Journal of General Plant Pathology (2025) 91:109–114
https://doi.org/10.1007/s10327-025-01217-4

OTHERS

Antifungal activity of traversianal, a diterpenoid aldehyde from *Cercospora* sp. ME202, against the rice blast fungus, *Pyricularia oryzae*

Masatoshi Ino[1] · Junichi Kihara[2] · Atsushi Ishihara[3] · Makoto Ueno[1,2]

Received: 10 November 2024 / Accepted: 24 December 2024 / Published online: 15 January 2025
© The Author(s) 2025

Abstract
Here, we evaluated the antifungal activity of traversianal, a diterpenoid derived from *Cercospora* sp. ME202, against *Pyricularia oryzae*, which causes rice blast. Traversianal had concentration-dependent inhibitory activity against conidial germination and inhibited hyphal growth in a TLC bioassay. Staining conidia for viability with fluorescein diacetate and propidium iodide showed that the fungicidal activity was associated with cell membrane damage over time. Pretreatment of rice leaves with traversianal before inoculation decreased the number of blast lesions. These results suggest that traversianal has potential for controlling rice blast.

Keywords Antifungal compound · *Cercospora* Sp. · *Pyricularia oryzae* · Rice blast disease · Traversianal · *Oryza sativa*

Rice blast, caused by the fungus *Pyricularia oryzae*, which can infect all aboveground parts of rice plants, is responsible for 10–30% of annual rice yield losses worldwide (Skamnioti and Gurr 2009; Talbot 2003; Wilson and Talbot 2009). Although synthetic chemical fungicides are the major means for managing rice blast (Asibi et al. 2019; Rajeswari et al. 2024), excessive use of fungicides leads to fungicide resistance in the fungus (Yamaguchi et al. 2002; Tenni et al. 2021). Therefore, it is crucial to search for alternative antifungal compounds with novel modes of action.

The extensive range of secondary metabolites produced by microorganisms are a rich resource for mining compounds such as antibiotics and bioactive compounds that can be used as pharmaceuticals and pesticides (Demain 2009; Keswani et al. 2020; Sparks et al. 2023). Fungi produce secondary metabolites with diverse chemical structures that may have novel mechanisms of action as antimicrobial agents (Xu et al. 2021).

The largest class of natural products comprise the terpenoids, which likely are the most structurally diverse and have the most important biological properties of all natural products (Gershenzon and Dudareva 2007). Natural diterpenoids derived from microorganisms are among the most valuable sources for novel bioactive compounds (Saha et al. 2022), and many are active against plant pathogenic fungi such as *Botrytis cinerea*, *Colletotrichum coccodes*, and *Phytophthora infestans* (Bi and Yu 2016; Han et al. 2018).

We previously reported that traversianal (Fig. S1), a diterpenoid aldehyde produced by the fungus *Cercospora* sp. ME202 isolated from *Trifolium incarnatum*, had antifungal activity against *Colletotrichum orbiculare* (Ino et al. 2024), which causes cucumber anthracnose. To further evaluate the antifungal activity of traversianal against other plant pathogenic fungi, in the present study, we tested traversianal against *P. oryzae*.

Traversianal, purified from the culture filtrate of *Cercospora* sp. ME202 using silica gel column chromatography and high-performance liquid chromatography (HPLC) as previously described (Ino et al. 2024), was tested for inhibition of conidial germination of *P. oryzae* on glass slides and thin-layer chromatographic (TLC) bioassays as previously described (Ino et al. 2024). In brief, 1.0×10^5 conidia/ml were mixed with 1–30 ppm of traversianal in water with

✉ Makoto Ueno
makoto-u@life.shimane-u.ac.jp

[1] The United Graduate School of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan

[2] Laboratory of Plant Pathology, Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan

[3] Faculty of Agriculture, Tottori University, 4-101 Koyama Minami, Tottori 680-8553, Japan

DMSO (max: 0.3%) and placed on glass slides in a moist chamber at 25 ± 2 °C for 24 h, then the number of germinated conidia among 300 conidia for each treatment were counted using a light microscope (BA210E; Shimadzu, Kyoto, Japan). The percentage of germinated conidia was calculated as Number of germinated conidia/300 conidia) × 100. For the TLC bioassay, 100 µl of 30 ppm traversianal was spotted on a TLC plate (Silica 12 Gel 60; Merck KGaA, Darmstadt, Germany) and developed in chloroform–ethanol–water (40/10/1, v/v/v). The dried plate was sprayed with molten potato sucrose agar containing 20 ppm chloramphenicol and $>1.0\times10^7$ conidia/ml, then incubated at 25 ± 2 °C for 7 days, then any mycelial inhibition zones were measured.

On the slides, conidial germination was inhibited by traversianal in a concentration-dependent manner, with over 70% reduction at concentrations above 10 ppm compared with the control, and complete inhibition at 30 ppm (Fig. 1a). The percentage germination after treatment with traversianal at 10 ppm was 25.6 ± 9.8%, 3.7 ± 2.5% at 20 ppm and 0% at 30 ppm compared with 98.4 ± 2.4% after the control treatment with 0.3% DMSO (Fig. 1b). On the TLC plate, traversianal was detected in the mycelial inhibition zone at Rf 0.91 (Fig. 2).

The time course of the fungicidal activity of traversianal against *P. oryzae* was followed by assessing cell viability using double staining with fluorescein diacetate (FDA) and propidium iodide (PI); green fluorescence from FDA indicates viability, and red fluorescence from PI indicates compromised cell membrane integrity (Firstencel et al. 1990; Ji et al. 2018). Conidia of *P. oryzae* (1.0×10^5 conidia/ml) were suspended in traversianal (30 and 40 ppm) in 0.4% DMSO, and 24 µl was placed on glass slides in a moist chamber at 25 ± 2 °C. DMSO (0.4%) was used as the control. After 0, 12, 24, 36 and 48 h, FDA (200 µg/ml; 3 µl) and PI (100 µg/ml; 3 µl) were added to each slide. After 10 min, the conidia were examined using a fluorescence microscope (BZ-X700 all-in-one; 17 KEYENCE Corp., Osaka, Japan) with a GFP filter and Texas RED filter to count conidia fluorescing green and those fluorescing red. The percentage viability was calculated as (Number of conidia fluorescing green / Total number of conidia fluorescing green or red) × 100. After 48 h, most conidia in the control (0.4% DMSO) fluoresced green (viable), whereas a large fraction of conidia treated with traversianal (30 or 40 ppm) fluoresced red (non-viable) (Fig. 3a). Over time, the viability of control conidia remained constant, but decreased in the two traversianal treatments (Fig. 3b) as shown by a decrease in the number of cells with green fluorescence and increase in those with red fluorescence. Thus, traversianal had fungicidal activity against *P. oryzae* and progressively induced damage to the cell membrane of conidia.

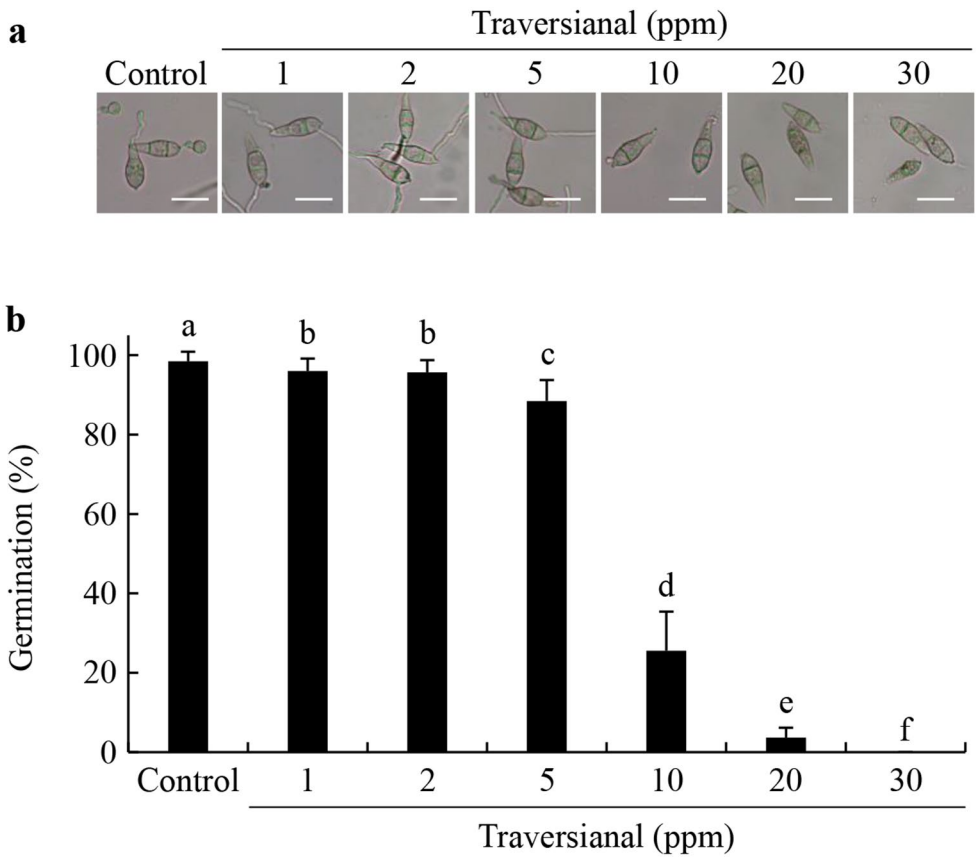

Fig. 1 Effect of 24-h incubation in various concentrations of traversianal on conidial germination of *Pyricularia oryzae*. Conidial suspensions (1.0×10^5 conidia/ml) in traversianal (1, 2, 5, 10, 20, and 30 ppm with 0.3% DMSO) or 0.3% DMSO (control) on glass slides were incubated in a moist chamber at 25 ± 2 °C for 24 h. (**a**) Light micrographs of conidia. Scale bar: 20 µm. (**b**) Mean (± SD) percentage germination ($N=900$ conidia [300/experiment, 3 experiments]). Different letters above the bars indicate a significant difference between means (Tukey's HSD test, $P<0.05$)

Fig. 2 Thin-layer-chromatography (TLC) bioassay to detect antifungal activity of traversianal against *Pyricularia oryzae*. Silica gel TLC plate was spotted with traversianal at 30 ppm (100 µl) and developed in chloroform–ethanol–water (40:10:1, v/v/v), then sprayed with molten potato sucrose agar containing > 1.0×10^7 conidia/ml and incubated at 25 ± 2 °C for 7 days

To test the preventive effect of traversianal (30 and 40 ppm) against rice blast, rice seeds (*Oryza sativa* L. cv. Koshihikari) were germinated in tap water, then five were sown in Sun soil (Nagata Co., Ltd., Shimane, Japan) in Petri dishes (5 seeds in each of six dishes). The dishes were then placed in a plastic case (20 × 10 × 5 cm) and immersed in uniconazole (0.25 ppm Sumiseven; Sumitomo Chemical, Osaka, Japan), a plant growth regulator, as described previously (Ashizawa and Zenbayashi 2005); distilled water was added as needed to maintain the solution level. Plants were grown to the four-leaf stage in an incubator as described above for the pathogen. The plants were then sprayed with traversianal (30 or 40 ppm) or 0.4% DMSO (as a control) (2 ml per five plants) and kept at 25 ± 2 °C for 24 h, then sprayed with a conidial suspension of *P. oryzae* (1.0×10^5 conidia/ml; 2 ml per five plants). Plants were placed in a moist chamber for 24 h at room temperature in the dark, then transferred to an incubator at 25 ± 2 °C with 12 h light/12 h dark. After 7 days, all blast lesions were counted. Traversianal at 40 ppm gave the best protection, with 48.7 ± 29.1 lesions/plant (mean ± SD), 55% fewer than on the control (mean 109.0 ± 52.6 lesions/plant) (Fig. 4). Traversianal at 30 ppm also reduced lesions to 36% fewer (69.3 ± 32.8 lesions/plant) than on the control.

Means in Figs. 1 and 3, and 4 were analyzed for significant differences among treatments using Tukey's HSD test ($P < 0.05$) in SPSS Statistics ver. 22.0 for Windows (IBM, Armonk, NY, USA).

Traversianal was active against brine shrimp (*Artemia salina*) with LC100 at 2.5×10^{-6} M, lethal against snails (*Biomphalaria* sp.) at > 2.0×10^{-7} M, and had modest hemolytic activity against human red blood cells at 5×10^{-7} M (Stoessl et al. 1989). However, it caused no harm to chicks after oral crop intubation with 100 mg/kg and was not toxic in standard growth and germination inhibition tests on bacteria and fungi according to Stoessl et al. (1989). However, knowledge on the bioactivity of traversianal remains limited and unclear.

In our previous study, traversianal inhibited conidial germination of *Colletotrichum orbiculare* at concentrations above 2 ppm and was fungicidal at 5 and 10 ppm (Ino et al. 2024). In the present study, traversianal also had antifungal activity against *P. oryzae*, although the concentration required for complete inhibition of conidial germination was 30 ppm, approximately 6-fold higher than required for *C. orbiculare*. Their different sensitivities to traversianal in terms of the concentration and exposure time required for fungicidal activity might be due to differences in their conidial characteristics, such as size and septation.

In the preventive efficacy tests, traversianal reduced the number of lesions by approximately 55% at 40 ppm compared to the control and was not phytotoxic. Thus, pretreatment with traversianal could partially protect rice plants from pathogen infection for at least 24 h, suggesting it has potential as a new control agent against *P. oryzae*. Further studies are essential to optimize treatment protocols and elucidate its antifungal mechanisms as are comprehensive toxicological evaluations using diverse organisms (including mammals, birds, and aquatic species) to scientifically validate its safety profile.

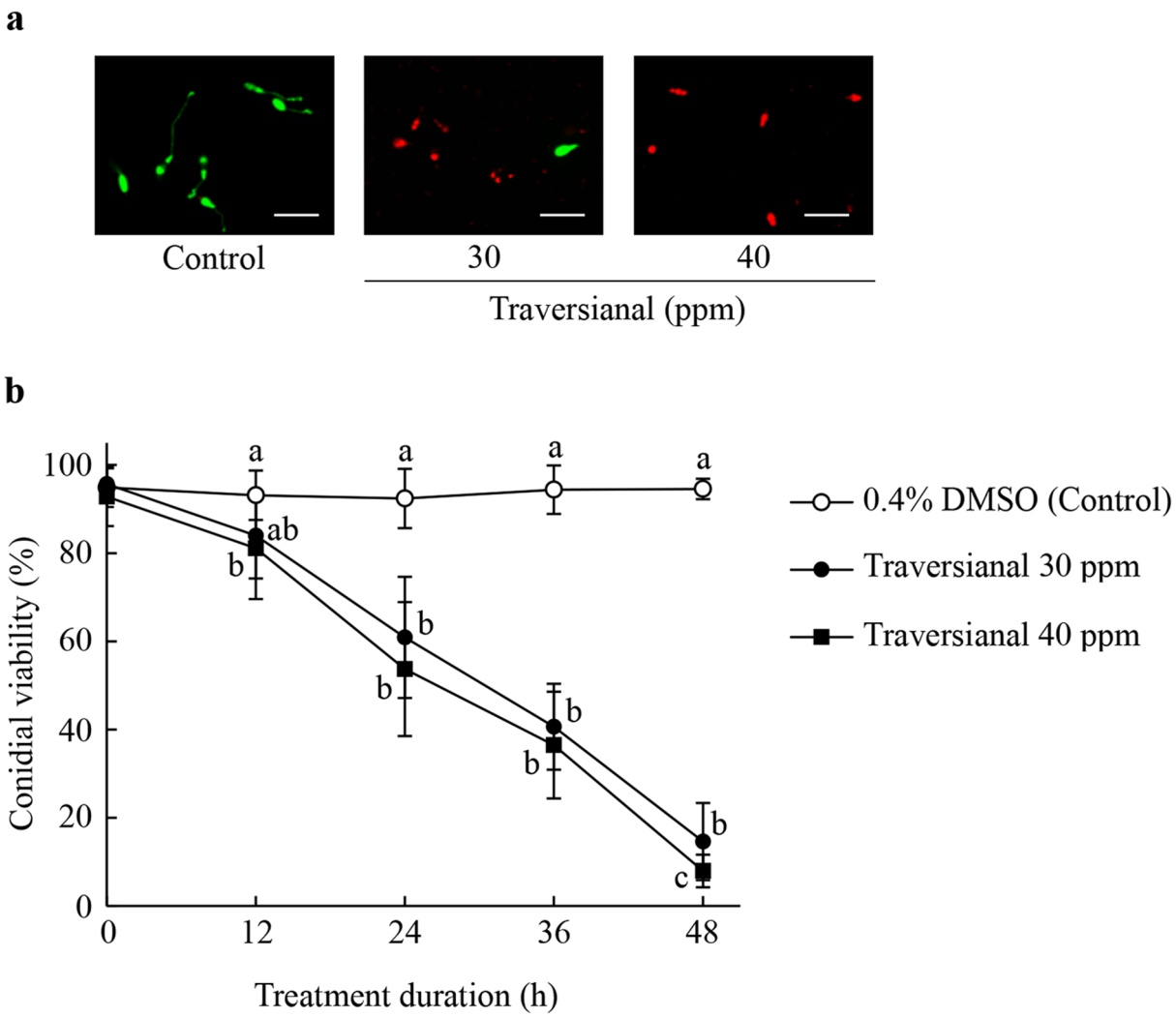

Fig. 3 Time course of fungicidal activity of traversianal against *Pyricularia oryzae* monitored for viability using fluorescein diacetate (FDA) and propidium iodide (PI). Conidia of *P. oryzae* (1.0×10^5 conidia/ml) in 30 or 40 ppm traversianal in 0.4% DMSO or 0.4% DMSO (control) were incubated on glass slides in a moist chamber at 25 ± 2 °C. After 0, 12, 24, 36 and 48 h, FDA and PI were added at final concentrations of 20 and 10 µg/ml, respectively. After 10 min, conidia were examined with a fluorescence microscope to count green-fluorescing conidia (viable) in. (**a**) Conidia after 48 h. Scale bar: 50 µm. (**b**) Mean (± SD) percentage of viable conidia in three experiments in five randomly selected fields of view using a 10× objective lens per experiment. Different letters above the bars indicate a significant difference between means (Tukey's HSD test, $P < 0.05$)

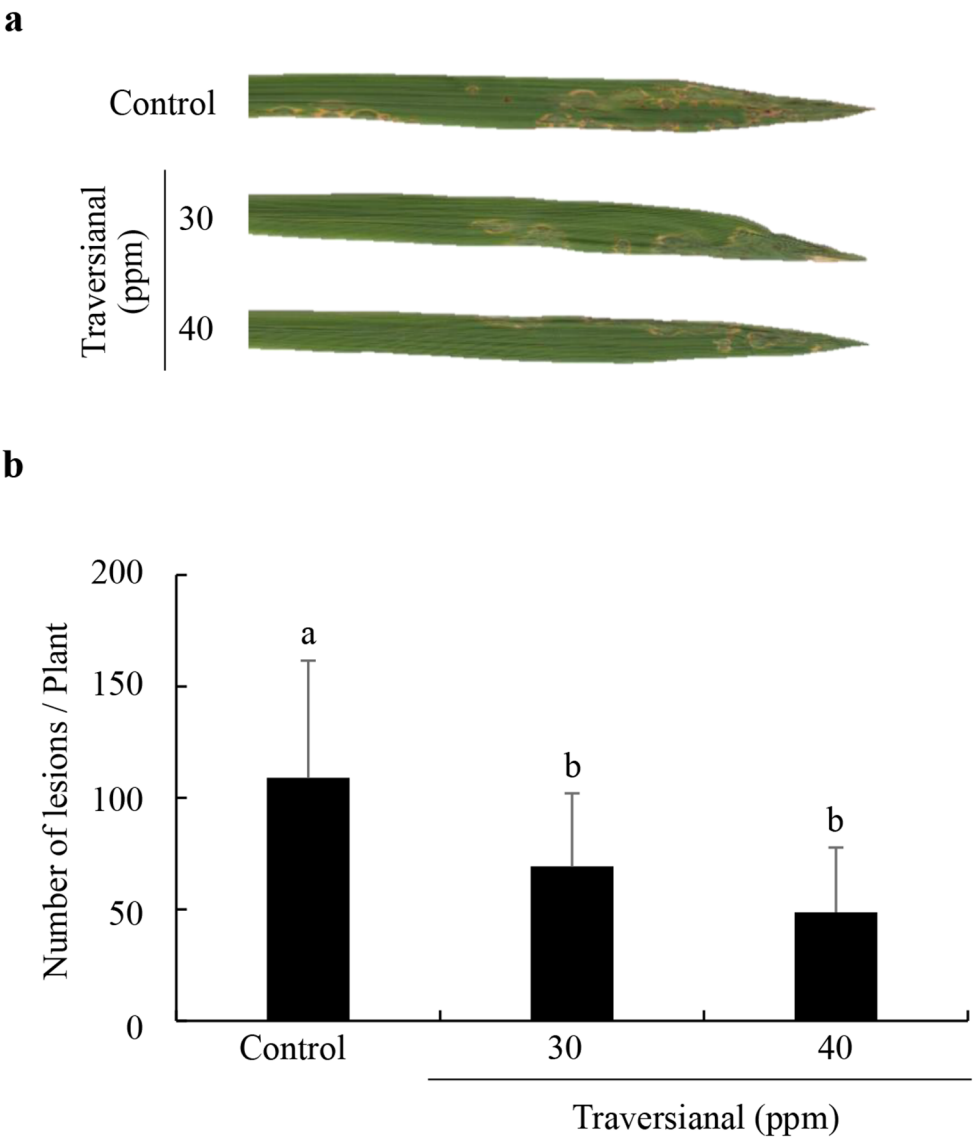

Fig. 4 Effect of traversianal on number of blast lesions at 7 days after inoculation of rice seedlings (4-leaf stage) that were pretreated with traversianal (30 or 40 ppm in 0.4% DMSO) 24 h before they were sprayed with 1.0×10^5 conidia/ml of *Pyricularia oryzae*. The control was pretreated with 0.4% DMSO. (**a**) Leaves with blast lesions and (**b**) mean (±SD) number of blast lesions on five rice plants per treatment in three experiments. Different letters above bars indicate a significant difference between means (Tukey's HSD test, $P < 0.05$)

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10327-025-01217-4.

Acknowledgements The authors thank Shimane University for providing financial support for the study and for publishing this report.

Author contributions Conceptualization: MI, JK, MU; formal analysis and research: MI, MU, AI; original draft preparation: MI, MU, AI; review and editing: MI, MU, AI; funding acquisition: MU; supervision: KJ, MU, AI.

Funding Open Access funding provided by Shimane University. This study was funded by Shimane University, Japan.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Ashizawa T, Zenbayashi KS (2005) Improved efficiency in differentiating races of rice blast fungus using a multi-cell tray and plant

growth regulator (in Japanese with English summary). Jpn J Phytopathol 71:11–15. https://doi.org/10.3186/jjphytopath.71.11

Asibi AE, Chai Q, Coulter JA (2019) Rice blast: a disease with implications for global food security. Agron 9:451. https://doi.org/10.3390/agronomy9080451

Bi YH, Yu ZG (2016) Diterpenoids from *Streptomyces* sp. SN194 and their antifungal activity against *Botrytis Cinerea*. J Agric Food Chem 64:8525–8529. https://doi.org/10.1021/acs.jafc.6b03645

Demain AL (2009) Antibiotics: natural products essential to human health. Med Res Rev 29:821–842. https://doi.org/10.1002/med.20154

Firstencel H, Butt TM, Carruthers RI (1990) A fluorescence microscopy method for determining the viability of entomophthoralean fungal spores. J Invertebr Pathol 55:258–264. https://doi.org/10.1016/0022-2011(90)90061-A

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414. https://doi.org/10.1038/nchembio.2007.5

Han JW, Oh M, Lee YJ, Choi J, Choi GJ, Kim H (2018) Crinipellins a and I, two diterpenoids from the basidiomycete fungus *Crinipellis Rhizomaticola*, as potential natural fungicides. Molecules 23:2377. https://doi.org/10.3390/molecules23092377

Ino M, Kihara J, Ishihara A, Seki K, Tanaka T, Yoshikiyo K, Ueno M (2024) Identification of antifungal compound isolated from *Cercospora* sp. ME202 and evaluation of its inhibitory activity against *Colletotrichum orbiculare*. J Gen Plant Pathol 90:35–41. https://doi.org/10.1007/s10327-023-01153-1

Ji DC, Chen T, Ma DY, Liu JL, Xu Y, Tian SP (2018) Inhibitory effects of methyl thujate on mycelial growth of *Botrytis Cinerea* and possible mechanisms. Postharvest Biol Technol 142:46–54. https://doi.org/10.1016/j.postharvbio.2018.04.003

Keswani C, Singh HB, García-Estrada C, Caradus J, He YW, Mezaache-Aichour S, Glare TR, Borriss R, Sansinenea E (2020) Antimicrobial secondary metabolites from agriculturally important bacteria as next-generation pesticides. Appl Microbiol Biotechnol 104:1013–1034. https://doi.org/10.1007/s00253-019-10300-8

Rajeswari E, Prasad MS, Vidya Sagar B, Madhav MS, Bhushan BT, Bhushan B, Rao YR (2024) Management of rice blast with modern combination fungicides against *Magnaporthe oryzae*. Vegetos 37:321–328. https://doi.org/10.1007/s42535-023-00611-7

Saha P, Rahman FI, Hussain F, Rahman SMA, Rahman MM (2022) Antimicrobial diterpenes: recent development from natural sources. Front Pharmacol 12:820312. https://doi.org/10.3389/fphar.2021.820312

Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease. Trends Biotechnol 27:141–150. https://doi.org/10.1016/j.tibtech.2008.12.002

Sparks TC, Sparks JM, Duke SO (2023) Natural product-based crop protection compounds—origins and future prospects. J Agric Food Chem 71:2259–2269. https://doi.org/10.1021/acs.jafc.2c06938

Stoessl A, Cole RJ, Abramowski Z, Lester HH, Towers GHN (1989) Some biological properties of traversianal, a strongly molluscicidal diterpenoid aldehyde from *Cercospora Traversiana*. Mycopathologia 106:41–46. https://doi.org/10.1007/BF00436925

Talbot NJ (2003) On the trail of a cereal killer: exploring the biology of *Magnaporthe Grisea*. Annu Rev Microbiol 57:177–202. https://doi.org/10.1146/annurev.micro.57.030502.090957

Tenni D, Sinetti A, Waldner M, Torriani SFF, Romani M (2021) First report of QoI resistance in Italian population of *Pyricularia oryzae*. J Plant Dis Prot 128:1705–1709. https://doi.org/10.1007/s41348-021-00494-3

Wilson RA, Talbot NJ (2009) Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. Nat Rev Microbiol 7:185–195. https://doi.org/10.1038/nrmicro2032

Xu K, Li XQ, Zhao DL, Zhang P (2021) Antifungal secondary metabolites produced by the fungal endophytes: Chemical diversity and potential use in the development of biopesticides. Front Microbiol 12:689527. https://doi.org/10.3389/fmicb.2021.689527

Yamaguchi J, Kuchiki F, Hirayae K, So K (2002) Decreased effect of carpropamid for rice blast control in the west north area of Saga Prefecture in 2001 (Abstract in Japanese). Jpn J Phytopathol 68:261

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.