

Chemical composition and antifungal activity of the volatile oil from *Epichloë gansuensis*, endophyte-infected and non-infected *Achnatherum inebrians*

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Endophytic fungi that belong to the genus *Epichloë* (including species previously classified in the genus *Neotyphodium*) have been found in many cool-season grasses (subfamily Pooideae) [1]. *Achnatherum inebrians* (Hance) Keng (drunken horse grass, DHG) is a toxic perennial bunchgrass, distributed mainly throughout the harsh conditions of alpine or sub-alpine grasslands in the northwest of China. This species is usually infected by the fungal endophyte *Epichloë gansuensis* [1,2], which apparently enhances its resistance to the abiotic and biotic stress [3].

The dried above-ground plant samples (100 g) of endophyte-infected (E+) or endophyte-free (E–) DHG and dried mycelia of *E. gansuensis* were ground to a powder, so as to pass through a 1-mm sieve, and then subjected to hydro-distillation, with the samples steam distilled for 3 h using a Clevenger-type collector. The resulting hydro-distillate of samples were extracted through ethyl acetate to obtain the oil. The oils were dried over anhydrous sodium sulphate (Na₂SO₄), then rotary evaporated, leaving a concentrate of the essential oil (a faint yellow color). The essential oil was stored in –20°C refrigerator under N₂ in a sealed vial until required. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out to determine the composition of the essential oil from *A. inebrians* and *E. gansuensis*. Identification of the compounds was performed by the retention indices (RI), and authentic reference com-

pounds, peak matching library searches, as well as a database of the National Institute of Standards and Technology (NIST). Identification of the oil compounds is expressed as percent peak area relative to total peak area from the MS analyses of the whole extracts [4].

The volatile oil yield of E+ *A. inebrians* was 0.11% (g/g fresh weight) by hydro-distillation. Sixty-two compounds were identified in the E+ plants. The main components were heptadecane (9.06%); 2',3',4'-trimethoxyacetophenone (7.82%); octadecane (5.95%); tetracontane (5.63%); octadecane, 3-ethyl-5-(2-ethylbutyl) (4.95%), etc. The volatile oil yield of E– *A. inebrians* was 0.09% (g/g fresh weight) by hydro-distillation. Sixty compounds were identified in the E– plants. The main components were heptadecane (10.28%); pentadecane, 2,6,10-trimethyl (7.17%); *tert*-hexadecanethiol (5.59%); hexadecane (5.72%) and 1,2-benzenedicarboxylic acid, *bis*-(2-methylpropyl) ester (5.12%). There were 31 compounds identified in both E+ and E– plants. Of these, the main components were heptadecane; hexadecane; octadecane; 3-ethyl-5-(2-ethylbutyl); heptacosane and *tert*-hexadecanethiol. The content of pentadecane; tetradecane, 2,6,10-trimethyl; hexadecanoic acid, methyl ester; tetratetracontane; octadecane, 3-ethyl-5-(2-ethylbutyl) and ethyl *iso*-allocholate were greater in the E+ plants. The content of 17-pentatriacontene; nonadecane, and pentadecane, 2,6,10-trimethyl were greater in the E– plants. The volatile oil yield of *E. gansuensis* was 0.09% (g/g fresh weight) by hydro-distillation. There were 34 compounds

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identified in the *E. gansuensis* culture solution. The main components were 1,3-dioxolane (75.63%); 1-hexacosene (1.83%) and eicosane (1.54%). Seven compounds were shown as common to both the E+ and E- plants and to *E. gansuensis*.

Inhibitory activity of the DHG volatile oil on mycelial growth of the fungal pathogens was measured according to the method of Zhang et al. [5]. *Alternaria alternata* and *Bipolaris sorokiniana* were isolated from *A. inebrians*. *Curvularia lunata*, *Fusarium avenaceum*, *Fusarium solani* and *Trichoderma viride* were isolated from *Poa pratensis*. Colonies of *A. alternata*, *B. sorokiniana*, and *C. lunata* were inhibited significantly ($P < 0.05$) by E+ (vs. E-) volatiles under an oil concentration from 0.02 to 2 mg mL⁻¹ and

showed increased inhibition with increasing oil dose, while colonies growth of *A. alternata*, *B. sorokiniana*, and *C. lunata* were stimulated at 0.01 mg mL⁻¹ concentration. *F. avenaceum*, *F. solani*, and *T. viride* also showed some degree of growth inhibition by the volatile oils, the difference between the respective E+ and E- oil treatments being significant ($P < 0.05$) except at the lowest concentration of 0.01 mg mL⁻¹ (Figure 1).

Although the presence of *E. gansuensis* apparently affected some qualitative and quantitative differences in the production of DHG volatile compounds, this oil should also be more fully studied for possible important associations within DHG. For example, what may be the implications for DHG oil components in regulating the growth of *E. gan-*

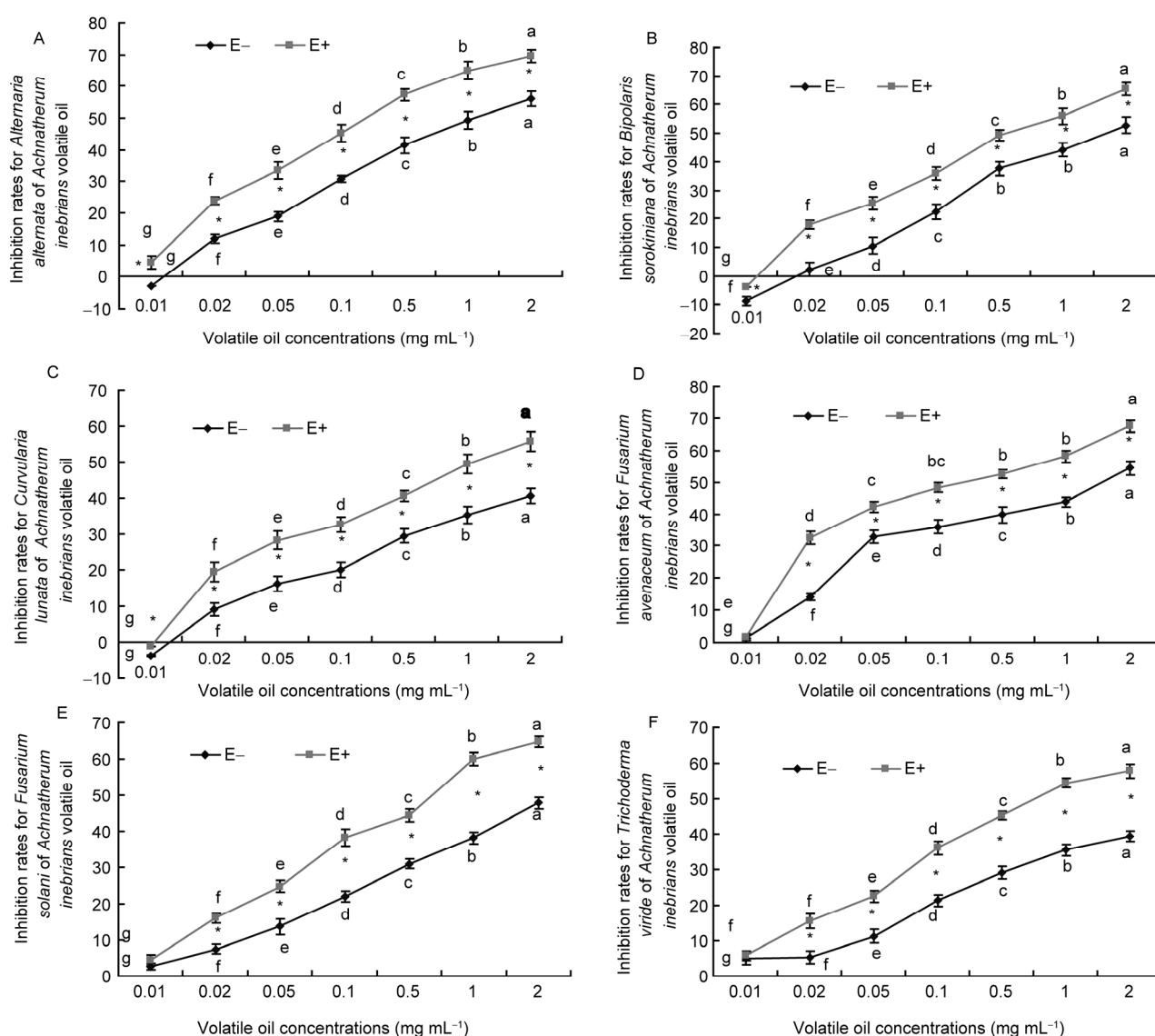


Figure 1 Inhibition rates for 6 different fungi using (E+/E-) *Achmatheterum inebrians* volatile oils. Each treatment was for all three samples ($n=3$). Values are mean±standard error (SE), with bars indicating SE. Columns with non-matching letters indicate a significant difference at $P < 0.05$ (Duncan test). The asterisk (*) indicates significant difference ($P < 0.05$) between E+ and E- volatile oil treatments at the same concentrations.

suensis to prevent mutualism from becoming parasitism? The specific volatile compounds that affect fungal pathogens and the antifungal mechanisms of DHG volatile oil also need to be further clarified.

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