# Control of powdery mildew disease in *Abelmoschus esculentus* by metabolites of halotolerant fungus *Alternaria alternata*

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## Abstract

Powdery mildew is a widespread and economically important disease on many crop plants like okra, carrots, beets, squash, tomatoes, cucumbers, radishes, etc., caused by one of the prime phytopathogen *Podosphaera xanthii*. In this study, crude metabolites of halophilic fungi *Alternaria alternata* were used against the *P. xanthii* in a dose-dependent manner, and different concentrations like 50, 100, 150, 200, and 250 mg $mL^{-1}$ were analyzed by disc diffusion method. Among them, high antifungal activity of 16 mm in diameter was exhibited by 250 mg concentration. Based on the study, it is put forth that halophilic fungi from extreme environments produce highly stable active constituents, which can be utilized to replace chemical fungicides.

**Keywords:** *Alternaria alternata*, Halophilic fungi, Okra, *Podosphaera xanthii*, Phytopathogens.

## Introduction

*Abelmoschus esculentus* (L.) Moench, one of the most common vegetables in India, is an excellent source of magnesium, folate, fiber, vitamins A, B, C, K1, proteins (lectin), and minerals (Sathish and Eswar, 2013). It is packed with antioxidants, including flavonoids, isoquercetin, and polyphenols, which lower blood sugar, lessen the risk of blood clots and oxidative damage, and protect against inflammation in the brain because of its special capacity which can enter the brain. The oil content of okra seeds ranges from 20 to 40%, with up to 47.4% of the oil being linoleic acid. Okra seeds are also a significant source of polyunsaturated fatty acids, which are vital for human nutrition (Gemede *et al*., 2014). The anti-fatigue activity of okra seed is caused by reducing blood lactic acid and urea nitrogen, improving hepatic glycogen storage, and promoting antioxidant capability by decreasing level of malondialdehyde and enhancing levels of superoxide dismutase and glutathione peroxidase (Xia *et al*., 2015). In streptozotocin-induced diabetic rats, *A. esculentus* has antihyperlipidemic and anti-diabetic properties (Sabitha *et al*., 2011). Lectin, a protein molecule derived from *Abelmoschus esculentus*, has shown anticancer activity against skin fibroblast (CCD-1059 sk) and human breast cancer cells (Monte *et al*., 2014). *A. alternata* possess the compound rubrofusarin B exhibited antifungal efficacy against pathogenic yeast, *Candida albicans* (Shaaban *et al*., 2012). *A. alternata* metabolites are also known for their herbicidal activity against parthenium weed (Javaid and Adrees, 2009). Various other fungal species such as *Drechslera* spp. (Akbar *et al.*, 2014), *Alternaria citri* (Javaid *et al*., 2022), *Trichoderma pseudokoningii*, *Penicillium italicum* and *Aspergillus* spp. (Khan and Javaid, 2020, 2021, 2022) are also known for their herbicidal and antifungal metabolites.

One of the most dangerous plant diseases is powdery mildew, which significantly reduces crop yields in several crops (Kiss, 2003). Powdery mildew is a dangerous disease affecting the leaves, stems, and fruits of numerous plant crops that is caused by *P. xanthii*. Many disease-causing pests or biological organisms such as pests, viruses, and fungal pathogens approach the okra for energy utilization (Choi, 2018). Powdery mildew-affected plants have a "flour-dusted" appearance. The disease initially manifests as round, powdery white spots that can occur on leaves, stems, and occasionally fruits. Eventually, it spreads to the entire upper leaf surface. The older leaves are first affected, and over time, the leaves gradually become yellow and dry off. Although rarely lethal, if unregulated, it can eventually deprive the plants of vital nutrients and water, leading to catastrophic damage. Plants can become weaker, bloom less frequently, and grow more slowly when infected. Generally, it causes mild damage, such as leaves turning yellow, withering, or deformed. Various disease management methods and synthetic fungicides have been used with more toxic effects which readily show not only the harmful effects on the soil but certainly kill the beneficial microbes that support plant growth and development. For instance, Fungicides based on copper harm the number of bacteria that fix nitrogen. Fungicidal residues such as apron, arrest, and captan often stay in the soil, interacting with living things and influencing the N-fixation process in the legume-*Rhizobium* relationship. By applying triarimol and captan, the frequency of *Aspergillus* species that stimulate plant growth and development can be reduced. On the other hand, common fungicides such as mancozeb, chlorothalonil, and boscalid listed as carcinogens 72% of them, are linked to cancer. Plant growers face a dilemma where they must risk their lives to preserve plant health, given the harmful impacts of chemical residues discovered in edibles (Meena *et al*., 2020).

It is the challenge to provide the possible substitution in controlling powdery mildew disease by strong natural bioactive compounds that can ease the farmers or planters to handle the product with low cost, eco-friendly with much efficacy (El-Saadony *et al*., 2022). There is convincing evidence that bioactive metabolites from extremophiles (Microbes in extreme environments) show promising biological activity, one such is halophiles, which have evidenced that halophilic or halotolerant fungi attract attention with their remarkable biological activities against pathogenic microbes, the reasons encounter the adaptation mechanisms of its genome cascades under different stress conditions like pH, temperature, dissolved oxygen, salinity, etc., which may be the reason for high lead stable proteins or peptide molecule with high biological activity (Santhaseelan *et al*., 2022). One such halotolerant fungus is *A. alternata* which causes black spot disease in the leaves of tomatoes. However, this fungus also exists as a good source for many active metabolites that can trigger many biological activities, which able to tolerate high salt concentrations, up to 20% of NaCl, and synthesize many novel metabolites known for its remarkable peptide molecules which ensures the possible substitution for synthetic fungicides. Unique peptides such as perylenequnones alterperylenol and dihydroalterperylenol from *Alternaria* sp. had antifungal activity on *Valsa ceratosperma*, a plant pathogen infecting apples and pears plant (Okuno *et al*., 1983). Tenuazonic acid, produced by *A. alternata* acts as a potential bioherbicide agent to control *Lantana camara*, and is toxic for most livestock to graze (Sanodiya *et al*., 2010). In this study, concerning the organic fungicides, the active secondary metabolites from the halotolerant fungi *Alternaria alternata* were used against *P. xanthii* phytopathogens, in replacing the chemical fungicides.

## Materials and Methods

### Temperature

The temperature of the sediment was recorded by using a standard thermometer.

### Hydrogen ion concentration

At a ratio of 1:2.5, the pH of the sediment sample was determined in the diluted sample (sediment: water ratio).

### Salinity

Using a hand refractometer, the sediment sample's salinity was determined by diluting it with distilled water (Atago, Japan).

### Collection of *Alternaria alternata*

The sediment sample was collected in the Tuticorin area (Latitude 8º48’N and Longitude 78º11’E) (Urani saltpan) on the southeast coast of India. 30 g of sample silt was roughly placed on a magnetic shaker for 10 minutes after being deposited into 100 mL of sterile seawater. For 14 days, 1 mL of the slurry was plated onto malt, yeast, glucose, peptone, or potato dextrose agar supplemented with 200 mg $L^{-1}$ of chloramphenicol. Following an enumeration, the colonies were separated into malt extract agar slants. Isolates were kept in soft agar vials covered in sterile liquid paraffin after being repeatedly streaked over malt extract agar plates to purify them. Organisms were identified using microscopic observations by lactophenol cotton blue staining methods.

### Extraction and characterization of crude bioactive metabolites

On a modest scale, fermentation was used to cultivate the isolated halophilic fungus *A. alternata*. After inoculating a 500 mL flask with 250 mL of autoclaved potato dextrose broth (PDB) adjusted with 20% NaCl, a fungal disc (6 mm in diameter) of 10-day-old cultures was maintained in a rotatory shaker at room temperature for a duration of 10 days. Filtration was used to remove the mycelium from the fermented culture broth following the incubation periods. The culture filtrates were extracted with ethyl acetate thricely, resultant layers were mixed and the solvent was removed by evaporation with a rotator vacuum distillation system (Li *et al*., 2005).

### Collection of pathogenic fungus

The pathogen P. xanthii was isolated from the infected leaves of okra plants. To get rid of the surface soil, tap water was used to wash the plant material. Using a sterile blade, small pieces of 1 to 2 mm in size were removed from the junction of the diseased leaf region. The sections were then aseptically cleaned three times using distilled water that had been sterilized. After removing any excess moisture from the bits using sterilized filter paper, they were transferred to Petri plates that were sterilized and contained autoclaved potato dextrose agar medium supplemented with streptomycin (30 mg $L^{-1}$). The plates were then incubated at 27±1 °C in a biological oxygen demand (BOD) incubator, and the growth of mycelial cells was checked every day. After being detected, the fungal growth was preserved for further use. The fungal morphological traits were observed.

***in vitro* fungicidal assay**

The crude extracts were assessed for antifungal activity against the pathogenic fungus *P. xanthii* using well diffusion methods (Reller *et al*., 2009). Initially, *P. xanthii* was cultured on PDA plates and incubated for five days at 30 °C (Khalil and Hashem, 2018; Hashem *et al*., 2021). The inoculum was adjusted to $10^7$ spores $mL^{-1}$ and a loopful of fresh hyphal conidial culture was transferred to sterilized phosphate buffer solution, pH 7.0. Additionally, 1 mL of the spore suspension was spread evenly onto malt extract agar plates. Using a sterile cork borer, six-millimeter wells were cut. Individual wells were then filled with varying quantities of crude extracts (50, 100, 150, 200, and 250 mg $mL^{-1}$) and incubated for two hours at 4 °C. Using amphotericin B as the standard, the plates were incubated at 30 °C for three days. The inhibitory zones were identified and noted following incubation.

## Results and Discussion

The physicochemical conditions of the salterns favor the fungi growth and can withstand high extremity. During the sampling period (May), a temperature of 34 ℃ was observed in the soil sample, and a pH of 7.6 was recorded in the crystallizer pond (Table 1). The salinity of 160% was probably recorded during the summer, which is a particularly high salinity. In addition, the heat from the sun causes the brine water in shallow saltern ponds to evaporate quickly, accelerating the crystallization process (Helan and Kalaiselvam, 2013). Additionally, the salinity of the crystallizer pond was maintained at 300–600%, with water masses that are consistently above 250%. This could be because of the evaporation of water, which causes gypsum and other minerals to precipitate. Eventually, NaCl precipitates and the salinity rises above 300 PSU (Oren, 2002). During the summer season, *Aspergillus* and *Penicillium* sp. (Michael and Kalaiselvam, 2018) were reported as dominant in crystallizer as well as in evaporator pond, which highly correlated with our study.

In general, *A. alternata*, a plant pathogenic fungus that causes leaf spots, and blights on many plants' part, especially it targets tomatoes, commonly called *Alternaria* stem canker of tomato, could ultimately resist many fungicides. Mainly it resides on seeds and seedlings and is often spread by spores as they become airborne and land on plants. In this study, the active toxic peptides from the pathogenic strain *A. alternata* could act against the target pathogen *P. xanthii, and* may eradicate the mycelium in powdery form all over the leaf plates. In this study, fungal strains such as *Aspergillus niger, Penicillium chrysogenum, Cladosporium cladosporoides*, *A. alternata,* and *Aureobasidium pullulans* were identified using Microscopic and Macroscopic examination. Since, *A. alternata* is the preference, which can be screened in sterile liquid paraffin-covered soft agar vials, pure cultures of *A. alternata* were kept after being cultivated on malt extract agar plates.

The percentage of crude extracts (1.7 g) was collected and tested for antifungal activity against *P. xanthii*, according to the results (Table 1 & Fig 1). The maximum inhibitory zone was observed at the concentration of 250 mg $mL^{-1}$ with a zone of 16mm in diameter. In this study, it is evident that crude peptides from the *A. alternata* could be marked for organic fungicides. Only meager work was carried out as *Penicillium oxalicum* was used as a biocontrol agent in controlling the powdery mildew of strawberries (De Cal *et al*., 2008). Many phytocompound studies exhibit its effects against *P. xanthii*, likely, black seed oil (Hafez, 2008), *Curcuma longa* (Fu *et al.*, 2018), phenolic compounds from *Nostoc muscorum* (Cyanobacterium) possess strong activity against *Aspergillus niger*, *A. flavus, Penicillium* sp., and *Fusarium microsporium* - common fungal adherence (Righini *et al*., 2022). Enormous works related to botanicals like phenol, alkaloids, flavonoids, terpenoids, etc., were designed to check the activity but only meager work has been done based on mycotherapy. However, no work has been carried out on halophilic fungi-derived peptides. Thus, this prime work paves an idea for utilizing the fungal-based metabolite interaction against this harmful pathogen. Since, the MIC zone exhibits comparatively low to standard, in the future it may undergo strain improvement with genetic modification/alteration in the nutrient medium to improve the organic-based fungicide in place of toxic chemicals.

## Conclusion

The growing global population necessitates careful management of plant resources, and advancements in plant health management are essential to ensuring that humans can continue to benefit from nature. More diversity is needed for successful plant health management since new methods for managing plant diseases in an eco-friendly manner are more important than ever. The outcome of the projects reflects the promising alternative to chemical fungicides by developing fungal-based organic fungicides which are eco-friendly for the management of the plant pathogenic fungi P. xanthii and its effect of powdery mildew in Red Burgundy Okra. Substitutions as biocontrol agents in agricultural fields to reduce the disease of okra and to obtain better growth and yield for local farmers

## Author's contributions

SP and MHSR conceived the idea. The literature was searched, and the manuscript was written by SP, AA, and SRK. Correction, editing, and supervision by SP, MHSR and EGW.

## Conflict of interests

The authors declare no conflict of interest.

**Table 1:** MIC of crude metabolites of *Alternaria alternata* against *P. xanthii*.

| S. No | Concentration (mg $mL^{-1}$) | Minimum inhibitory concentration (mm) |
|---|---|---|
| 1 | 50 | - |
| 2 | 100 | 12 |
| 3 | 150 | 14.2 |
| 4 | 200 | 15.1 |
| 5 | 250 | 16 |
| 6 | Standard | 19 |

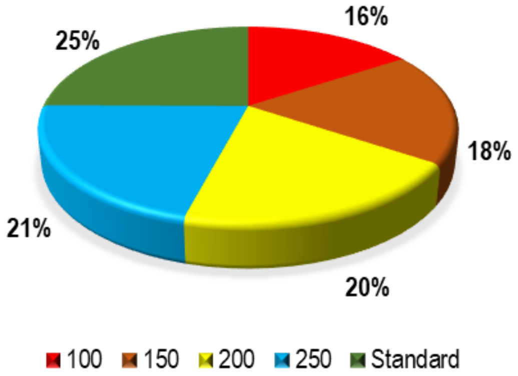


**Fig. 1:** Inhibition range of fungal crude metabolites against *P. xanthii*.

## References

Akbar M, Javaid A, Ahmad E, Javed T, Clary J (2014). Holadysenterine, a natural herbicidal constituent from *Drechslera australiensis* for management of *Rumex dentatus. J. Agric. Food Chem.*, **62**: 368-372.

Bharat NK, Sharma J, 2014. Occurrence of *Fusarium* Wilt of Tomato under Protected Conditions in Himachal Pradesh, India. *Int. J. Bioresour. Stress Manag.*, **5**: 285-287.

Choi IY, 2018. First Report of Powdery Mildew Caused by *Podosphaera xanthii* on Okra in Korea. *Plant Dis*., **102**: 1663.

De Cal A, Redondo C, Sztejnberg A, Melgarejo P, 2008. Biocontrol of powdery mildew by *Penicillium oxalicum* in open-field nurseries of strawberries. *Biol. Control*, **47**: 103-107.

El-Saadony MT, Saad AM, Soliman SM, Salem HM, Ahmed AI, Mahmood M, El-Tahan AM, Ebrahim AAM, Abd El-Mageed TA, Negm SH, Selim S, Babalghith AO, Elrys AS, El-Tarabily KA, AbuQamar SF, 2022. Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. *Front. Plant Sci*. **13**: 923880.

Fu W, Liu J, Zhang M, Li J, Hu J, Xu L, Dai G, 2018. Isolation, purification and identification of the active compound of turmeric and its potential application to control cucumber powdery mildew. *J. Agric. Sci.*, **156**: 358-366.

Gemede HF, Negussie R, Gulelat DH, Ashagrie Z, 2014. Nutritional quality and health benefits of okra (*Abelmoschus Esculentus*): A review. *Global J. Med. Res.*, **14**: 29-37.

Hafez YM, 2008. Effectiveness of the antifungal black seed oil against powdery mildews of cucumber (*Podosphaera xanthii*) and barley (*Blumeria graminis* f. sp. *hordei*). *Acta Biol. Szeged.*, **52**: 17-25.

Hashem AH, Khalil AMA, Reyad AM, Salem SS, 2021. Biomedical applications of mycosynthesized selenium nanoparticles using *Penicillium expansum* ATTC 36200. *Biol. Trace Elem. Res.*, **199**: 3998-4008.

Helan M, Kalaiselvam M, 2013. Antibacterial activity of halophilic fungi isolated from solar saltern at Tuticorin. *World J. Pharm. Pharmaceut. Sci.*, **2**: 536-545.

Javaid A, Adrees H, 2009. Parthenium management by cultural filtrates of phytopathogenic fungi. *Nat. Prod. Res.*, **23**: 1541-1551.

Javaid A, Jabeen T, Khan IH, Jabeen K, Akbar M, 2022. Herbicidal potential of *Alternaria citri* Ellis and Pierce metabolites against *Parthenium hysterophorus* L. *Allelopathy J.* **55**: 25-34.

Khalil AMA, Hashem AH, 2018. Morphological changes of conidiogenesis in two *Aspergillus* species. *J. Pure Appl. Microbiol.*, **12**: 2041-2048.

Khan IH, Javaid A, 2020. *In vitro* biocontrol potential of *Trichoderma pseudokoningii* against *Macrophomina phaseolina*. *Int. J. Agric. Biol.*, **24**: 730-736.

Khan IH, Javaid A, 2021. *In vitro* screening of *Aspergillus* spp. for their biocontrol potential against *Macrophomina phaseolina*. *J. Plant Pathol.*, **103**: 1195-1205.

Khan IH, Javaid A, 2022. DNA cleavage of the fungal pathogen and production of antifungal compounds are the possible mechanisms of action of biocontrol agent *Penicillium italicum* against *Macrophomina phaseolina*. *Mycologia*, **114**: 24-34.

Kiss L, 2003. A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Manag. Sci.*, **59**: 475-483.

Li H, Qing C, Zhang Y, 2005. Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. *World J. Microbiol. Biotechnol.*, **21**: 1515-1519.

Meena RS, Kumar S, Datta R, Lal R, Vijayakumar V, Brtnicky M, Sharma MP, Yadav GS, Jhariya MK, Jangir CK, Pathan SI, Dokulilova T, Pecina V, Marfo TD, 2020. Impact of agrochemicals on soil microbiota and management: A review. *Land*, **9**: 34.

Michael HSR, Kalaiselvam M, 2018. Diversity of halophilic mycoflora habitat in saltpans of Tuticorin and Marakkanam along southeast coast of India. *Int. J. Microbiol. Mycol.*, **7**: 1-17.

Monte LG, Santi-Gadelha T, Reis LB, Braganhol E, Prietsch RF, Dellagostin OA, Lacerda RRE, Gadelha CAA, Conceic FR, Pinto LS, 2014. Lectin of *Abelmoschus esculentus* (okra) promotes selective antitumor effects in human breast cancer cells. *Biotechnol. Lett.*, **36**: 461-469.

Okuno T, Natsume I, Sawai K, Sawamura K, Furusaki A, Matsumoto T, 1983. Structure of antifungal and phytotoxic pigments produced by *Alternaria* species. *Tetrahedron Lett.* **24**: 5653-5656.

Oren A, 2002. Diversity of halophilic microorganisms: Environments, phylogeny, physiology and applications. *J. Ind. Microbiol. Biotechnol.*, **28**: 56-63.

Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ, 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin. Infect. Dis.*, **49**: 1749-1755.

Righini H, Franciosos O, Martel Quintana A, Roberti R, 2022. Cyanobacteria: a natural source for controlling agricultural plant diseases caused by fungi and oomycetes and improving plant growth. *Horticulturae*, **8**: 58.

Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K, 2011. Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench. in streptozotocin-induced diabetic rats. *J. Pharm. Bioallied. Sci*., **3**: 397-402.

Sanodiya BS, Thakur GS, Baghel RK, Pandey AK, Bhogendra G, Prasad KS, Bisen PS, 2010. Isolation and characterization of tenuazonic acid produced by *Alternaria alternata*, a potential bioherbicidal agent for control *Lantana camara*. *J. Plant Prot. Res.*, **50**: 133-139.

Santhaseelan H, Dinakaran VT, Dahms HU, Ahamed JM, Murugaiah SG, Krishnan M, Hwang JS, Rathinam AJ, 2022. Recent antimicrobial responses of halophilic microbes in clinical pathogens. *Microorganisms*, **10**: 417.

Sathish D, Eswar A, 2013. A Review on: *Abelmoschus esculentus* (Okra). *Int. Res. J. Pharm. Appl. Sci*., **3**: 129-132.

Shaaban M, Shaaban KA, Abdel-Aziz MS, 2012. Seven naphtho-γ-pyrones from the marine-derived fungus *Alternaria alternata*: Structure elucidation and biological properties. *Org. Med. Chem. Lett*., **2**: 6.

Xia F, Zhong Y, Li M, Chang Q, Liao Y, Liu X, Pan R, 2015. Antioxidant and anti-fatigue constituents of okra. *Nutrients*, **7**: 8846-8858.