DOI 10.1007/s10600-023-04204-3
*Chemistry of Natural Compounds, Vol. 59, No. 6, November, 2023*

# TWO NEW ANTIFUNGAL AROMATIC SESQUITERPENES FROM THE LEAVES OF CIGAR TOBACCO

**Guang-Hai Zhang,[1] Chen-Xi Zhang,[2] Wan-Long Yang,[1,3] Mei-Feng Bao,[2] Jun-Ru Shen,[1,3] Xu-Bing Tang,[1,3] Xiao-Hui He,[1,3] Yu-Ping Wu,[1*] and Guang-Hui Kong[1*]**

*Two new aromatic sesquiterpenes, (1-isopropyl-6-methoxy-3-methylnaphthalen-7-yl)methanol (**1**) and 1-isopropyl-6-methoxy-3,7-dimethylnaphthalene (**2**), as well as five known ones (**3–7**) were isolated from the leaves of cigar tobacco. Their structures were determined mainly using spectroscopic methods, including extensive 1D and 2D NMR techniques. Interestingly, the anti-fungi activity test revealed that compounds **1** and **2** showed potential anti-*Golovinomyces cichoracearum *(the main pathogen of tobacco powdery mildew disease) activity with inhibition rates of 55.4% ± 4.8, and 76.2% ± 5.1 respectively compared with the negative control. These rates are lower than that of a positive control (with an inhibition rate of 90.5% ± 5.3).*

**Keywords**: cigar tobacco, aromatic sesquiterpenes, antifungal activity.

Tobacco (*Nicotiana tabacum* L.) is one of the most widely planted economic crops worldwide [1]. The economic importance of tobacco encouraged researchers to investigate this species in detail [2, 3]. Moreover, *Nicotiana* species, in particular *N. tabacum*, are ‘non-food’ crops amenable to transformation, and are often used as molecular farming to produce a wide array of valuable small molecules as well as pharmaceutically important compounds, such as antibodies and recombinant proteins [3–7]. In recent years, many bioactive naturally small molecule metabolites have also been reported from this genus, including diterpenoids, sesquiterpenoids, flavonoids, coumarins, furans, alkaloids, sterols, etc. [3, 8–14].

Sesquiterpenes are an important class of natural products possessing three isoprene-derived units widely distributed across plants, marine organisms, and microbes [15, 16], and their basic framework can be classified based on the number of carbon rings [17]. Many sesquiterpenes showed a wide range of biological activities such as antitumor, antibiotic, antiviral, immunosuppressive, insecticidal, antifungal, and similar [18, 19]. In tobacco, sesquiterpenes are also important kinds of metabolites, and more than 80 sesquiterpenes had been reported in tobacco [2, 3]. They have strong biological activities, including cytotoxicity, antiviral, antibacterial [9, 20–22], and also have ecological roles in the *Nicotiana* species including phytoalexins or in plant–herbivore interactions [3, 10].

Cigar tobacco (an important subspecies of *N. tabacum*) is an important economic crop, which has been widely planted in Yunnan Province in recent years. Owing to the secondary metabolites of plants being significantly impacted by the geographical environment, the high altitude and extensive sunlight environment in Yunnan caused cigar tobacco to have its unique flavor and aromatic character [23, 24]. Therefore, the cigar tobacco in different geographical parts of Yunnan became a good resource for local chemists to search for new and bioactive natural products. To discover more novel bioactive sesquiterpenes from cigar tobacco, we focused on the leaves of Yunxue-39 (a variety of cigar tobacco planted in Yunnan), and isolated two new (**1** and **2**) as well as five known (**3–7**) aromatic sesquiterpenes. Interestingly, the anti-fungal activity test revealed that compounds **1** and **2** showed potential anti-*G. cichoracearum* (the main pathogen of tobacco powdery mildew disease) activity.

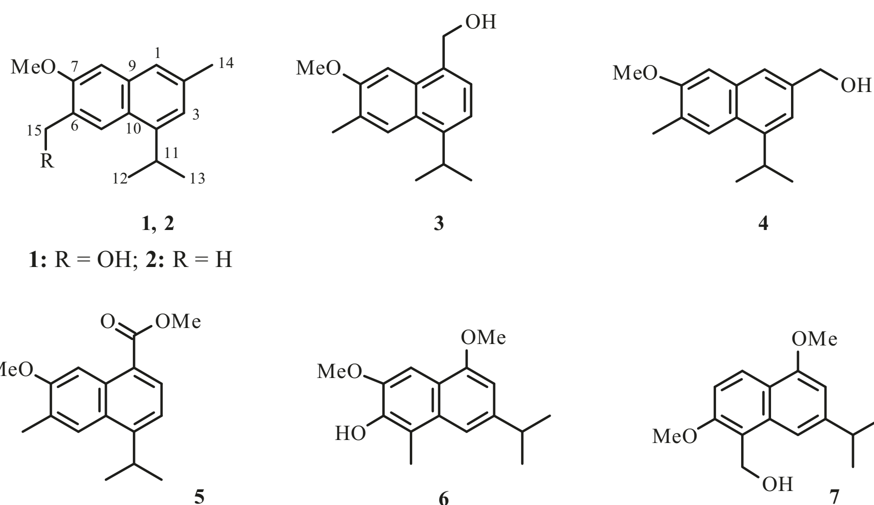
---

1) Yunnan Academy of Tobacco Agricultural Sciences, 650021, Kunming, P. R. China, e-mail: yn_cigar_team@163.com; ypwumm@163.com; 2) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute; of Botany, Chinese Academy of Sciences, 650201, Kunming, P. R. China; 3) Yunnan Oriental Tobacco Co., Ltd., 678000, Baoshan, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2023, pp. 926–929. Original article submitted March 21, 2023.

0009-3130/23/5906-1097 ©2023 Springer Science+Business Media, LLC 

TABLE 1. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR Data of Compounds **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

| C atom | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 7.52 (d, J = 2.2) | 124.5 (CH) | 7.50 (d, J = 2.2) | 124.1 (CH) |
| 2 | – | 135.5 (C) | – | 135.7 (C) |
| 3 | 6.84 (d, J = 2.2) | 126.2 (CH) | 6.85 (d, J = 2.2) | 126.7 (CH) |
| 4 | – | 141.6 (C) | – | 141.2 (C) |
| 5 | 7.74 (s) | 126.9 (CH) | 7.65 (s) | 125.8 (CH) |
| 6 | – | 136.4 (C) | – | 132.5 (C) |
| 7 | – | 152.3 (C) | – | 154.3 (C) |
| 8 | 7.04 (s) | 108.1 (CH) | 6.97 (s) | 107.4 (CH) |
| 9 | – | 132.7 (C) | – | 131.8 (C) |
| 10 | – | 128.5 (C) | – | 127.5 (C) |
| 11 | 3.26 (m) | 32.3 (CH) | 3.26 (m) | 32.5 (CH) |
| 12,13 | 1.33 (d, J = 6.8) | 23.4 ($CH_3$) | 1.32 (d, J = 6.8) | 23.3 ($CH_3$) |
| 14 | 2.37 (s) | 21.5 ($CH_3$) | 2.38 (s) | 21.7 ($CH_3$) |
| 15 | 4.57 (s) | 62.0 ($CH_2$) | 2.27 (s) | 15.6 ($CH_3$) |
| 7-OMe | 3.76 (s) | 56.2 ($CH_3$) | 3.75 (s) | 56.2 ($CH_3$) |




A 70% aqueous acetone extract prepared from the leaves of cigar tobacco was subjected repeatedly to column chromatography and preparative RP-HPLC to afford two new sesquiterpenes, (1-isopropyl-6-methoxy-3-methylnaphthalen-7-yl)methanol (**1**) and 1-isopropyl-6-methoxy-3,7-dimethylnaphthalene (**2**), together with five known analogues (**3–7**). The $^{1}H$ and $^{13}C$ NMR data of **1** and **2** were listed in Table 1. The new compounds were confirmed by the search for the newly updated SciFinder database (an electronic database for chemical structure published by the American Chemical Society). The known compounds were identified as nicosesquite A (**3**) [25], nicosesquite B (**4**) [25], 4-isopropyl-7-methoxy-6-methylnaphthalene-1-carboxylate (**5**) [9], 7-isopropyl-3,5-dimethoxy-1-methylnaphthalen-2-ol (**6**) [26], and (3-isopropyl-1,6-dimethoxynaphthalen-5-yl)-methanol (**7**) [26] by the comparison with the literatures.

Compound **1** was obtained as a pale-yellow gum. Its molecular formula ($C_{16}H_{20}O_2$) was established by the quasimolecular ion peak observed in HR-ESI-MS at *m/z* 267.1370 $[M + Na]^+$ (calcd for 267.1361), and suggesting seven degrees of unsaturation. Its UV spectrum showed absorption maxima at 215, 254, and 305 nm, and the IR spectrum showed absorption bands at 3412, 1618, 1572, and 1459 $cm^{-1}$, indicating the presence of a hydroxy group and aromatic rings. Its $^{1}H$ NMR spectrum exhibited signals for four aromatic protons at δ [7.52 (d, J = 2.2 Hz), 6.84 (d, J = 2.2 Hz), 7.74 s, and 7.04 s], one methyl group at δ [2.37 (3H, s) and two methyl groups combined signal at δ 1.33 (6H,d, J = 6.8 Hz], one methoxy group at δ 3.76 (s), one methine proton at δ 3.26 (m), and one hydroxymethyl proton at δ 4.57 (s). The $^{13}C$ and HSQC NMR spectra data of **1**, together with the above $^{1}H$ NMR signals, suggested the presence of one tetrasubstituted naphthalene nucleus (C-1–C10, H-1, H-3, H-5, and H-8) [9, 26], one isopropyl group (C-11–C-13, H-11, and $H_6$-12, 13) [27], one methyl group (C-14 and $H_3$-14), one hydroxymethyl group (C-15, and $H_2$-15), and one methoxy group ($\delta_C$ 56.2 q, $\delta_C$ 3.76 s).

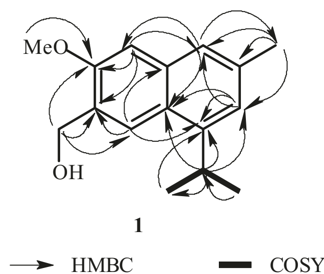



Fig. 1. Key HMBC a correlations and $^1H–^1H$ COSY of **1**.

In addition, the existence of a tetrasubstituted naphthalene nucleus can be confirmed by the HMBC correlations (Fig. 1) from H-8 to C-1, C-6, C-10, from H-5 to C-7, C-4, C-9, C-10, from H-1 to C-8, C-10, and from H-3 to C-4, C-10, whereas the existence of the isopropyl group was verified by the $^1H–^1H$ COSY correlations (Fig. 1) of H-11/$H_6$-12, 13, and the HMBC correlations from $H_6$-12, 13 to C-11, from H-11 to C-12, 13.

As the nucleus and the key substituent were determined, the position of the substituent can also be determined by further analysis of its HMBC correlations. The HMBC correlations from H-11 and $H_6$-12, 13 to C-4 indicated that the isopropyl group was located at C-4. The hydroxymethyl group located at C-6 was confirmed by the HMBC correlations of $H_2$-15 to C-5, C-6, and C-7, and from H-5 to C-15. The methyl group located at C-2 was confirmed by the HMBC correlations of $H_3$-14 with C-1, C-2, C-3, and of H-1 and H-3 with C-14. Finally, the methoxy group located at C-7 was confirmed on account of the HMBC correlations from the methoxy proton ($\delta_H$ 3.76 s) to C-7. Thus, the structure of **1** was elucidated, and given the systematic name of (1-isopropyl-6-methoxy-3-methylnaphthalen-7-yl)methanol.

1-Isopropyl-6-methoxy-3,7-dimethylnaphthalene (**2**) was also isolated as a pale-yellow gum and it gave a $[M + Na]^+$ peak at *m/z* 251.1418, consistent with the molecular formula of $C_{16}H_{20}O$. The $^1H$ and $^{13}C$ NMR data (Table 1) of **1** and **2** showed high similarity. The obvious chemical shift differences resulting from the hydroxymethyl group in **1** were replaced by a methyl group in **2**. The HMBC correlations from H-11 to C-3, C-4, C-10, from $H_6$-12, 13 to C-4 indicated that the isopropyl group was located at C-4. The methoxy group located at C-7 was confirmed on account of the HMBC correlations from methoxy proton ($\delta_H$ 3.75 s) to C-7. In addition, two methyl groups located at C-2 and C-6 can also be confirmed by further analysis of its HMBC correlations. Therefore, the structure of **2** was established as shown.

As certain of the sesquiterpenes exhibit potential antifungal activity [28, 29], and the fungi *G. cichoracearum* is the main pathogen of tobacco powdery mildew disease [30], compounds **1** and **2** were tested for their anti-*G. cichoracearum* activity.

The anti-fungal activity was tested according to previous literatures [29, 30], and chlorothalonil was used as a positive control. The results revealed that compounds **1** and **2** showed potential anti-*G. cichoracearum* (DC.) activity with inhibition rates of 55.4% ± 4.8 and 76.2% ± 5.1 respectively. These rates are lower than that of the positive control (with an inhibition rate of 90.5% ± 5.3).

## EXPERIMENTAL

**General**. UV spectra were obtained using a Shimadzu UV-1900 spectrophotometer. A Bio-Rad FTS185 spectrophotometer was used for scanning IR spectra. $^1H$, $^{13}C$, and 2D NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as an internal standard. Spectra were described using the atomic numbering of the skeleton given in the structures of **1** and **2**. ESI-MS and HR-ESI-MS analyses were measured on an Agilent 1290 UPLC/6540 Q-TOF mass spectrometer. Semipreparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 mm × 25 cm) or Venusil MP $C_{18}$ (2.0 mm × 25 cm) columns. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Qingdao, China), LiChroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, USA), or MCI gel (75–150 μm, Mitsubishi Chemical, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% $H_2SO_4$ in ethanol and heating.

**Plant Material**. The leaves of Yunxue-39 (a variety of cigar tobacco) were collected from the fermentation plant of Yuanjiang County, Yuxi Prefecture, Yunnan Province in 2021. The specimen (21-09-357) had been deposited in the herbarium of the Kunming Institute of Botany.

**Extraction and Isolation**. The air-dried tobacco leaves (4.2 kg) were crushed to 30 mesh, and were extracted four times with 70% aqueous acetone (3 × 8 L) at room temperature and filtered. The extract (162 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$–$CH_3OH$ gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. Further separation of fraction B (9:1, 20.8 g) by silica gel column chromatography, eluted with $CHCl_3$–$(CH_3)_2CO$ (9:1–2:1), yielded mixtures B1–B6. Subfraction B2 (8:2, 4.36 g) was subjected to silica gel column chromatography using petroleum ether–acetone and semipreparative HPLC (50–55% MeOH–$H_2O$, flow rate 12 mL/min) to give **1** (16.4 mg) and **2** (18.5 mg).

**Anti-fungal Activity Assays**. The anti-fungal activity was tested according to previous literatures [29, 30]. The fungus, *Golovinomyces cichoracearum* (DC.) V. P. Gelyuta was obtained from a diseased tobacco plant in a greenhouse at Kunming, P. R. China, and it was morphologically identied by Dr. W. L. Yang (Yunnan Academy of Tobacco Agricultural Sciences). The fungus was propagated on the sterile tissue culture bottle seedlings using *N. tabacum* cv. HD (a tobacco variety widely cultivated in China) as the host. In addition, another batch of tissue culture seedlings was cultured, and tobacco plants at the stage of 4–5 true leaves were used in an antifungal assay.

The propagated fungus was collected from tobacco leaves on the ultra-clean workbench, and prepared to a conidial suspension of the pathogen ($1 \times 10^5$ conidia/mL) in 0.1% Tween-20 aqueous solution. This pathogen solution was sprayed on sterile seedlings of tobacco plants at the stage of 4–5 true leaves (the sprayed amount is 1.0 mL per plant). After 6 h (waiting until the moisture on the tobacco leaves had evaporated), 250 μg/mL of tested compounds solution (dissolved in 0.1% of Tween-20 aqueous solution) was evenly coated on the tobacco leaves using a fine brush. The cap of the tissue culture bottle was resealed, and the tobacco was cultivated under the tissue culture lamp. After 7 days, the tobacco seedlings were tacked out, and the leaves with similar size and growth position were selected to count the infected spot area. Tween-20 aqueous solution (0.1%) was used as a negative control, and chlorothalonil ($C_8C_{l4}N_2$, CAS NO. 1897-45-6, a commercial antifungal pesticide for powdery mildew disease in China) was used as a positive control. The inhibition rates were calculated according to the formula:

$$\text{Inhibition rate (\%)} = [(C - T)/C] \times 100\%,$$

where C is the average infected spot area of the negative control, T is the infected spot area of the treatment. All results are expressed as the average of three parallel treatments.

**(1-Isopropyl-6-methoxy-3-methylnaphthalen-7-yl)methanol (1)**, $C_{16}H_{20}O_2$, obtained as pale-yellow gum. UV (MeOH, $\lambda_{max}$, nm) (log ε): 305 (3.61), 254 (3.52), 215 (4.08). IR (KBr, $\lambda_{max}$, $cm^{-1}$): 3412, 3060, 2962, 1618, 1572, 1459, 1368, 1247, 1231, 1155, 937. $^1H$ (500 MHz) and $^{13}C$ (125 MHz) NMR data, see Table 1. ESI-MS *m/z* 267 $[M + Na]^+$; HR-ESI-MS *m/z* 267.1370 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO_2$, 267.1361).

**1-Isopropyl-6-methoxy-3,7-dimethylnaphthalene (2)**, $C_{16}H_{20}O$, obtained as a pale-yellow gum. UV (MeOH, $\lambda_{max}$, nm) (log ε): 298 (3.64), 250 (3.57), 215 (4.13). IR (KBr, $\lambda_{max}$, $cm^{-1}$): 3064, 2968, 1615, 1564, 1452, 1373, 1251, 1239, 1158, 902; $^1H$ (500 MHz) and $^{13}C$ (125 MHz) NMR data, see Table 1. ESI-MS *m/z* 251 $[M + Na]^+$; HR-ESI-MS *m/z* 251.1418 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO$, 251.1412).

## ACKNOWLEDGMENT

This project was supported financially by China Tobacco Monopoly Bureau Grants and Yunnan Provincial Tobacco Monopoly Bureau Grants (110202103018, 2022530000241002).

## REFERENCES

1. M. Camlica and G. Yaldiz, *Grasas. Y. Aceites.*, **72** (1), e389 (2021).
2. A. Rodgman and T. A. Perfetti, *The Chemical Components of Tobacco and Tobacco Smoke*, Second Edition, CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2013.
3. F. L. Bideau, M. Kousara, L. Chen, L. Wei, and F. Dumas, *Chem. Rev.*, **117**, 6110 (2017).

4. D. A. Ullisch, C. A. Muller, S. Maibaum, J. Kirchhoff, A. Schiermeyer, and S. Schillberg, *J. Biosci. Bioeng*., **113** (2), 242 (2012).
5. M. Drapal, E. M. A. Enfissi, and P. D. Fraser, *J. Plant Physiol*., **265**, 153486 (2021).
6. P. Marconi, M. Alvarez, S. Klykov, and V. Kurakov, *Bioprocess. Int*., **12** (1), 42 (2014).
7. F. Garabagi, E. Gilbert, A. Loos, M. D. Mclean, and J. C. Hall, *Plant. Biotechnol. J.*, **10** (9), 1118 (2015).
8. G. Y. Yang, J. M. Dai, Z. J. Li, J. Wang, F. X. Yang, X. Liu, J. Li, Q. Gao, X. M. Li, Y. K. Li, W. G. Wang, M. Zhou, and Q. F. Hu, *Arch. Pharm. Res*., **45**, 572 (2022).
9. P. S. Yang, S. Y. Tang, C. B. Liu, L. Ye, F. M. Zhang, P. He, Z. H. Liu, Y. K. Chen, M. M. Miao, Q. P. Shen, and J. Q. Wang, *J. Asian Nat. Prod. Res*., **21** (2), 109 (2019).
10. N. Song, L. Ma, W. G. Wang, H. H. Sun, L. Wang, I. T. Baldwin, and J. S. Wu, *J. Exp. Bot*., **70** (20), 5895 (2019).
11. Q. F. Hu, Y. Y. Ma, H. Y. Liu, J. M. Dai, F. X. Yang, J. D. Zhang, J. Wang, X. M. Li, X. Liu, J. Li, Y. K. Li, W. G. Wang, M. Zhou, and G. Y. Yang, *Chem. Biol. Technol. Agric*., **9**, 88 (2022).
12. M. I. Sifola, L. Carrino, E. Cozzolino, L. D. Piano, G. Graziani, and A. Ritieni, *Sustainability*, **13** (4), 1 (2021).
13. T. Zhou, F. X. Yang, B. B. Cai, C. B. Liu, J. Ling, W. S. Kong, G. Y. Yang, and X. Liu, *Chem. Nat. Compd*., **57**, 1019 (2021).
14. L. F. Zhang, H. S. Wang, W. Xiong, W. Kai, M. X. Liu, C. P. Zheng, Y. P. Wu, G. Y. Yang, Y. K. Li, Q. F. Hu, and G. H. Kong, *Chem. Nat. Compd*., **58**, 1114 (2022).
15. H. Z. Shu, C. Peng, L. Bu, L. Guo, F. Liu, and L. Xiong, *Phytochemistry*, **192**, 112927 (2021).
16. B. Chopra and A. K. Dhingra, *Phytother. Res*., **35** (9), 4660 (2021).
17. D. E. Cane, *Compr. Nat. Prod. Chem*., **2**, 155 (1999).
18. S. Sut, F. Maggi, M. Nicoletti, V. Baldan, and S. Dall′Acqua, *Curr. Med. Chem.*, **25** (18), 1241 (2018).
19. S. L. Liu, L. Zhou, H. P. Chen, and J. K. Liu, *Phytochemistry*, **195**, 113048 (2022).
20. Q. P. Shen, X. M. Xu, C. B. Liu, W. Zhao, N. J. Xiang, Y. K. Chen, M. M. Miao, Z. H. Liu, and G. Y. Yang, *Nat. Prod. Res*., **30** (22), 2545 (2016).
21. S. Z. Shang, W. Zhao, J. G. Tang, X. M. Xu, H. D. Sun, J. X. Pu, Z. H. Liu, M. M. Miao, Y. K. Chen, and G. Y. Yang, *Fitoterapia*, **108**, 1 (2016).
22. J. Chappell and R. Nable, *Plant. Physiol*., **85**, 469 (1987).
23. H. W. Sun, K. Zhang, J. Long, X. W. Zhang, J. W. Zhang, J. Y. Cai, X. W. Ma, X. Feng, Y. B. Shu, J. Wei, and Y. C. Liu, *Chin. J. Mod. Agr. Sci. Tech*., **14**, 211 (2020).
24. H. W. Wang, X. Zhang, H. Y. Suo, X. Zhao, and J. Q. Kan, *J. Sens. Stud*., **34** (1), e12475 (2019).
25. J. Y. Cai, J. M. Dai, W. Xiong, H. P. Wang, W. Zhao, S. C. Qin, Y. Li, H. C. Cai, Q. F. Hu, W. G. Wang, and S. Z. Shang, *Chem. Nat. Compd*., **59**, 483 (2023).
26. S. Z. Shang, W. Zhao, J. G. Tang, J. X. Pu, D. L. Zhu, L. Yang, H. D. Sun, G. Y. Yang, and Y. K. Chen, *Phytochem. Lett*., **17**, 173 (2016).
27. A. M. Ciko, M. Jurin, R. O-Rakovac, D. Gao-Soka, and I. Jerkovi, *Algal Res*., **56**, 102330 (2021).
28. T. Fujimura, S. Sato, T. Tajima, and M. Arai, *Plant. Pathol*., **65** (8), 1358 (2016).
29. M. Quaglia, M. Fabrizi, A. Zazzerini, and C. Zadra, *Plant. Physiol. Biochem*., **52**, 9 (2012).
30. H. C. Wang, S. J. Yang, D. Q. Xu, X. J. Chen, X. D. Hu, S. H. Shang, and J. X. Shi, *Chin. J. Plant. Prot*., **38** (6), 123 (2012).