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