



EFFECT OF DIFFERENT CHEMICAL INDUCERS ON MYCELIAL GROWTH OF *Neoscytalidium dimidiatum*

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Abstract: *Neoscytalidium dimidiatum* has become one of the most aggressive fungal pathogen that cause economical damage to plants with changing climatic conditions. Pathogen causes disease symptoms including dieback, canker, blight, root rot, leaf spot, and fruit rot at a wide range of plant species and significant yield losses and damages. Few studies have been conducted on the efficiency of different chemical fungicides against the pathogen, but no effective control method has been found. Also, comprehensive studies on different control methods were needed due to the disadvantages in the use of chemical fungicides. The aim of the study was to evaluate the effects of chitosan (1, 1.5, 2 mg/ml), methyl jasmonate (MeJA; 0.01, 0.1, 1 mM) and acibenzolar-S-methyl (BTH; 0.01, 0.1, 1 mM) on mycelial growth of *N. dimidiatum*. The results showed statistically significant differences among the inhibition rates of chemical inducers against *N. dimidiatum*, but also among different doses of chemical inducers as compared to control. Chitosan at 2 mg/ml concentration was the most effective with the inhibition rate of 45.2%, followed by 1.5 mg/ml and 1 mg/ml doses of chitosan that inhibited mycelial growth at the rates of 44.6 and 37.9%, respectively. BTH was the second most effective treatment after chitosan with the inhibition rate of 18.9% at 1 mM dose, while MeJA was sufficiently ineffective in inhibiting the mycelium growth of *N. dimidiatum* at the concentrations tested. The results indicated that chitosan could be an alternative to fungicides due to its high level of effectiveness and non-toxicity.

Keywords: Chemical inducer, Disease control, Mycelial growth, *Neoscytalidium dimidiatum*

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1. Introduction

Neoscytalidium is a fungal genus that has emerged as a significant agricultural threat with global warming. The pathogen has a wide range of host species including trees, crops, vegetables, and shrubs and causes disease symptoms such as canker, blight, dieback, leaf spot, root rot, and fruit rot (Derviş and Özer, 2023). High temperatures, drought and plant stress promote infection by the pathogen and can significantly reduce the quality and value of fruit in some crops (Hong et al., 2020; Derviş and Özer, 2023). *Neoscytalidium* have been reported recently by many researchers from Türkiye and around the world (Derviş et al., 2020; Ören et al., 2022a; Ören et al., 2022b; Güney et al., 2022; Zaeimian and Fotouhifar, 2023; Çaplık et al., 2024; de Lima Costa et al., 2024).

Three species of this genus including *N. dimidiatum*, *N. novaehollandiae* and *N. orchidacearum* were first identified as plant pathogenic species (Crous et al., 2006; Phillips et al., 2013; Huang et al., 2016; Suwannarach et al., 2018). However, *N. dimidiatum* was recognized as a single species due to high nucleotide similarity between the species and the other two species were accepted as synonyms (Zhang et al., 2021; Crous et al., 2021). The fungus forms blackish-brown pycnidia and doliiform,

oblong-obtuse, 0–2-septate, dark brown, powdery arthroconidia originating from aerial mycelium (Phillips et al., 2013). *Neoscytalidium dimidiatum* could infiltrate host plants through wounds, natural openings, and directly penetrate young plants via the formation of appressoria.

The high adaptability of the pathogen possessed important challenges in disease control. The most common and effective control strategy against *N. dimidiatum* was the use of chemical fungicides nowadays (Moral et al., 2019; Al Raish et al., 2020). However, the adverse effects of fungicides on human and environment, the absent of registered chemicals against the pathogen in some country and the risk of fungicide resistance in the future revealed the necessary of alternative control methods. The use of various synthetic non-toxic chemical compounds helps to inhibit pathogen development and to develop effective control methods by activating natural defense mechanisms in plants (Li et al. 2009; Varghese and Thomas, 2023). Among these compounds with non-toxic properties, chemicals such as chitosan, BTH (Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester) and jasmonic acid are known to play a role as secondary inducers in the activation of related genes and signaling mechanisms (Métraux, 2001; Walters et al. 2007). They were also used as a biological control agent



against various plant pathogenic fungi, bacteria, and viruses (Johnson and Temple, 2016; Thomas-Sharma et al. 2017; Gutiérrez-Martínez et al. 2017). The inducers provide effective protection against various pathogens by regulating the defense-related enzymes such as peroxidase, β -1,3-glucanase, chitinase, catalase, superoxide dismutase, PR proteins and phytoalexin metabolism in the host plant, and showing antifungal activity against pathogens (Andrade et al., 2013; Gutiérrez-Martínez et al., 2017; de Souza et al., 2018; Varghese and Thomas, 2023). Various chemical ingredients have been used by the researchers in the control of *N. dimidiatum*, but the use of methyl jasmonate (MeJA), chitosan and Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester or acibenzolar-S-methyl (BTH) in disease management has not been adequately addressed (Du et al., 2019; Kılınc and Güldür, 2020; Sür and Oksal, 2021; Sakçı et al., 2022; Abdul-Karim et al., 2023; Mohammadi et al., 2024).

This study aimed to investigate the effects of chitosan, jasmonic acid or methyl jasmonate (MeJA) and Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester or acibenzolar-S-methyl (BTH) applications on mycelial growth of *N. dimidiatum* and to examine their potential as an alternative to chemical fungicides.

2. Materials and Methods

Neoscytalidium dimidiatum isolate M1, identified as pathogen in a previous study was utilized in the assay (Ören et al., 2022a). The isolate was grown on potato dextrose agar (PDA; Merck, Darmstadt, Germany) medium for seven days and stored on filter paper in 4 °C.

2.1. Preparation of Chitosan, Methyl Jasmonate and Acibenzolar-S-methyl

Chitosan (HiMedia; 75%-85% deacetylation degree, Pennsylvania, United States) with low molecular weight was used in the study. According to the manufacturer's protocol, the stock solution of chitosan at 10 mg/ml concentration was firstly prepared and adjusted to pH 5.6 using 0.1 M NaOH. The application doses of chitosan were adjusted to 1.0, 1.5, and 2.0 mg/ml by adding sterile distilled water. Stock solutions (1 mM) of methyl jasmonate 95% (MeJA; Sigma-Aldrich, Massachusetts, United States) and acibenzolar-S-methyl (BTH; Syngenta) were prepared considering the molecular weights of commercial products and the solutions were diluted to different doses ranging from 0.1 to 0.01 mM. These concentrations were selected considering the studies that reported that defense mechanisms were activated and disease development was prevented in different research (Johnson and Temple, 2016; Gutiérrez-Martínez et al., 2017; Thomas-Sharma et al., 2017; Palacioğlu, 2024).

2.2. Evaluation of the Inhibitory Effects of Chitosan, MeJA and BTH on Mycelial Growth of *Neoscytalidium dimidiatum*

Potato dextrose agar medium was prepared separately in 250 ml Erlenmeyer flasks for each concentration of the

chemical inducers, autoclaved at 121 °C for 20 min, and cooled to 50 °C. Different concentrations of each of compounds were mixed with sterile PDA medium and poured in Petri dishes about 20 ml in each dish. Agar disks with a diameter of 4 mm were taken from the fungal culture of the *N. dimidiatum* isolate grown for 7 days and placed on each PDA medium containing different chemical inducers. Petri dishes were incubated at 25 °C for 7 days. All treatments were performed as 3 replicates for each concentration. PDA medium without any chemical inducers was used as control. At the end of the incubation period, the diameters of the fungal colonies in each Petri dishes were measured from both directions and arithmetic mean was calculated to evaluate the effects of chemical inducers on mycelium growth of *N. dimidiatum*.

2.3. Statistical Analysis

Significant differences between mean values of mycelial growth were determined by analysis of variance (one-way ANOVA) using Least Significant Difference (LSD) method ($P < 0.05$). The effectiveness of each compound at different concentrations was calculated using the Abbott formula (Karman, 1971).

3. Results and Discussion

Neoscytalidium dimidiatum is an important fungal pathogen that has a wide range of host and caused dieback, canker, blight, root rot, leaf spot, and fruit rot diseases in agricultural areas. The pathogen has recently caused serious economic losses due to climate change. Many studies have been conducted to assess the efficiency of various control methods, including some chemical fungicides, biocontrol agents and essential oils (Xian et al., 2018; Noegrohati et al., 2019; Taguiam et al., 2020; Ratanaprom et al., 2021; Sür and Oksal, 2021; Sakçı et al., 2022; Riska et al., 2023). This study aimed to investigate *in vitro* the efficiency of chitosan, MeJA, and BTH as alternative control methods against *N. dimidiatum*. These chemical inducers depending on their doses inhibited mycelial growth of the pathogen and significant differences were observed among both inducers and application doses (Figure 1). The results indicated that chitosan treatment was the most effective on pathogen development. Inhibition rates on mycelial growth increased with increasing chitosan dose (Figure 2). The application of 2 mg/ml chitosan allowed mycelial growth of 2.89 cm, while the growth at 1 mg/ml and 1.5 mg/ml doses were 3.27 cm and 2.92 cm, respectively (Figure 1). In MeJA and BTH applications, the lowest mycelial development was observed at 1 mM dose. BTH application was the second effective application, allowing lower mycelial growth than MeJA treatment. Mycelial growth values in BTH application ranged from 4.27 to 4.98 cm. Mycelial growth observed in Petri dishes treated with MeJA ranged from 5.05 to 5.21 cm.

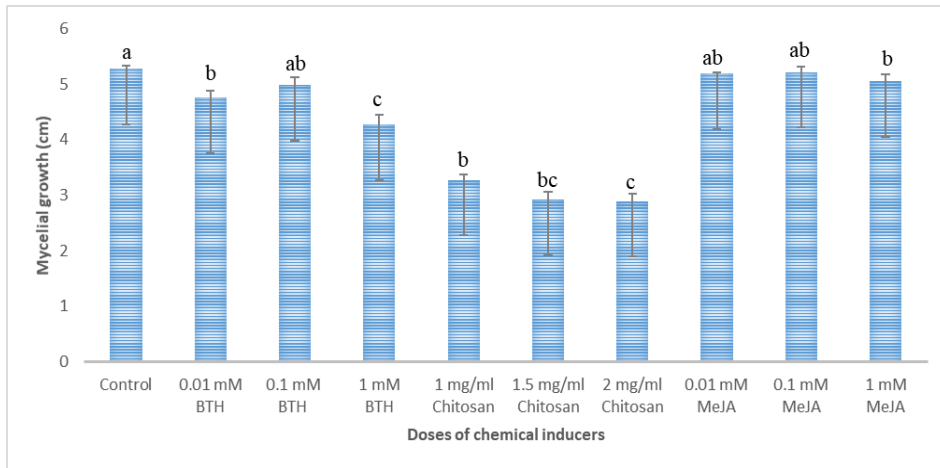


Figure 1. Effects of BTH, chitosan and MeJA on the mycelial growth of *Neoscytalidium dimidiatum* seven days after incubation.

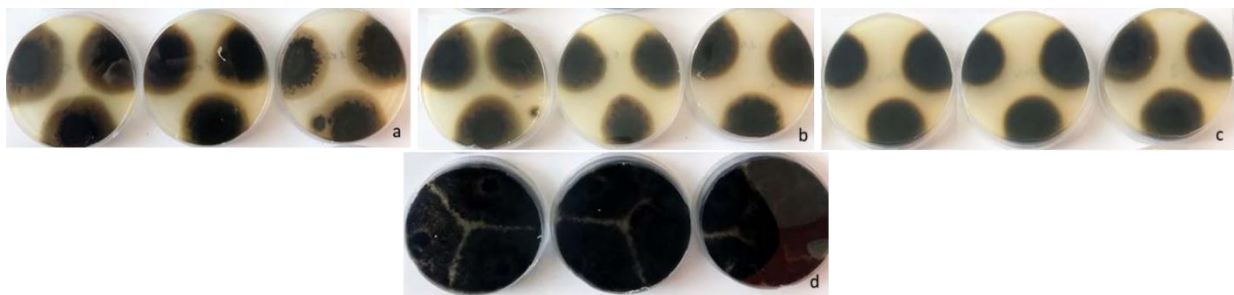


Figure 2. Effects of chitosan at 1 mg/ml, 1.5 mg/ml and 2 mg/ml on mycelial growth *Neoscytalidium dimidiatum* (a: 1 mg/ml, b: 1.5 mg/ml, c: 2 mg/ml, d: control).

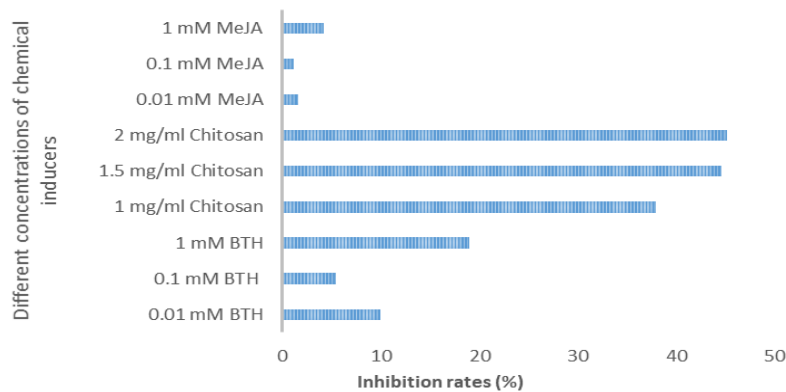


Figure 3. Inhibition rates (%) resulted from chitosan, BTH and MeJA applications at different doses on mycelial growth of *Neoscytalidium dimidiatum*.

When the inhibition rates of chemical inducers after incubation were examined, it was observed that the highest effect was provided by the 2 mg/ml chitosan (Figure 3). The application was quite effective on mycelial growth and provided 45.2% inhibition as compared to the control. This was followed by 1.5 and 1 mg/ml chitosan doses with the inhibition rates of 44.6% and 37.9%, respectively. Inhibition rates of BTH applications varied between 5.4 and 18.9%. MeJA showed less effect than chitosan and BTH with inhibition rates ranging from 1.1 to 4.2%. Similarly, Mohammadi et al. (2024) who determined the

effects of six different doses (100, 250, 500, 1000, 1500, 2000 ppm) of chitosan nanoparticles on *N. novaehollandiae* (syn. *N. dimidiatum*) *in vitro* conditions, reported that radial growth of the pathogen varied between 0 and 5.88 mm, and mycelial growth inhibited completely at doses of 1500 ppm and above. The researchers also stated that mycelial growth was inhibited by 7.1 to 60.34% at doses ranging from 100 ppm to 1000 ppm, respectively, while 100% inhibition was observed at the doses of 1500 and 2000. Similar findings were reported by Chun and Chandrasekaran (2019), who observed that 5.0 mg/ml CS and CNPs

concentration maximally inhibited of radial mycelial growth of *Fusarium andiyazi* on tomato by 54.8% and 73.81%, respectively. Dodgson and Dodgson (2017), who 0.5% chitosan had a similar effect to fungicides in preventing anthracnose in cucumber. In an another study, conducted by Abdul-Karim and Aljarah (2023), the antifungal effect of kaolin and MgO nano-particles at 0.5%, 1% and 2% concentrations were investigated on *N. dimidiatum*. MgO nano-particles completely inhibited fungal growth at all doses, while 1 and 2% kaolin applications inhibited by 35.69 and 37.08%, respectively. Evaluating *in vitro* antifungal activity of alginate-stabilized Cu₂O-Cu nanoparticles against *N. dimidiatum* causing brown spot disease on dragon fruits, Du et al. (2019) reported that 25.1, 22.5 and 100% inhibition on the pathogen growth was obtained at 15, 22.5 and 3 ppm doses, respectively. Similar results were obtained by Sür and Oksal (2021), who investigated the *in vitro* efficacy of seven different fungicides against *N. dimidiatum*, the causal agent of sudden shoot dryness in apricot trees. They reported that cyprodinil+fludioxonil at doses of 30 and 100 µg/mL, and floupiram+tebuconazole at doses of 10, 30 and 100 µg/mL completely inhibited the mycelium growth. The researchers also indicated that the efficacy rates of the other five fungicides used varied between 0 and 98.02%. In another study, the *in vitro* activities of five fungicides against *N. novaehollandiae* responsible for cancer and death symptoms in almonds were examined and the highest activity was obtained with fluazinam (EC₅₀;0.002 µl ml⁻¹), thiophanate-methyl (EC₅₀;0.3 µl ml⁻¹), and tebuconazole (EC₅₀;0.4 µl ml⁻¹) treatments. The lowest effect was found in the application of trifloxystrobin (EC₅₀; 19.5 µl ml⁻¹) (Sakçı et al., 2022). Chemical compounds like chitosan, jasmonic acid and BTH are among the basic compounds that play a key role in the induction of plant resistance mechanisms against diseases and provides effective protection against many significant fungi species such as *Fusarium* spp., *Alternaria* spp., *Botrytis* spp. and *Phytophthora* spp. These inducers provided resistance in plants by affecting spore germination, germ tube elongation, mycelial growth of the pathogen and inducing defense related genes and pathways (Xu et al., 2007; Li et al., 2009; Al-Hetar et al., 2011; Silva et al., 2014; Siddiqi and Husen 2019).

4. Conclusion

Chitosan was identified as the most effective compound in inhibiting the mycelial growth of *N. dimidiatum* under *in vitro* conditions, achieving up to 45.2% inhibition, followed by BTH at a 1 mM dose with an inhibition rate of 18.9%. However, the tested doses of MeJA were insufficient in inhibiting the fungal growth. Using higher doses of MeJA and investigating different combinations of the compounds may be more useful in inhibiting fungal growth. Chitosan among the compounds could be evaluated as an alternative to fungicides due to its high level of effectiveness and non-toxic, environmentally-friendly properties.

Author Contributions

The percentage of the author(s) contributions is presented below. All author(s) reviewed and approved the final version of the manuscript.

	G.P.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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