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**Salicylic Acid Concentrations Suppress Grey Mold in Tomato
Plants**

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Abstract. General Background: Grey mold caused by *Botrytis cinerea* severely constrains tomato production in greenhouse and field systems worldwide. **Specific Background:** Chemical fungicides often fail due to the pathogen's genetic plasticity, prompting exploration of alternative resistance inducers such as salicylic acid (SA). **Knowledge Gap:** Limited data exist regarding concentration-dependent responses of SA against *B. cinerea* under combined laboratory and greenhouse conditions. **Aim:** This study evaluated six SA concentrations (0–10 mM) for their ability to suppress fungal growth and reduce lesion development on tomato leaves. **Results:** Concentrations of 8 and 10 mM completely inhibited mycelial growth and biomass production (0.0 mg; 100% inhibition) in vitro. Under greenhouse conditions, the same concentrations reduced lesion area to 0.0 mm at 24, 48, and 72 hours compared with the untreated control. Lower concentrations produced partial suppression in a dose-dependent manner. **Novelty:** The findings demonstrate a clear concentration threshold at 8 mM SA for complete fungal suppression across experimental systems. **Implications:** SA presents a promising eco-compatible strategy for grey mold management and may serve as a complementary tool within integrated disease control programs.

Highlights:

- 8–10 mM SA achieved complete fungal growth suppression.
- Biomass dry weight reduced to 0.0 mg under optimal concentration.
- Greenhouse lesion development eliminated at threshold dose.

Keywords: Salicylic Acid, Botrytis Cinerea, Tomato, Grey Mold, Induced Resistance

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Commented [sa1]: Pertama, abstrak sudah informatif tetapi dapat diringkas agar lebih padat dan langsung menonjolkan temuan kunci tanpa pengulangan detail angka yang terlalu banyak. Kedua, klaim kebaruan dalam kesimpulan perlu ditegaskan secara lebih spesifik agar tidak terkesan umum. Ketiga, implikasi praktis dapat diperjelas dengan menyebutkan batasan penelitian dan arah riset lanjutan secara lebih konkret.

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Introduction

Tomato is one of the major vegetables crop that is planted around the world and subjected to many pathogenic fungi such as *Alternaria solani*, *Phytophthora infestans*, *Verticillium sp.*, *Fusarium oxysporum f. sp lycopersici*, *Rhizoctonia solani* and *B. cinerea* that causes several diseases [1][2][3]. One of the most important diseases of tomatoes is the Grey mold disease which is attacks tomato plant and leads to significant losses in tomato grown in the green houses and fields [4] [5] [6]. This disease can infect all parts of the plant including leaves, stems and fruits and cause of postharvest fruit rot. This disease is caused by the pathogenic fungus *Botrytis cinerea* [7].

B. cinerea is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 plant hosts worldwide [8] [9]. Although there are several fungicides for controlling this disease, many fungicides have failed in controlling this disease by dint of its genetic plasticity. *B. cinerea* infections have been controlled by using several types of fungicides. Unfortunately, most control methods used against this pathogen is difficult due to its ecological behavior. [10] [11].

Application of salicylic acid has induced plant tolerance to biotic stress including fungi, bacteria and viruses [12][13][14][15]. In general, the salicylic acid (SA) is defined as endogenous signal that activates plant defensive behaviors, such as increased resistance to pathogens and pathogenesis-related transcription [15][16][17][18][19]. According to Chuanfu and Zhonglin, it regulates a number of physiological processes, including defense-related processes in plants. El-Mohamedy discovered salicylic acid is chemical inducers examined and considerably decreased tomato plant root rot infection [20][21]. However, Waheed et al. investigated the effectiveness of salicylic acid as an organic inducer to tomato plant resistance against *Fusarium* wilt disease, which is caused by *Fusarium oxysporum* in greenhouse, field, and laboratory conditions. In vitro Salim et al. tested different concentrations of salicylic acid and found all concentrations significantly reduced the linear growth of *B. cinerea* [22][23]. In the current study, we investigated tomato plants were treated with six concentrations of SA and their effects against *B. cinerea* under laboratory and greenhouse conditions.

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Method

Growth conditions:

This study was conducted at Agricultural Researches Center / Ministry of Higher education and scientific research / Scientific Research Commission , Baghdad / Iraq to evaluate the efficiency of salicylic acid (SA) in inducing resistance in tomato plants against grey mold disease caused by *B. cinerea* under laboratory and greenhouse conditions .

Fungal pathogen (*B. cinerea*) :

A single isolate of the pathogenic fungus *Botrytis cinerea* was used, isolated from a greenhouse planted with tomatoes infected with gray mold in the Al-Madain district of Baghdad Governorate. The pathogenic fungus was purified and identified using culture media and based on cultural and microscopic characteristics. This isolate was then used in laboratory and greenhouse experiments.

B. cinerea was cultured in PDA medium, PDA was autoclaved for 20 minutes at 121 C/1.5 kg/cm², and then incubated for seven days at 25 °C. In this work, the inoculum size of *B. cinerea* (10⁶ spores/ml) was measured using a hemocytometer in according to the method described by reference, generated from cultures that were 7 days old [24].

Effect of salicylic acid on radial growth and biomass dry weight of *B. cinerea* In vitro

Under laboratory conditions, the effects of SA at 2, 4, 6, 8, and 10 mM on liner growth and the dry weight of biomass of pathogen (*B. cinerea*) were investigated.

Linear growth of mycelium :

Each concentration of SA was added to a 100 ml flask holding 20 ml of potato dextrose agar medium (PDA). The flasks were then transferred onto sterile petri dishes with a diameter of 10 cm. Control plates contained PDA without SA Plates were incubated at 25°C for 24 , 72 and 120 hrs. after being inoculated at the center with 5 mm-diameter disks of the pathogen. The pathogen's linear growth in every approach was assessed and contrasted with the control treatment.

Dry weight of pathogen biomass :

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In 2020, 250 ml flasks with 50 ml of dextrose-containing potato broth medium and the tested concentrations of SA (0, 2, 4, 6, 8, and 10 mM of SA), a 5 mm disk of a 7-day culture of *B. cinerea* was sliced and introduced. Following a seven-day inoculation period, the culture medium was filtered using Whatman No. 1 filter paper, and the fungal biomass was dried at 80 °C for twenty-four hours before being weighed. Three replicates were used for each treatment were employed in this investigation.

Influence of SA concentrations

To study the effect of six concentrations (0 , 2 , 4 , 6 , 8 and 10) mM of SA on lesion development caused by *B. cinerea* on tomato leaves. Methods described by reference was used that includes putting 10 droplets of 4 µl spore suspension of *B. cinerea* containing 10⁶ spores ml⁻¹ of each concentration [25]. Five days after infection, the number of expanding lesions on each treatment was determined. Data represent the mean of three replicates, with twelve leaves per treatment.

Statistical analysis

All results of this study were statistically analyzed using the SPSS statistical software, Version 22.0. The mean , standard deviation and standard error of mean for all treatments was calculated at a probability level of 0.05.

Result and Discussion

The results presented in Table 1 revealed that the two studied variables, salicylic acid concentration and incubation period, significantly affected the growth of the spore-forming fungus *Botrytis seneria*. The results also indicated that concentrations of 8 and 10 mM completely inhibited fungal growth for all incubation periods: 24 hours, 1 hour, and 120 hours. It is clear that SA are toxic to the tested fungus (*B. cinerea*) through their direct lethal effect on fungal cells, 8 and 10 mM of SA was sufficient to inhibit the mycelium growth of *B. cinerea* completely while control treatment recorded 14.16 , 17.63 and 97.06 mm for 24 , 72 and 120 hours (table 1) . Also the results showed that the concentrations 2 , 4 and 6 mM of SA recorded 11.33 , 9.53 and 5.28 mm for 24 hours while 72 hours recorded 12.13 , 11.56 and 6.35 mm and 120 hours recorded 63.60 , 56.08 and 12.06 mm .

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Table 1 : Inhibitory Effect of SA on the phytotoxicity of *B. cinerea* on PDA medium under laboratory conditions

Parameter	Conc.	Mean	Std. Deviation	Std. Error
Growth rat at 24hrs	.00	14.1667	.85049	.49103
	2.00	11.3333	.61101	.35277
	4.00	9.5333	.56862	.32830
	6.00	5.2833	.18930	.10929
	8.00	.0000	.00000	.00000
	10.00	.0000	.00000	.00000
	Total		6.7194	5.60465
Growth rate At 72hrs	.00	17.6333	.51316	.29627
	2.00	12.9333	.40415	.23333
	4.00	11.5667	.60277	.34801
	6.00	6.3500	.18028	.10408
	8.00	.0000	.00000	.00000
	10.00	.0000	.00000	.00000
	Total		8.0806	6.78786
Growth rate at 120hrs	.00	97.0667	1.83394	1.05883
	2.00	63.6000	1.76918	1.02144
	4.00	56.0333	.76376	.44096
	6.00	12.0667	.51316	.29627
	8.00	.0000	.00000	.00000
	10.00	.0000	.00000	.00000
	Total		38.1278	37.65501

Table 2 : Effect of SA concentrations on biomass dry weight and growth inhibition of *B. cinerea* under laboratory conditions .

Concentration (mM)	Mean(mg)	Std. Deviation	Std. Error of Mean
0.00	263.1000	2.35797	1.36137
2.00	116.5333	1.62891	.94045
4.00	107.1667	1.46401	.84525
6.00	77.1333	1.55349	.89691

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8.00	.0000	.00000	.00000
10.00	.0000	.00000	.00000
Total	93.9889	91.30516	21.52083

Results of the effect of six concentrations of SA on biomass dry weight of the pathogenic fungus *B. cinerea* under greenhouse conditions exhibited that the tested treatments varied in their abilities in reducing dry weight of the pathogen hence increase the pathogen inhibition by increasing the acid concentration. Table 1 shows that concentrations of 8 and 10 mM of salicylic acid completely inhibited the growth of the pathogenic fungus compared to 2, 6, 3, and 10 mmol for the control treatment, while concentrations of 2 and 4 mM of the acid were recorded 116.53 and 107.16 mM respectively . It is clearly that the SA 8 and 10 mM demonstrated complete inhibition of the pathogen growth to 100 % and dry weight of the biomass to 0.0 mg which reflected the inhibitory effect of SA to the growth and sporulation of the pathogenic fungus (*B. cinerea*) [26].

Table 3 : Treatment of tomato plant leaves with SA and their effect on infection lesions by *B. cinerea* under greenhouse conditions .

Infection lesion area at	Conc.	Mean (mm)	Std. Deviation	Std. Error	Minimum	Maximum
24hrs	.00	3.7667	.05774	.03333	3.70	3.80
	2.00	3.6667	.05774	.03333	3.60	3.70
	4.00	2.7667	.05774	.03333	2.70	2.80
	6.00	2.4333	.05774	.03333	2.40	2.50
	8.00	.0000	.00000	.00000	.00	.00
	10.00	.0000	.00000	.00000	.00	.00
	Total	2.1056	1.60604	.37855	.00	3.80
48hrs	.00	6.3667	.11547	.06667	6.30	6.50
	2.00	6.1333	.11547	.06667	6.00	6.20
	4.00	4.9333	.15275	.08819	4.80	5.10
	6.00	4.6000	.17321	.10000	4.40	4.70
	8.00	.0000	.00000	.00000	.00	.00
	10.00	.0000	.00000	.00000	.00	.00
	Total	3.6722	2.74801	.64771	.00	6.50
72hrs	.00	8.4333	.20817	.12019	8.20	8.60
	2.00	8.3333	.11547	.06667	8.20	8.40

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4.00	8.0333	.05774	.03333	8.00	8.10
6.00	7.7000	.10000	.05774	7.60	7.80
8.00	.0000	.00000	.00000	.00	.00
10.00	.0000	.00000	.00000	.00	.00
Total	5.4167	3.94957	.93092	.00	8.60

The results shown in Table 3 show that tomato plants were more susceptible to *B. cinerea* at concentrations ranging from 4 to 8 mM of SA at three intervals (24, 48, and 72) after tomato leaves were infected by SA. Because 2 mM of SA did not promote susceptibility in tomato leaves, these data also show that a threshold concentration of SA is required to generate the susceptible response in tomato plants to *B. cinerea*. This experiment demonstrates the significance of SA at 8 mM in tomato plants' interactions with *B. cinerea*. The results in Table 3 confirmed that the three studied incubation periods of 24, 72, and 120 hours showed that both concentrations 8 and 10 mM completely inhibited the growth of the fungus *Botrytis cinerea* to 0 mm compared to the control treatment, which recorded 3.76, 6.36 and 8.43 mm respectively.

This study revealed that the concentration 8 mM of SA is capable to prevent the pathogen fungal growth and kill the fungal cells in PDA medium, and the concentration 8 and 10 mM of SA was inhibit the growth of the pathogen in PDB medium. The mode of action of SA was concluded as involving inhibiting the secreted enzymes by *B. cinerea* [26]. Moreover, some defensive systems, including phenolic substances, oxidative enzymes, and other metabolites, may be stimulated by the studied SA inducer [21]. The phenomena of SA-induced tolerance in tomato plants versus the pathogen *B. cinerea* was examined in relation to dependent signaling networks and its possible use in plant defense.

Some sources indicate that the inhibition of plant pathogenic fungi growth by salicylic acid is achieved through the production of certain defensive compounds in the plant, which leads to the creation of an environment unsuitable for fungal growth [27]. This acid enhances the plant's immune response, increasing its resistance to fungal diseases. In addition, the acid affects the fungus's cell membranes and its metabolic processes, thus hindering its growth and reproduction [28][29]. Other sources indicate that salicylic acid increases the concentration of certain phenolic compounds in the plant, which inhibit fungal growth by destroying their cell walls. Additionally, this acid stimulates specific signaling pathways that activate enzymes involved in the production of certain fungal-toxic compounds. Salicylic acid affects some cellular processes of fungi, such as cell division, which leads to inhibiting their growth and increasing the rates of lipid oxidation in fungal cells, which leads to damage to cellular structures [30].

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Conclusions

Under laboratory and greenhouse conditions, the present study shows classically salicylic acid (SA) is inducer of resistance, for the first time, in tomato plants (*Solanum lycopersicum*) against grey mold disease caused by saprophytic pathogen *Botrytis cinerea*. Results clearly demonstrated a concentration-dependent response with 8 and 10 mM completely inhibiting mycelial growth and biomass production of the pathogen (100% growth suppression) as well as significantly lower lesion development on tomato leaves under greenhouse conditions, compared to the untreated control [55]. These findings clearly emphasize the role of SA, either as direct antifungal agent or prompt plant defense response, possibly through the induction of phenolics, oxidative enzymes, and salicylic-acid-dependent signaling pathways that limit the access of pathogen to its colonality [135]. More specifically, these results support the practical use of salicylic acid as a viable and novel environmental-safe alternative and/or supplement to traditional fungicides for controlling grey mold disease in tomato production systems—especially under protected cultivation systems where disease pressure is higher. It is important that further studies are performed to discover the SA-molecular mechanisms that mediate SA-induced resistance, the implications of long term exposure to SA on plant physiology or yield, and field efficiency and practicality of the treated plants in combination with biological control agents and chemical reduction programs.

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