In vitro Antifungal Activity of *Menta Piperita* Extract Against *Venturia inaequalis*

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Abstract

Apple is vulnerable to different types of diseases, including the Apple scab, caused by *Venturia inaequalis*. The cause of the apple scab disease is the fungus Venturia inaequalis. Lamiaceae family member mint was used in the experiments. Examining the effectiveness of mint plant extracts obtained by grinding against fungi was the goal of the research study. Venturia unqualified. The fungus mycelium enlargement inhibition was assessed under in vitro conditions using different plant extracts of 5 %, 10 %, 15 %, 20 %, and 25 %, a total of 3 isolates were processed for this purpose. The samples were inoculated on PDA media. After an incubation period of 7 days, the diameter of colonies was measured, and calculates the % inhibition zone ratios and compared to the controls. The inhibition effects of the mint extract were greater than the controls. The highest antifungal effect was detected at 20% and 25% doses of mint extract and showed a significant fungicidal effect on them. They could be used as fungicides in integrated and biological control of the apple scab, which is thought to be one of the most serious diseases of apples (Malus Domestica) in the world. The mint extract decreased the mycelium growth of B1-017, N8-017, and P7-017 to varying degrees. In conclusion, it was found that mint extract can be another method in the management of apple scab disease caused by *V. inaequalis*.

Key words: *Menta piperita*, extracts, antifungal, *Venturia inaequalis*

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1. Introductions

A sweet fruit produced by *Malus Domestica* is an apple (apple tree). If apple trees are grown from seed, they are big. The most widely cultivated species in the genus Malus is the apple tree, which is grown all over the world. More than 7,500 apple cultivars with various desired traits are known to exist. Baluchistan is the main producer of apples in Pakistan, while Khyber Pakhtunkhwa (KP) ranks second and contributes 25% of the country's apple growth. While some farmers also plant during the monsoon season, apple production typically begins in December and January. The primary factor in the production of apples is rain. Because the tree would not produce fruit without rain, apple-producing areas are not located on plane lands; instead, their gardens are situated in [1].

The apple scab disease is caused by *Venturia inaequalis*. In apples, this pathogen caused the disease, the most important disease which badly affects apple production [2].

The first symptoms of apple scab disease are found in the leaves, blossoms, and developing fruits of infected trees. Farmers often used preventive practices such as biocontrol treatments and targeted fungicides to stop the incidence and spread of apple scab disease [3].

The fungus which causes apple scab survives during winter chiefly in fallen leaves. They will develop over the winter, accumulating an inoculum for the beginning of a new cycle in the spring [4]. Environmental factors also have an impact; for example, in dry weather, fewer primary infections may occur and growers may use longer spray intervals. [5]. Plant extracts with no negative effects on humans or animals could be a potential substitute for traditional methods of treating apple scabs [6]. The plant extracts with fungicidal effects include the extract of mint (*mentapipreta).* Mint contains different bioactive compounds which have antifungal effects against the scab ascospores [7].

Mint oil is the subject of preliminary research due to its potential for use as a short-term treatment for irritable bowel syndrome and its alleged traditional medical applications for minor ailments. When applied topically, peppermint oil and leaves provide cooling relief from itching, muscle pain, and nerve pain. They can also be used as a fragrance [8].

2. Materials and methods

The proposed study was carried out at the Department of Microbiology and Biotechnology Abasyn University Peshawar from November 2019 to April 2020. The samples were isolated and collected from Swat, Khyber Pakhtunkhwa, Pakistan.

2.1. Sample Collection

The samples of apple scabs were collected from three different locations of swat, namely Biha, Nalkot, and Pushtunai. The samples were labeled bearing specie name, (*Frances*, *pink lady*, *golden delicious*) location, and date. The infected leaves showing symptoms of apple scab were collected and cut infected lesion portions along with some healthy portions. Some healthy portions are used for the purpose of media. In LFH surface sterilization has been done with 1% mercuric chloride solution for 15 to 20 Sec. and then washed three times in distilled water for 30 Sec.

2.2. Sample processing

The sterilized pieces of leaves scab lesions were placed on PDA media. PDA media is a versatile, nutrient-rich medium that is used to grow the fungus V. Inaequalis. PDA media is well-liked because it can support the growth of a wide range of fungi and is rich in nutrients.

2.3. Media preparation

For the preparation of the media. 200g of peeled potato, 15g of dextrose, and 20g of agar were combined with 1L of distilled water to make it. The medium and plates underwent a 15-minute autoclave at 121C. once the media has been autoclaved, it was allowed to cool in a laminar flow hood (LFH). while protecting it from solidifying. The cool media was poured into each plate and was left for solidification in a sterile environment. The sterilized pieces of leaves scab lesions were placed on a PDA medium under aseptic conditions. All the plates were covered and labeled and incubated at 28c for 7 days. After 10 days of incubation, PDA media showed signs of fungus development. The process of further purification to identify a single fungal strain involved sub-culturing distinct colonies on brand-new PDA media.

2.4. Identification of the fungus

Identification of fungus was done from foliar symptoms olive-brown velvety texture on leaves that is the visual identification. Identification was also be done by preparing slide from lesion area of leaves that is microscopic identification of fungus.

2.5. Sub-culturing of the fungus

For the sub-culturing of the fungus ultraviolet light (UV) has been on for one hour(hr.)then surface sterilization was done with 70% ethanol. Add streptomycin to the media to prevent the growth of bacteria and shake it well. Pour the media into Petri plates and allowed the media to be cooled down. The pure samples of fungus were placed on PDA media under aseptic conditions. 2- 2 plates were used for each culture. All the plates were covered and labeled and incubated at 28c for 7 days. After 10 days of incubation, PDA media showed signs of fungus development.

2.6. Menthapiperita extract preparation

Mint plants were collected from the field along with stems and leaves. Then weigh it on a digital balance. Wash the mint soaked all mint in water to remove dust particles and contaminants. After washing the *Menthapiperita* were shaded dry and then grind it. Add a small amount of water during grinding *Menthapiperita*. Once the grinding was completed then filter all the extract with the help of a mason cloth in a sterilized jar. Tightly close the jar and stored in the refrigerator overnight.

3. Antifungal activity

The antifungal activity of extract (*Mentapiperita*) was passed out to assess its effect on the mycelial growth of *V. inaequalis*. After sterilization of PDA media, plant extract were added at different concentration (5%, 10%, 15%, 20%, 25%) into 30 mL PDA media and poured it in Petri plates. A control plate was also used. Control plates contained only PDA. By measuring the diameter of the colony in two directions at right angles, the pathogen's mean radial mycelial growth was calculated. According to the described procedures, the media was ready. Only medium and pathogen isolates are present on the control plate.

3.1. Procedure for antifungal activity

After sterilizing a flask and some plates, produce a suspension of the antibiotic streptomycin in one of the flasks using distilled water. Then added the suspension in media for the purpose to inhibit bacterial growth. Then took 18 plates for all three cultures. 6- 6 plates were used for each separate isolate. For a single culture 1 plate was used for control and the remaining 5 plates were used for different concentrations of mint i:e 5%, 10%, 15%, 20%, and 25%. Then 30 ml media were used for different concentrations of mint which are calculated for each concentration.

For 5% we took 1.5ml extract in the pipette and put it in the flask then also took 28.5ml media through the pipette and put it in the same flask. Gently shake the flask and quickly pour in the Petri plate and allowed the plate to cool down the media.

For 10% we took 3ml extract in the pipette and put it in the flask then also took 27ml media through the pipette and put it in the same flask. Gently shake the flask and quickly pour in the Petri plate and allowed the plate to cool down the media.

For 15% we took 4.5ml extract in the pipette and put it in the flask then also took 25.5ml media through the pipette and put it in the same flask. Gently shake the flask and quickly pour the flask into the Petri plate and allowed the plate to cool down the media.

For 20% we took 6ml extract in the pipette and put it in the flask then also took 24ml media through the pipette and put it in the same flask. Gently shake the flask and quickly pour the flask into the Petri plate and allowed the plate to cool down the media.

For 25% we took 7.5ml extract in the pipette and put it in the flask then also took 22.5ml media through the pipette and put it in the same flask. Gently shake the flask and quickly pour the flask into the Petri plate and allowed the plate to cool down the media.

For the control plate, only the media are poured into the plate which is free from the extract and allowed the plate to cool down.

When all the plates are cooled and the media are solidified then made 3 wells in each plate along with the control. Then fix pathogenic isolate in these wells. Once the plates were completed they

covered all the plates and label them. Incubate all the plates in an incubator at 20c for 7 days. Check results after seven days and also measure the radial growth diameter for all the wells.

Repeat the same procedure for the other two isolates (N8-017), (P7-017).

Table 1: The family, scientific name, and popular name of each plant species that was employed in this study.

Scientific name	Common name	Family
Menthapiperita L.	Mint	Lamiaceae

Table 2: The plant extract treatment against *V. Inaequalis.*

	Application	concentration %
Plant extract	*Mint (Menthapiperita L.)*	5
		10
		15
		20
		25

3. Results and discussion

Tables 3, Table 4, and Table 5 display the effects of the tested plant extract on mycelium growth as a percentage of mycelium growth inhibition of B1-017, N8-017, and P7-017. When compared to the control, three different doses of mint extract—15%, 20%, and 25%—inhibit or kill the growth of fungus. The extract of mint exhibited antifungal properties. A high dose of mint against B1-017, N8-017, and P7-017 isolates was observed that showed the antifungal effect.

Table 3: Effects of plant extracts on mycelial growth of *V. Inaequalis.*

ISOLATE B7-017			
Medicinal plants	**Concentration (mL-1)**	**Colony diameter (mm)**	**% Effect**
Mint	5	3 5	
	10	13.24.4	
	15	11.53.8	
	20	7.6 2.5	
	25	6.4 **2.1**	
	control	18.6 6.2	

At 7 days following inoculation, the mean radial mycelial growth of V. inaequalis was calculated. Each observation is base on 6 replicate plates. + and ++ symbols specify that antifungal effects at shown concentrations are fungicidal and fungistatic respectively

The Efficacy of the mint extract against B1-017 isolate

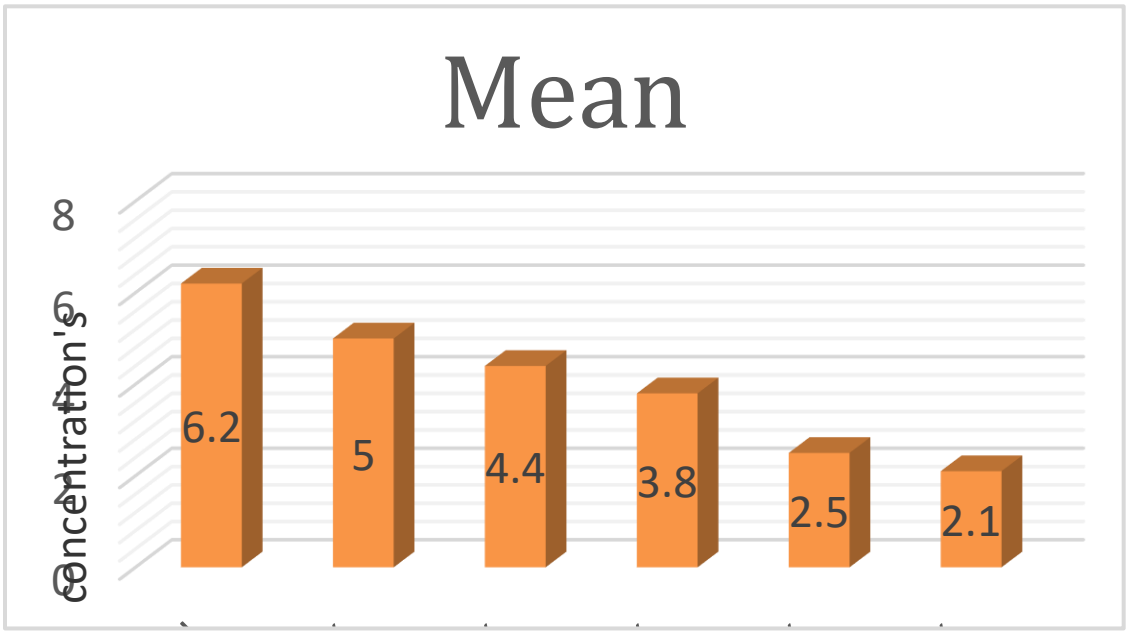

Figure 1: To checked the efficacy 15% dose shows fungistatic effect while 20% and 25% dose shows fungicidal effect.

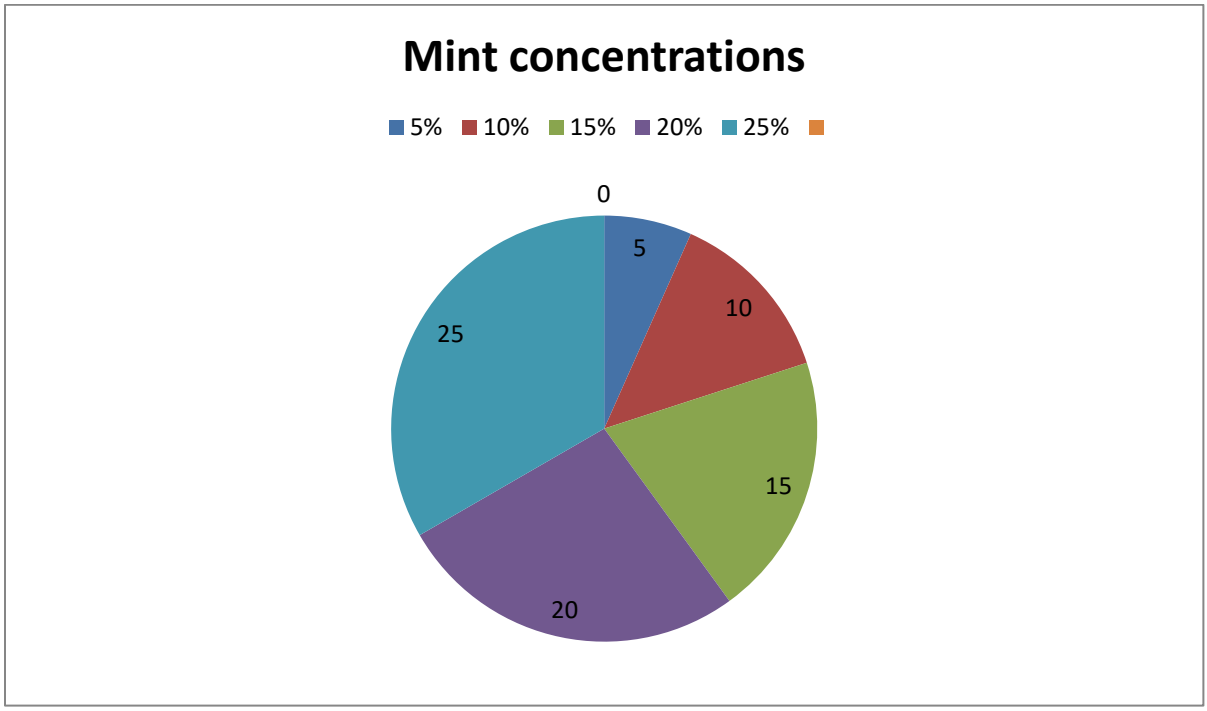

Figure 2. Mint concentrations. Dark blue shows 5%, orange shows 10%, grey shows 15%, yellow shows 20%, and light blue shows 25% concentrations.

Table 4: The plant extract effects on mycelial growth of *V. inaequalis.*

	ISOLATE N8-017		
Medicinal plants Effect	Concentration (mL-1)	Colony diameter (mm)	%
Mint 5.5	5	16.5	
	10	14.9	
4.9			
	15	12	
4			
	20	9.6	
3.2+			
	25	6.8	
2.2++			
	Control.	21.1	

At 7 days following inoculation, the mean radial mycelial growth of V. inaequalis was calculated. Six duplicate plates are used for each observation. The symbols + and ++ denote antifungal actions that are, respectively, fungicidal and fungistatic at the amounts given.

The Efficacy of mint extract against N8-017 Isolate

To check the efficacy 20% dose shows fungistatic effect and 25% dose shows fungicidal effect.

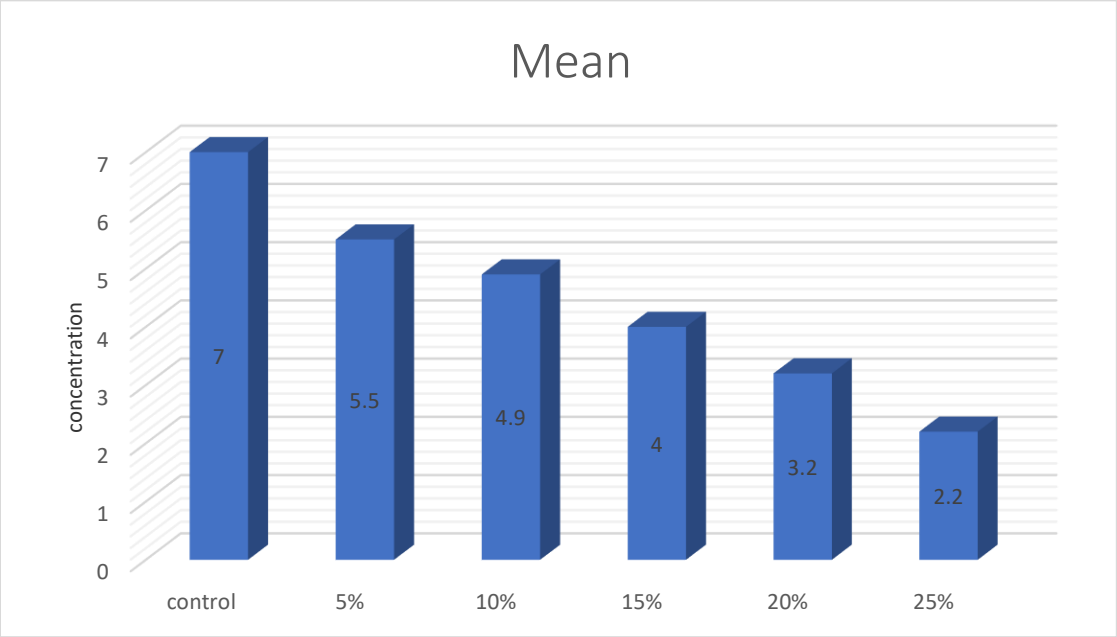

Fig 3: The Efficacy of mint extract against N8-017 Isolate

Table 5: The plant extracts effects on the mycelial growth of *V. inaequalis*.

Isolate P7-017			
Medicinal plants	Concentration (mL-1)	Colony diameter (mm)	% Effect
Mint	5	12.1	4
	10	10.8	3.6
	15	8.6	2.8+
	20	6.7	2.2++
	25	6.8	2.2++
	control	14.6	4.8

Seven days following inoculation, the mean radial mycelial growth of V. inaequalis was assessed. On six replicate plates, each observation is based. The + and ++ symbols denote that the antifungal actions at the quantities shown are, respectively, fungicidal and fungistatic.

The Efficacy of mint extract against P7-017 isolate

To check the efficacy. 15% dose shows fungistatic effect while 20% and 25% dose shows fungicidal effect.

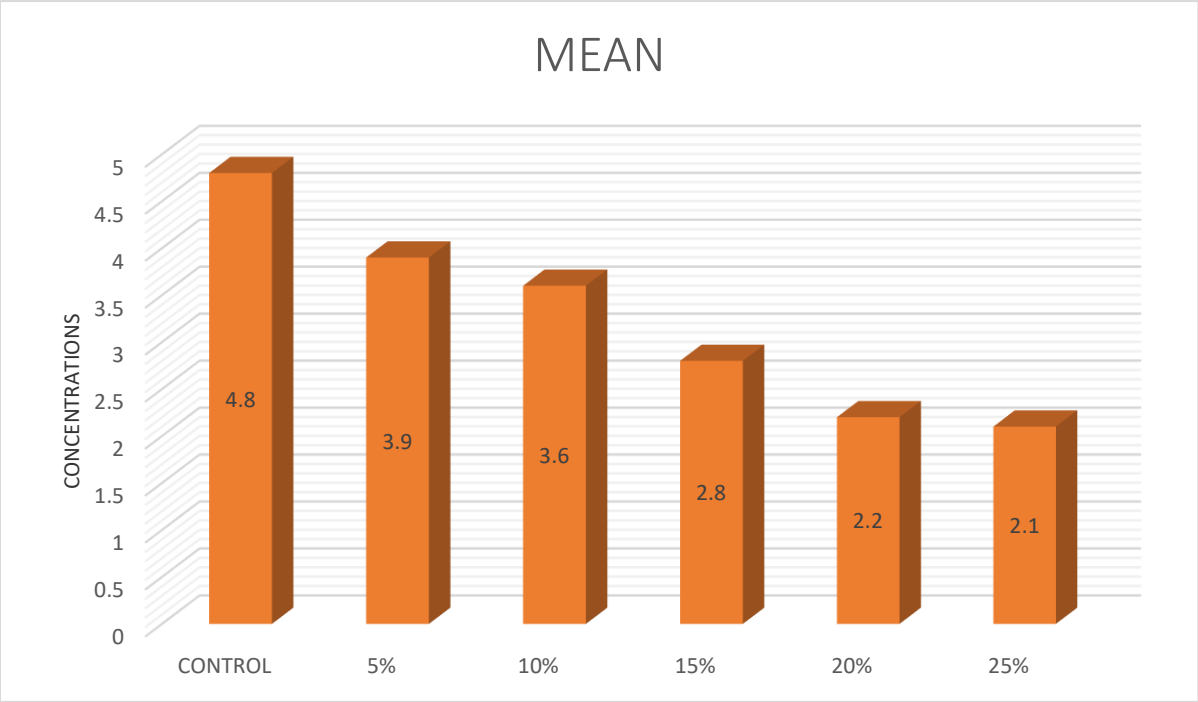

Figure 4: The Efficacy of mint extract against P7-017 isolate.

DISCUSSION

In research, the mint extract showed varying degrees of inhibitory effects against B1-017, N8-017, and P7-017 as compared to the control application. The three isolates' three strongest antifungal effects on colony formation were discovered at 20% and 25% concentrations of mint extract (Table 3, Table 4, and Table 5). Parallels between our findings and those of other related research were evident. Depending on the dosage increase that was attained, research using various doses of mint extracts reported having a stronger effect against fungus.

In vitro tests were performed to determine the antifungal activity against 8 phytopathogenic fungi of the methanol extract of 9 wild plant species. The wild marjoram extract demonstrated

the highest activity, completely inhibiting the mycelial growth of 6 of the 8 fungi as well as the spore germination of the 6 fungi (*B. cinerea*, *Alternaria solani*, Penicillium sp., *Cladosporium* sp., *Fusariumoxy* [9].

Mint, thyme, and lavender extracts all inhibited the growth of *R. solani* and Fusarium spp. mycelium to varying degrees in vitro tests to determine the antifungal effects of some plant extracts and essential oils against cotton damping-off agents; the highest fungi-toxic effect was noted at an 8% dose of thyme extract. The pathogens' mycelium growth was completely inhibited by all of the doses of thyme essential oil that was used, and they displayed a notable fungicidal effect [10].

Pagiotti et al., claimed that linalool, which is present in lavender essential oil, is responsible for its minor antifungal activity, which was discovered to be less than that of the other essential oils. Some plants with essential oils, such as thyme, have been shown to have strong antifungal activity in vitro against a variety of phytopathogenic fungi [11].

Soylu et al, eight different essential oils were tested in vitro at various concentrations for effectiveness against *P. cinnamomi*, *Pyrenochaeta Lycopersici*, and *V. dahliae*. The most effective essential oils were discovered to be thyme, oregano, peppermint, and coriander. The authors demonstrated the fungistatic + fungicidal activity of the oils and determined their activity. According to the research, mint essential oil significantly prevented the growth of a small number of soil-borne pathogens, including *F. oxysporum f.* sp. *lycopersici*, *F. solani var. coeruleum, V. dahlia*, *Pythiumultimum*, and *R. solani*, even at the lowest concentrations [12].

Kezdoglou et al, assessed the effectiveness of *F. oxysporum*, *Aspergillus niger*, *Penicillium expansum*, and *V. dahliae* against the essential oils of lavender, oregano, sage, and spearmint. The oregano and spearmint oils, as well as carvacrol and carvone, respectively, showed the most inhibitory action on mycelial development in the study, according to the researchers [13].

Researchers at the result of their study reported that essential oils collected from various aromatic plants have a broad spectrum against plant pathogens.

4. Conclusion

Our investigations employed a mint plant extract that had strong mortality effects and very few ascospores that sprouted as a result of the treatments. We may draw the conclusion that mint extracts utilized in lab settings have noticeable effects in comparison to the control. In a field experiment with apple crops, all of the extract concentrations studied in the lab were evaluated. These findings suggest that the extract's chemical constituent has potent fungicide properties, suggesting that it might be utilized to combat fungi in the integrated and biological management of the apple scab disease.

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