

Exploring Microbial Metabolites for Post Harvest Disease Control : A Case Study on Strawberry Anthracnose Disease

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ABSTRACT

Strawberry (*Fragaria × ananassa*) is a famous red fruit for their sweet flavor, juicy texture and distinctive aroma. Strawberry is highly perishable with a short shelf life that leads to quality deterioration, significant losses and economic challenges for growers and distributors. Addressing these challenges, it is essential to manage post-harvest pathogens. Among that anthracnose, caused by *Colletotrichum gloeosporioides*, poses a significant threat to strawberry cultivation worldwide. This study evaluates the efficacy of selected secondary metabolites isolated from the beneficial microorganism against *C. gloeosporioides*. Among four metabolites tested, guaiacol exhibited consistent and complete inhibition of fungal growth across 2000 and 1000 ppm, highlighting their strong antifungal properties whereas ethyl caprylate, oleic acid and isoamyl alcohol showed minimal inhibitory activity. These findings underscore the potential of guaiacol for managing strawberry anthracnose. Further investigation into their mechanisms of action and practical application in sustainable agriculture is required.

Keywords : Anthracnose, Antifungal activity, Secondary metabolites, Strawberry

STRAWBERRY is an important fruit crop scientifically known as *Fragaria × ananassa*, is renowned for its sweet flavor, vibrant red color and nutritional benefits. It belong to the Rosaceae family, which includes other economically important fruits like apples, pears and raspberries (Natsheh *et al.*, 2015). Strawberry is a popular fruit crop globally and have gained significant attraction in India due to its high market demand and profitability. Strawberry is widely cultivated across various regions of India with specific areas emerging as key production hubs due to its favorable agro-climatic conditions. The major strawberry-growing regions in India are Nainital (Uttarakhand), Dehradun (Uttarakhand), Mahabaleshwar (Maharashtra), Kashmir Valley (Jammu & Kashmir), Bangalore (Karnataka) and

Kalimpong (West Bengal). Recent advancements in agricultural practices, including the development of heat-tolerant varieties, improved irrigation techniques, and protected cultivation methods (*e.g.*, polyhouses and shade nets) have enabled the successful cultivation of strawberry in the plains of India. Key regions in the plains where strawberry cultivation has gained momentum are Pune, Nashik and Sangli in Maharashtra. These regions have adopted modern farming techniques to overcome challenges such as higher temperatures and humidity, which are not traditionally conducive to strawberry cultivation. During the 2020-21 growing season, strawberry cultivation in India covered approximately 3,000 ha with a total production of 20,000 MT (NHB, 2021).

This reflects the growing importance of strawberry as a high-value crop in Indian agriculture.

Strawberry is highly perishable due to its soft texture, high moisture content and thin skin, making it particularly vulnerable to mechanical damage and pathogen infections during harvesting, handling and storage (Rico *et al.*, 2019). These factors significantly reduce its shelf life, leading to substantial postharvest losses. Globally, postharvest losses in strawberry can reach up to 40 per cent, highlighting the need for improved storage, handling and disease management practices (Trinetta *et al.*, 2020). Among the various diseases affecting strawberry cultivation, anthracnose is a major fungal disease threatening strawberry cultivation worldwide with the potential to cause yield losses of up to 70 per cent, thereby significantly affecting the production (Aljawasim *et al.*, 2023).

Anthracnose affects multiple parts of the strawberry plant, initially appearing as irregular black or brown necrotic leaf lesions that can coalesce, causing extensive damage. Crown rot occurs when the pathogen penetrates into vascular tissue, leading to wilting, stunted growth and plant death. The disease also causes flower blight and fruit rot with sunken dark lesions on unripe or ripe fruits that expand rapidly under warm, humid conditions, resulting in soft rot and postharvest losses. The fungal spores spread easily *via* rain, irrigation, wind and human activity (Freeman and Katan, 1997). The severity of anthracnose has escalated over the past decade due to increased cultivation of susceptible cultivars, global trade of infected plant material and climatic conditions favoring pathogen growth (Chen *et al.*, 2019). Temperatures between 25 - 30°C create optimal conditions for *Colletotrichum* spp. and the pathogen's ability to remain latent in asymptomatic plants complicates disease detection and control.

The susceptibility of strawberry to anthracnose is believed to be polygenic and quantitatively inherited. Despite advancements in breeding, no resistant varieties have been developed, leaving the crop highly vulnerable to the disease (Dodds and Rathjen, 2010 and Amil-Ruiz *et al.*, 2011). Current management

strategies rely heavily on chemical fungicides, raising environmental concerns and contributing to the emergence of fungicide-resistant pathogen strains. In response, many developed nations have restricted or banned certain fungicides, highlighting the need for safer and more sustainable alternatives. Biopesticides, derived from natural organisms or their metabolites, offer an environment friendly approach to disease management. Metabolomics plays a crucial role in identifying and characterizing these microbial-derived compounds, facilitating their potential commercial application. Modern analytical techniques provide detailed insights into the mechanisms of these metabolites, complementing traditional disease control methods and improving their efficacy. As part of efforts to develop alternatives to chemical fungicides, microbial secondary metabolites have shown promising potential in managing anthracnose, offering a sustainable strategy to mitigate its impact on strawberry production.

MATERIAL AND METHODS

Collection and Isolation of Pathogen

The anthracnose pathogen was isolated from infected samples showing typical symptoms on leaves were obtained from ICAR-IIHR, Bengaluru (13°7' N, 77°29' E). Small sections of the infected fruit were surface sterilized by immersion in one per cent (w/v) sodium hypochlorite (NaOCl) solution for one minute. To remove excess traces of disinfectant, the sections were rinsed three times with sterile distilled water. These sterilized fragments were then placed on potato dextrose agar (PDA) plates and incubated at 28 ± 2°C. Emerging fungal colonies were sub-cultured onto fresh PDA. Single-spore isolation was preferred to purify the isolates (Li *et al.*, 2019).

Pathogenicity Test

The pathogenicity of the isolated pathogen was assessed on strawberry fruits under controlled conditions. The pathogen was first cultured on PDA plates and incubated at 25°C for 7-10 days to generate actively growing fungal colonies. Fresh, healthy strawberry fruits were surface sterilized with 70

per cent ethanol for 30 seconds, followed by rinsing with one per cent NaOCl for one min. and then with sterile distilled water. The fruits were air dried before inoculation. The sterilized fruits were placed in plastic boxes with moist absorbent cotton to maintain high humidity. Sterilized needle was used to make small pinpricks on the fruit surface. Hyphal plugs from the actively growing margins of the fungal cultures on PDA were placed over the wounded areas. The experiment followed a completely randomized design with three replicates. The plastic boxes were sealed and incubated at 25°C in a growth chamber under dark conditions. Symptom development was monitored. To confirm the identity of the pathogen, it was reisolated from the infected fruits and compared with the original isolate for morphological confirmation (Mo *et al.*, 2018).

***In vitro* Antifungal Activity of Secondary Metabolites against Anthracnose**

The four metabolites *viz.*, isoamyl alcohol, guaiacol, oleic acid and ethyl caprylate were identified through OHR-LCMS analysis from bacterial isolates IIHR_BCRBGA03 (PP422079), IIHR_BCRBC01 (PP422089), IIHR_BCRBGA02 (PP422078) and IIHR_BCRBB02 (PP422073) (Mounika *et al.*, Unpublished data). These secondary metabolites, known for their potential antagonistic activity against pathogens, were evaluated using the poisoned food technique. The metabolites were tested against *C. gloeosporioides* at three concentrations: 2000 ppm (0.2%), 1000 ppm (0.1%) and 500 ppm (0.05%). A medium without any metabolite additions served as the control and each treatment was replicated three times. Fungal growth was measured by recording colony diameters on treated and control plates over a 12-day incubation period. The percentage inhibition of mycelial growth was calculated as per protocol given by (Vincent, 1947). For comparison, the systemic fungicides *viz.*, thiophanate-methyl 70 per cent WG (Vantage) and tebuconazole 250 EC (25.9% w/w, Folicur) were included at their recommended doses (0.1%) as standard checks.

$$I = (C-T)/C \times 100$$

Where,

I - Per cent inhibition

C - Colony diameter of pathogen in control (cm)

T - Colony diameter of the pathogen in treatment (cm)

Experimental Design and Statistical Analysis

All experiments were conducted using a completely randomized design with each experiment being repeated at least three times to ensure reliability. Statistical analyses were performed using SPSS version 16.0. Analysis of variance (ANOVA) was used to assess the significance of differences between treatments ($P < 0.05$). Duncan's multiple range test was employed to identify significant differences among the treatment means ($P < 0.05$).

RESULTS AND DISCUSSION

Collection and Isolation of the Pathogen

The already characterized culture of *C. gloeosporioides* (ON009253) available in the Fruit Pathology Lab, Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru - 560089, Karnataka, India was used in the present study.

Pathogenicity Test

Strawberry fruits inoculated with the pathogen exhibited typical symptoms of brown to black, water-soaked spots that appeared on the fruit. As the infection advanced, these spots developed into firm, sunken brown to black lesions. In humid conditions, orange-colored spore masses could be observed within the lesions. Over time, the lesions may appear less sunken with a brownish tint. As the infection progressed, the lesions deepened, becoming more sunken and blackened, eventually causing the entire fruit to mummify (Fig. 1).



Fig. 1 : a) Pathogenicity test of *Colletotrichum gloeosporioides* on strawberry fruits and b) spore morphology

***In vitro* Antifungal Activity of Secondary Metabolites Against Anthracnose**

The results of the experiment indicate varying levels of effectiveness among the four tested metabolites in inhibiting the growth of *C. gloeosporioides* at a concentration of 2000 ppm. Guaiacol exhibited the highest inhibition (100%), effectively preventing pathogen growth, comparable to the performance of the fungicide checks, Tebuconazole and Thiophanate-methyl (Fig. 2 & Table 1). Among the tested secondary metabolites, guaiacol was categorized under scale 4 (76–100%), while the remaining metabolites belonged to scale 1 (1–25%) (Table 4).

In contrast, oleic acid showed the least inhibition (9.44%), followed by ethyl caprylate (14.63%) and isoamyl alcohol (22.22%), indicating their relatively weak effect on the pathogen. These metabolites were categorized under scale 1 (1–25%), signifying low effectiveness against *C. gloeosporioides* (Table 4).

At 1000 ppm, the four metabolites tested exhibited varying levels of effectiveness in inhibiting the growth of *C. gloeosporioides*. Guaiacol was the most effective, achieving 100 per cent inhibition of the pathogen. It provided absolute control, comparable to the fungicide checks, tebuconazole and thiophanate-methyl (Fig. 2; Table 2). Guaiacol was classified under scale 4 (76–100%), indicating its high effectiveness in managing strawberry anthracnose. In contrast, ethyl caprylate showed the lowest inhibition (8.70%), followed by oleic acid (11.85%) and isoamyl alcohol

(15.00%), indicating their minimal impact on the growth of *C. gloeosporioides*. These metabolites were categorized under scale 1 (1–25%), reflecting their limited effectiveness (Table 4).

At a concentration of 500 ppm, the four tested metabolites (Guaiacol, oleic acid, ethyl caprylate, isoamyl alcohol) exhibited a wide range of inhibitory effects against *C. gloeosporioides*. None of the secondary metabolites achieved complete inhibition of the pathogen (Fig. 2; Table 3). Ethyl caprylate showed the lowest inhibition (1.67%), followed by oleic acid (11.11%), isoamyl alcohol (15.74%) and guaiacol (17.59%). These metabolites had very limited effects on the pathogen and were thus categorized under scale 1 (1–25%), indicating low efficacy (Table 4).

Guaiacol demonstrated consistent and high efficacy at 2000 and 1000 ppm, achieving 100 per cent inhibition of *C. gloeosporioides*, making it the most promising metabolite for the control of strawberry anthracnose. In contrast, ethyl caprylate, oleic acid and isoamyl alcohol were largely ineffective, showing minimal inhibition across all tested concentrations. These findings suggest that guaiacol is the most suitable candidate for further exploration as a biocontrol agent against this fungal pathogen at 2000 and 1000 ppm.

Strawberry anthracnose is caused by several *Colletotrichum* species, including *C. fragariae*, *C. acutatum* and *C. gloeosporioides* (Denoyes-Rothan

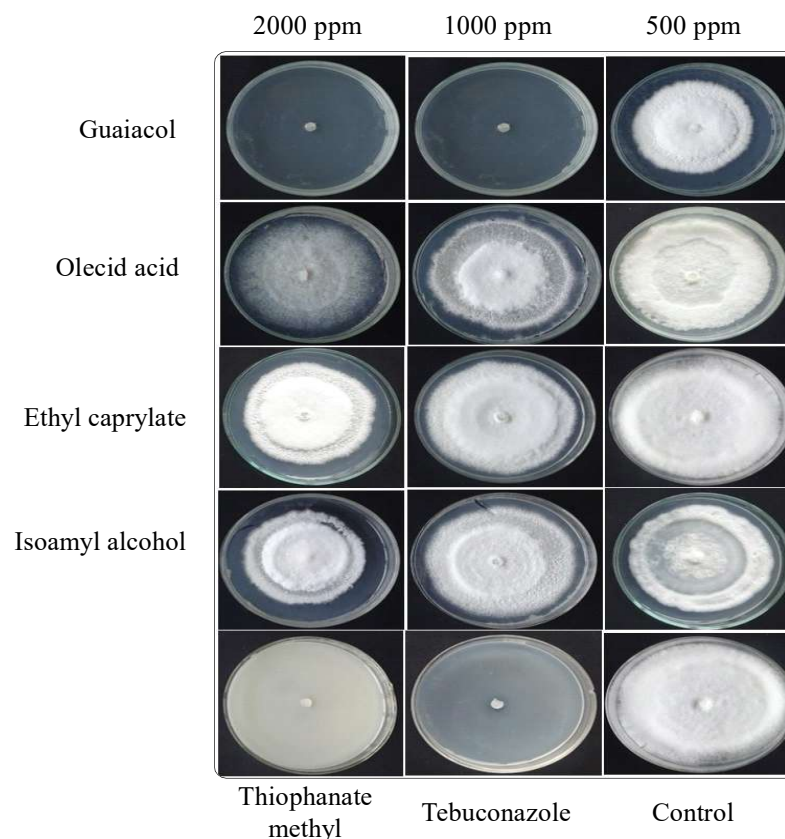


Fig. 2 : *In vitro* evaluation of microbial origin secondary metabolites at 2000, 1000 and 500 ppm against *C. gloeosporioides* after 12 days of incubation by poison food assay on PDA. Thiophanate methyl and tebuconazole as fungicide check at their recommended doses

TABLE 1
Antagonistic activity of secondary metabolites at 2000 ppm against *C. gloeosporioides*

Secondary metabolite	Per cent inhibition of <i>C. gloeosporioides</i>			
	3 DAI	6 DAI	9 DAI	12 DAI
Guaiacol	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
Oleic acid	16.12 ^d (23.53)	3.74 ^c (10.84)	8.41 ^c (16.85)	9.44 ^d (17.86)
Ethyl caprylate	51.11 ^b (45.62)	4.12 ^c (10.80)	7.35 ^c (15.72)	14.63 ^c (22.26)
Isoamyl alcohol	30.50 ^c (33.47)	10.51 ^b (18.49)	19.36 ^b (26.09)	22.22 ^b (28.07)
Tebuconazole	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
Thiophanate methyl	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
	Metabolite (A)	Days (B)	(A X B)	
C.D. @ 1%	2.22	1.81	4.444	
SE(m)±	0.779	0.636	1.558	
CV			4.80	

Values are the mean of three replications, figures in parentheses are arc-sine transformed. Values followed by same alphabet within a column do not differ significantly at $P < 0.05$

TABLE 2
Antagonistic activity of secondary metabolites at 1000 ppm against *C. gloeosporioides*

Secondary metabolite	Per cent inhibition of <i>C. gloeosporioides</i>			
	3 DAI	6 DAI	9 DAI	12 DAI
Guaiacol	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
Oleic acid	11.77 ^c (19.91)	6.40 ^b (14.49)	5.54 ^b (13.46)	11.85 ^{bc} (20.09)
Ethyl caprylate	27.55 ^b (31.55)	4.01 ^b (11.25)	5.76 ^b (13.70)	8.70 ^c (16.87)
Isoamyl alcohol	22.30 ^b (28.01)	5.26 ^b (12.89)	5.78 ^b (13.89)	15.00 ^b (22.69)
Tebuconazole	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
Thiophanate methyl	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
	Metabolite (A)	Days (B)	(A X B)	
C.D. @ 1%	1.87	1.53	3.74	
SE(m)±	0.66	0.54	1.31	
CV			4.20	

Values are the mean of three replications, figures in parentheses are arc-sine transformed. Values followed by the same alphabet within a column do not differ significantly at $P < 0.05$

et al., 2003 and Han *et al.*, 2016). These fungal pathogens have the potential to infect the entire strawberry plant, including the fruit, foliage and crown. Among these pathogens, *C. gloeosporioides* is particularly associated with crown rot, severely affecting both the fruit and crown regions, leading to

significant economic losses in strawberry production. The symptoms of anthracnose can sometimes be mistaken for other fruit diseases, such as Alternaria fruit rot, Phomopsis fruit rot and Rhizoctonia dry rot, or even abiotic damage like hail injury (Louws and Ridge, 2020).

TABLE 3
Antagonistic activity of Secondary metabolites at 500 ppm against *C. gloeosporioides*

Secondary metabolite	Per cent inhibition of <i>C. gloeosporioides</i>			
	3 DAI	6 DAI	9 DAI	12 DAI
Guaiacol	54.87 ^b (47.79)	19.20 ^b (25.74)	6.87 ^b (15.09)	17.59 ^b (24.61)
Oleic acid	8.53 ^d (15.87)	1.45 ^d (6.83)	2.22 ^c (8.47)	11.11 ^c (19.45)
Ethyl caprylate	23.00 ^c (28.57)	5.49 ^c (13.54)	2.46 ^c (8.59)	1.67 ^d (7.41)
Isoamyl alcohol	23.61 ^c (28.99)	5.82 ^c (13.70)	5.78 ^b (13.89)	15.74 ^{bc} (23.22)
Tebuconazole	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
Thiophanate methyl	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)	100.00 ^a (89.96)
	Metabolite (A)	Days (B)	(A X B)	
C.D. @ 1%	2.27	1.86	4.54	
SE(m)±	0.80	0.65	1.59	
CV			6.48	

Values are the mean of three replications, figures in parentheses are arc-sine transformed. Values followed by the same alphabet within a column do not differ significantly at $P < 0.05$

Biocontrol strategies are gaining prominence in disease management due to their advantages over chemical fungicides. The excessive use of synthetic fungicides has disrupted the natural microbiome,

TABLE 4
Growth inhibition category of secondary metabolites against *C. gloeosporioides*

Secondary metabolites	Growth inhibition category		
	500 ppm	1000 ppm	2000 ppm
Guaiacol	1	4	4
Oleic acid	1	1	1
Ethyl caprylate	1	1	1
Isoamyl alcohol	1	1	1

Values were categorized on a scale from 0 to 4, where 0 = no growth inhibition 1 = 1 to 25 %, 2 = 26 to 50 %, 3 = 51 to 75 % and 4 = 76 to 100%

leading to ecological imbalances, the emergence of fungicide-resistant pathogen strains and potential risks to human health. Several studies have demonstrated the efficacy of microbial antagonists in controlling anthracnose in various crops. For instance, anthracnose in mango has been effectively managed using epiphytic *Bacillus subtilis*, *B. siamense* and *B. velezensis* (Supriya *et al.*, 2024), while anthracnose in grapes has been controlled using *Bacillus* spp. and *Hanseniaspora* spp. isolated from grape surfaces (Patel *et al.*, 2022). The *Candida tropicalis* a yeast and *Alcaligenes fecalis* controlled the post harvest losses in mango (Sriram and Poornachanddra, 2013). These findings highlight the potential of microbial biocontrol agents in sustainable disease management.

Microbial secondary metabolites with antifungal properties offer a promising alternative to the heavy reliance on synthetic fungicides. The discovery of strobilurins, originally isolated from *Strobilurus tenacellus*, revolutionized crop protection and now accounts for approximately 25 per cent of the global fungicide market (Anke *et al.*, 1977). Similarly, *Trichoderma* species are known to produce several antifungal metabolites, including gliotoxin,

trichodermin and harzianic acid, which effectively inhibit plant pathogens (Harman, 2006).

The four compounds tested in the present study exhibited antimicrobial activity against various pathogens by inhibiting fungal growth through different modes of action. Among them, guaiacol demonstrated the highest efficacy, achieving complete inhibition of *C. gloeosporioides* at concentrations of 1000 and 2000 ppm. This indicates that guaiacol functions in a dose-dependent manner, with increasing concentrations resulting in stronger inhibition of the pathogen. The results suggest that guaiacol has significant potential as a natural biocontrol agent for the management of strawberry anthracnose, providing an effective and environmentally friendly alternative to conventional fungicides. Further studies on the mechanism of action, formulation development and field applications of guaiacol are warranted to optimize its use in sustainable strawberry disease management.

Guaiacol (2-methoxyphenol), widely used in industries for producing fragrances like eugenol, vanillin and artificial musk (Mohan *et al.*, 2006 and Hiscox & Boddy, 2017). Beyond its industrial applications, guaiacol has antifungal properties, particularly against *Fusarium graminearum*. In the present study the guaiacol is highly effective at 1000 and 2000 ppm as compared to 500 ppm. At 1.838 mM concentration, it efficiently inhibited mycelial growth, conidial formation, and germination in *F. graminearum*. Its antifungal mechanism was primarily attributed to its ability to disrupt fungal cell membranes by interfering with calcium ion (Ca^{2+}) transport channels, which are critical for maintaining cellular integrity. Additionally, guaiacol modulates oxidative stress within the fungus, as evidenced by reduced levels of malondialdehyde (MDA), a marker for oxidative damage and diminished activity of antioxidant enzymes like catalase, peroxidase and superoxide dismutase. These effects suggest that guaiacol operates through multiple mechanisms, including direct membrane disruption and alteration of the oxidative stress response, making it a potent

agent for fungal control with a multifaceted mode of action (Gao *et al.*, 2021).

Ethyl caprylate, oleic acid and isoamyl alcohol were largely ineffective against *C. gloeosporioides*, showing minimal inhibition across all tested concentrations. This limited efficacy suggests that these compounds may operate through mechanisms not effective against this particular pathogen, or that higher doses might be necessary to achieve control. Despite their antimicrobial properties, their specific mode of action might not align with the requirements for targeting *C. gloeosporioides*. Interestingly, the effectiveness of these compounds varies significantly across different fungal species. Oleic acid, specifically its derivative 3-(octadecyloxy) propyl ester, isolated from *Lepiota cristata* inflorescence, has demonstrated notable antifungal and antibacterial activity against *C. fulcatum*, *F. oxysporum* and *R. solani* (Abubacker and Devi, 2014). Ethyl caprylate, a fatty acid ester, generally exhibits lower antimicrobial activity compared to alcohols due to the absence of a reactive free hydroxyl group. However, it can be hydrolyzed by esterases in cells to release acids and alcohols, which are more potent antimicrobial agents (Ando *et al.*, 2015). This suggests that ethyl caprylate's effectiveness may depend on its conversion into more active forms within the target organism or environment. While isoamyl alcohol also displayed limited direct activity against *C. gloeosporioides*, further investigation into the potential synergistic effects of these compounds, higher concentrations, or their derivatives could uncover broader applications for managing other fungal pathogens.

In conclusion, guaiacol emerged as most effective metabolite at 2000 and 1000 ppm showing dose dependent effectiveness, demonstrating consistent and complete inhibition of *C. gloeosporioides*. These results highlight the potential of guaiacol as promising biocontrol agents for managing strawberry anthracnose. The ethyl caprylate, oleic acid and isoamyl alcohol were largely ineffective, showing minimal inhibition at all concentrations tested. These findings underscore the significant potential of

guaiacol as natural alternatives to chemical fungicides. The efficacy paves the way for further exploration and potential application in sustainable agricultural practices. This study also sheds light on the diverse antifungal properties of secondary metabolites derived from bioagents, contributing to the development of eco-friendly strategies for plant disease management.

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