Plant Growth Regulation (2026) 106:47
https://doi.org/10.1007/s10725-026-01453-4

ORIGINAL PAPER

GC-MS-based metabolomics reveals the impacts of water deficit stress and endophyte inoculation on the accumulation of tentatively identified hypoglycemic compounds in *Endostemon obtusifolius* (E. Mey. ex Benth.) N. E. Br

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Received: 18 January 2026 / Accepted: 24 March 2026
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Abstract

Endostemon obtusifolius, found in semi-arid areas of southern Africa, are commonly used to manage some human ailments, including diabetes. Interestingly, most of the ethnopharmacological potentials of *E. obtusifolius* remain largely unverified. Given its distribution, *E. obtusifolius* is prone to water deficit stress, which affects its growth and phytochemistry. Our previous findings showed that the inoculation of *E. obtusifolius* with endophytic species *Paenibacillus polymyxa* and *Fusarium oxysporum* enhances the growth attributes, stress tolerance and physiological parameters of the plant. Therefore, this study was aimed at evaluating the impact of individual and co-inoculation of *P. polymyxa* and *F. oxysporum* on the changes in some hypoglycemic compounds of *E. obtusifolius* subjected to different watering regimes using untargeted GC-MS metabolomics. Both inoculation (*Paenibacillus*, PI or *Fusarium*, FI) and co-inoculation (P+FI) significantly influenced the leaf metabolome of *E. obtusifolius*. Under the well-watered (WW) regime, P+FIWW treatment upregulated the highest number of hypoglycemic agents (caryophyllene, santalol-cis-α and n-pentadecanol). Compared to the control, FIWW significantly increased benzoic acid, α-pinene, *trans*-sesquisabine hydrate and aromanderene. Moreover, santalol, thunbergol and phyto-acetate were upregulated in the PIWW and P+FIWW under the well-watered treatments, whereas the co-inoculated (P+FISS) *E. obtusifolius* under severe water-deficit stress accumulated more α-phellandrene, α-pinene, and aromadendrene. Benzoic acid, on the other hand, was either unaffected or downregulated regardless of endophytic inoculation under the severe water deficit stress. Notably, the mild water deficit stress significantly enhanced the accumulation of hypoglycemic agents, and the effects were more marked in the PIMS and P+FIMS treatments. In conclusion, this study revealed that endophyte inoculation and water deficit stress have the potential to modulate the accumulation of bioactive compounds in medicinal plants.

Keywords Hypoglycemia · Water deficit · Endophyte · Medicinal plant

Communicated by Nicolas DESOIGNIES.

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Published online: 22 April 2026

Abbreviations

DIM	Dimension
FI	Inoculation of *Fusarium oxysporum*
GC-MS	Gas chromatography-mass spectrometry
H^+	Hydrogen ion
MS	Mild stress or 50% FC, and
$NADPH^+$	Nicotinamide adenine dinucleotide phosphate
NetCDF	Network Common Data Form
P + FI	Co-inoculation *Paenibacillus polymyxa* and *F. oxysporum*
P+FIMS	*Paenibacillus polymyxa* and *F. oxysporum* inoculated and moderately stressed
P+FISS	*Paenibacillus polymyxa* and *F. oxysporum* inoculated and severely stressed
P+FIWW	*Paenibacillus polymyxa* and *F. oxysporum* inoculated and well-watered
PCA	Principal component analysis
PI	Inoculation of *P. polymyxa*
PIMS	*Paenibacillus polymyxa* inoculated and moderately stressed
PISS	*Paenibacillus polymyxa* inoculated and severely stressed
PIWW	*Paenibacillus polymyxa* inoculated and well-watered
PLS-DA	Partial least squares-discriminant analysis
SS	Severe stress (SS) or 25% field capacity
UI	Uninoculated control
VIP	Variable importance in projection
WW	Soil water content- well-watered or 100% field capacity

Introduction

Abiotic stress is any physical, chemical or edaphic factor that may limit plant growth, development and productivity. Due to their sessile nature, plants are constantly exposed to varying degrees of abiotic stress, including water deficit, flooding, salinity, heat, light and heavy metal pollution (Zhang et al. 2023). In particular, water deficit is fast becoming the most dreadful environmental menace, causing huge agricultural losses in tropical, arid and semi-arid regions of the world, including southern Africa, where erratic rainfall is persistent (Nhemachena et al. 2020; Bhattacharya 2021). In plants, the negative impacts of water deficit stress are multidimensional, as it alters the morphological, physiological and chemical composition of affected plants (Bhattacharya 2021). Furthermore, due to the unabating scourge of climate change, the frequency and degree of water deficit stress are expected to increase in the coming decades, which in turn will make many plant species, including medicinal plants, vulnerable to other forms of biological stress (Khalid et al. 2023).

Depending on their taxonomic groupings, plants have developed complex mechanisms to cope with water-deficit stress. Plants may alter stomatal conductance and distribution, enhance transpiration efficiency, trigger hormonal and reactive oxygen species production, accumulate osmolytes and antioxidants, adjust their metabolic pathways or synthesise specific secondary metabolites (Ogbe et al. 2020; Seleiman et al. 2021). Plant secondary metabolites have ecophysiological relevance because they enhance plant fitness in their environments (Pant et al. 2021; Reshi et al. 2023). Specifically, under drought stress, secondary metabolites modulate resistance and sugar production in medicinal plants by influencing key biochemical pathways, including the Krebs cycle and the glycolytic pathway (Samsami and Maali-Amiri 2024). Interestingly, these diverse plant specialised metabolites have also been widely reported for their pharmacological relevance as anti-infectives, anticancer agents, and for the treatment of hyperglycemia (Albergaria et al. 2020). Plant-based hypoglycemic compounds are a special group of plant secondary metabolites of varying chemical structures that have shown promising antidiabetic properties. Plant-based hypoglycemic compounds are preferred alternatives to their orthodox counterparts owing to their safety records, affordability, effectiveness and structural complexity (Naveen and Baskaran 2018). Meanwhile, environmental factors, including water deficit, influence the biosynthesis and accumulation of secondary metabolites, such as hypoglycemic compounds, especially in medicinal plants (Zahedi et al. 2021). Thus, a sufficient understanding of the influence of climatic factors on the *in-planta* accumulation of pharmacologically active principles can provide valuable insight into the performance of medicinal plants under specific climatic conditions.

A couple of strategies have been devised to mitigate the negative consequences of periodic and recurring water-deficit stress on plants. Some recent techniques, including irrigation methods, mulching, contouring, soilless and film farming, development of drought-resistant crops, use of superabsorbent hydrogels, nanoparticles and biochar, and the application of exogenous growth regulators and synthetic hormones, have been reported (Seleiman et al. 2021). However, these techniques are very expensive and labour-intensive, and some have raised environmental and safety concerns (Ahluwalia et al. 2021; Chieb and Gachomo 2023). Alternatively, beneficial microbes, such as endophytic species, can enhance plant growth under harsh environmental conditions by producing phytohormones, extracellular enzymes, organic acids, and secondary metabolites (Ameen et al. 2024). Thus, inoculating plants with beneficial microbes is a veritable technique for enhancing

plant productivity under optimal and harsh environmental conditions (Srikanth et al. 2025). For example, under different water treatments, *Glycyrrhiza uralensis* inoculated with dark septate endophytic fungi (*Alternaria chlamydospore* and *Preussia terricola*) produced higher levels of active ingredients (glycyrrhizic acid and glycyrrhizin) compared to uninoculated plants (He et al. 2021). Plant secondary metabolite accumulation under stress conditions mediated by microbial inoculation can serve as antioxidants, cell wall-strengthening agents, pest and microbial antagonists, and protectors of cellular components (Yadav et al. 2021). In addition, the accumulation of these compounds in plants could help meet the ever-increasing demand for these bioactive principles, as they serve as lead molecules in the management of many health conditions, including diabetes (D'Amelia et al. 2021).

Metabolomics analysis has become a significant bioanalytical tool used to monitor plant stress and their responses to various stressors (Salam et al. 2023). It has also been effectively employed to identify different suites of primary and secondary metabolites produced by plants in various organs (Ma et al. 2024), as well as for drug discovery and quality control of natural products (Alarcon-Barrera et al. 2022). It has also been used to investigate and facilitate the identification and characterisation of hypoglycemic agents of medicinal plants (Ahmad et al. 2024). Interestingly, it has also been utilised to understand plant metabolomic responses to water deficit stress when inoculated with endophytic species (Shen et al. 2025). Wu and colleagues reported that untargeted metabolomic analysis of *Pinus taeda* seedlings inoculated with *Serendipita indica* under drought stress increased the accumulation of certain flavonoids and organic acids relative to drought-stressed, uninoculated seedlings (Wu et al. 2024).

Endostemon obtusifolius (E. Mey. ex Benth.) N. E. Br., a member of the Mint family (Lamiaceae), thrives in the semi-arid areas of South Africa, where it constantly faces varying degrees of water scarcity. The fragrant leaves of this plant serve dual purposes: as a culinary ingredient and as a traditional medicinal herb. Research has shown that *E. obtusifolius* crude extracts possess antidiabetic and antioxidant properties (Ogbe et al. 2023). However, the plant remains underexplored as its bioactive principles, as far as we know, have not been identified, isolated or characterised.

Furthermore, in a previous study from our group, we reported that two compatible endophytic species (*Fusarium oxysporum* and *Paenibacillus polymyxa*) isolated from a mother plant (*E. obtusifolius*) enhanced the growth and biological activity of *E. obtusifolius* under varying water regimes (Ogbe et al. 2024). Here, we aimed to use a GC-MS-based untargeted metabolomics approach to examine the effects of water deficit stress and endophyte inoculation on tentatively identified hypoglycemic compounds of *E. obtusifolius*.

Materials and methods

Cultivation of stem cuttings

Endostemon obtusifolius seldom produces seeds; thus, the plant was raised using stem cuttings as described in our previous study (Ogbe et al. 2024).

Inoculation of host plants with endophytes

For each treatment, ten approximately equal-sized (15 cm) stem cuttings were inoculated with previously isolated, identified and mutualistic endophytic species (Ogbe et al. 2023). The two endophytic species, the bacterium *P. polymyxa* (OL61995) and the fungus *F. oxysporum* (MZ598577), were chosen for this study owing to their drought stress-resistant records (Ogbe et al. 2023). *Paenibacillus polymyxa* spore suspension (10^7 CFU/mL) or *F. oxysporum* (7×10^5 spore number) conidial suspension were prepared, then 10 mL of each suspension was inoculated into the designated *E. obtusifolius* stem cuttings using the drench method, while the uninoculated plants (control) received an equal volume of sterile distilled water (Ogbe et al. 2024). To confirm the colonization of the stem cuttings, root samples in each inoculated group of plants were carefully harvested without destroying the plants, five weeks after inoculation. The inoculated species were re-identified on plates relying on their morphological features. A factorial experiment with two combining factors:

i. Inoculation of either *P. polymyxa* (PI) or *F. oxysporum* (FI), co-inoculation (P+FI), and uninoculated control (UI);
ii. Soil water content- well-watered (WW) or 100% field capacity (FC), mild stress (MS) or 50% FC, and severe stress (SS) or 25% FC.

There were twelve treatments in total, with each treatment replicated ten times, and the plants (ten-week-old stem cuttings) were then arranged in a completely randomized fashion. Six weeks after inoculation, the plants were subjected to water deficit stress for 8 weeks.

Gas chromatography-mass spectroscopy (GC-MS) analysis and data processing

Sample preparation

After the water deficit exposure, the leaves of the plants in each treatment were immediately harvested and quenched under liquid nitrogen. The samples were then stored at -80 °C, followed by freeze-drying to remove the water content of the plant samples (Isha et al. 2020). In triplicate, 2 g of leaf powder from each treatment were extracted with 20 mL of ethyl acetate, placed on a mechanical shaker for 18 h and sonicated in ice for half an hour. Thereafter, the crude extracts were filtered through Whatman No. 1 filter paper under vacuum, and the resulting extracts were concentrated at 40 °C using a rotary evaporator. The extracts were stored in a -80 °C freezer until they were needed.

GC-MS analysis

To investigate the metabolome differences in the twelve treatments of this study, the ethyl acetate crude extracts from each treatment were analyzed using GC-MS, adhering to the standard methods outlined by Yarazari and Jayaraj (2022). In summary, the analysis was conducted using a Shimadzu QP-2010 SE Gas Chromatograph, paired with an Agilent 5973 Mass Selective Detector, and managed by Agilent ChemStation software. A Zebron ZB-5MSplus capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) was employed. Ultra-pure helium served as the carrier gas, with a linear velocity of 37 cm/s and a flow rate of 1.0 mL/min. Each analyte (3 µl) was injected into the column, with the injector temperature maintained at 250 °C. The initial temperature of the oven was set at 60 °C and programmed to rise by 10 °C per min until reaching 280 °C, with a 3-min hold at each step. The mass spectrometer (MS) functioned in electron ionization mode at 70 eV, with an electron multiplier voltage of 1859 V. Additional MS settings included an ion source temperature of 230 °C, a quadrupole temperature of 150 °C, a solvent delay of 4 min, and a scan range of 50–700 amu. Compounds were tentatively identified by directly comparing the analyte's mass spectrum at a specific retention time with reference standards from the 2011 National Institute of Standards and Technology (NIST) library. The area percentage of each component was determined by comparing its average peak area to the total area recorded.

Data processing

Raw chromatogram datasets obtained from the GC-MS analysis were initially converted into network common data form (NetCDF) format (.cdf) using the GC-MS solution software from a Shimadzu QP-2010 SE Gas Chromatography. The NetCDF data were subsequently processed for peak recognition, filtering, and alignment using the XCMS and RStudio (Smith et al. 2006). The GC-MS spectra were also processed and formatted on Microsoft (*xlsx), where removal of outliers and basic calculations were performed.

Multivariate and univariate analysis

After data curation on RStudio and Microsoft (*xlsx), multivariate analyses, namely, principal component analysis (PCA), supervised and robust partial least squares-discriminant analysis (PLS-DA) were carried out on Metaboanalyst 6.0 (https://www.metaboanalyst.ca/). Metabolomics fold change (FC) analyses and T-tests were then combined to ascertain specific significant feature-based changes. Thereafter, a univariate analysis was carried out using Statistical Package for Social Sciences (SPSS®, version 29.0, IBM®, Armonk, USA). The level of significance was determined according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Thereafter, the data were transformed into graphic figures using GraphPad Prism® (version 10.6.0).

Results and discussion

The GC-MS untargeted metabolomics is a common technique for detecting and identifying bioactive constituents in medicinal plants. It is also applied to elucidate variations in secondary metabolites resulting from different metabolic responses to environmental stresses such as heat, salinity, drought, and nutrient stress. In this study, GC-MS-based untargeted metabolomics was employed to effectively demonstrate changes in metabolic pathways related to specific bioactive compounds of *E. obtusifolius*, particularly those with hypoglycemic properties (Fig. 1; Table 1).

Despite potential differences indicated by the total ion chromatogram (TIC) of the treatments, an unsupervised PCA of the metabolomic profiles was performed to observe possible interactions between the WW samples. The PCA score plot showed three distinct main clusters [cluster I (UIWW), cluster II (PIWW and P+FIWW) and cluster III (FIWW)] along the PC1 (28.7%) and PC2 (41.1%) axes, which accounted for 69.8% total variation (Fig. 1B). Furthermore, under the WW regimes, the inoculation of *E. obtusifolius* with endophytes resulted into 69.23% increase in the quantity of secondary metabolites of our interest (Fig. 1A). Most of the metabolite upregulations under the well-watered conditions were attributed to FI and P+FI inoculations. For instance, while FI was most potent at increasing α-pinene, *trans*-sesquisabine hydrate, α-phellandrene and

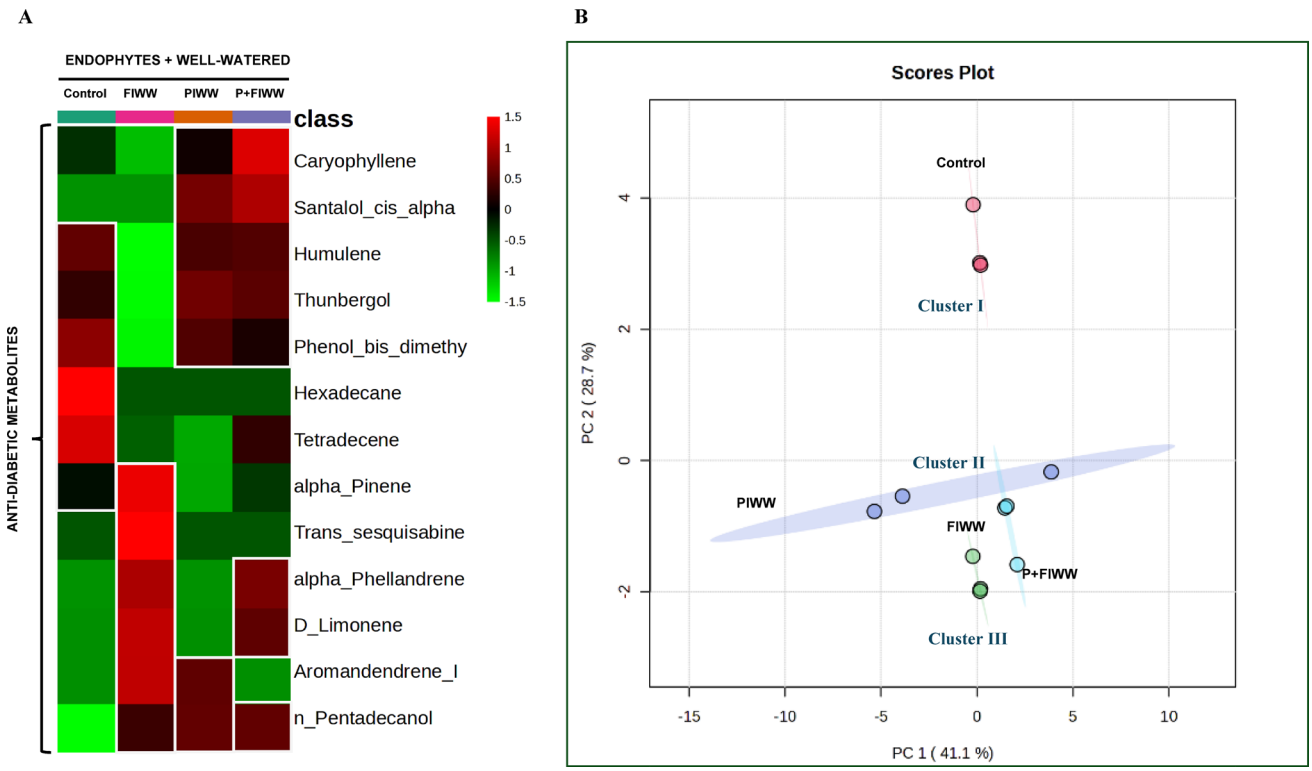


Fig. 1 Principal component analysis (PCA) on some tentatively identified hypoglycemic compounds of endophytes inoculated *Endostemon obtusifolius* (E. Mey. ex Benth.) N. E. Br. under well-watered conditions. **A**–heatmap of some tentatively identified hypoglycemic compounds triggered by endophytes generated using previously reported secondary metabolites, and **B**–Unsupervised PCA scores plot of endophytes inducing changes in some tentatively identified hypoglycemic compounds. *Abbreviations*: Control (UI), uninoculated; FIWW, *Fusarium oxysporum* inoculated well-watered; PIWW, *Paenibacillus polymyxa* inoculated well-watered and P+FIWW, *Paenibacillus polymyxa* and *Fusarium oxysporum* inoculated and well-watered

Table 1 Tentatively identified metabolites from ethyl acetate crude extracts of *Endostemon obtusifolius* leaves, showing their retention time, similarity index, class of compound and their hypoglycemic references

S/*N*	Compound	Retention time (min)	Molecular formula	Similarity index (%)	Class of compound	Hypoglycemic potential reference
1	α-pinene	7.06	$C_{10}H_{16}$	96	Monoterpene	(Santos et al. 2023)
2	Benzoic acid	9.44	$C_7H_6O_2$	97	Phenolics	(Debnath et al. 2020)
3	Caryophyllene	12.00	$C_{15}H_{24}$	96	Sesquiterpene	(Maurya et al. 2021)
4	Humulene	12.39	$C_{15}H_{24}$	95	Sesquiterpene	(Maurya et al. 2021)
5	Santalol	15.62	$C_{15}H_{24}O$	81	Sesquiterpene alcohol	(El Hachlafi et al. 2024)
6	$_D$-limonene	7.06	$C_{10}H_{16}$	94	Monoterpene	(Praveen Kumar et al. 2020)
8	Hexadecane	13.70	$C_{16}H_{34}$	93	Lipids	(Mahmood et al. 2020)
9	Phytol acetate	16.34	$C_{22}H_{42}O_2$	91	Diterpene	(Sajid et al. 2020)
10	Phenol,2,4-bis(1,1-dimethylethyl)	12.91	$C_{14}H_{22}O_2$	96	Phenolics	(Benoite and Vigasini 2021)
11	Thunbergol	23.73	$C_{20}H_{34}O$	86	Diterpene	(Fayez et al. 2022)
13	*Trans*-sesquisabinene hydrate	11.46	$C_{15}H_{26}O$	77	Sesquiterpene	(Maurya et al. 2021)
14	Aromandendrene	12.44	$C_{15}H_{24}$	83	Sesquiterpene	(Mahdizadehdehosta et al. 2025)
15	α-phellandrene	6.75	$C_{10}H_{16}$	94	Monoterpene	(Dahab et al. 2024)
16	3,7,11,15-tetramethyl-2-hexadecen-1-ol	16.34	$C_{20}H_{40}O$	91	Diterpene	(Adam et al. 2024)

aromanderene, the synergistic effect of the two endophytes (P+FI) triggered caryophyllene, santalol-*cis*-α and n-pentadecanol biosynthesis. These *in planta* chemical changes induced by endophytes were responsible for the separations observed among the treatment groups under well-watered conditions. Since most of the changes (increase) in hypoglycaemic compounds were ascribed to the application of FIWW, results from metabolomics FC analyses and t-tests were combined to ascertain significant feature changes (Fig. 2A). Compared to the control (UIWW), FIWW again proved to significantly induce FC in α-pinene, *trans*-sesquisabinene hydrate, aromanderene and α-phellandrene. These secondary metabolites were then subjected to univariate analyses, in which FIWW significantly increased benzoic

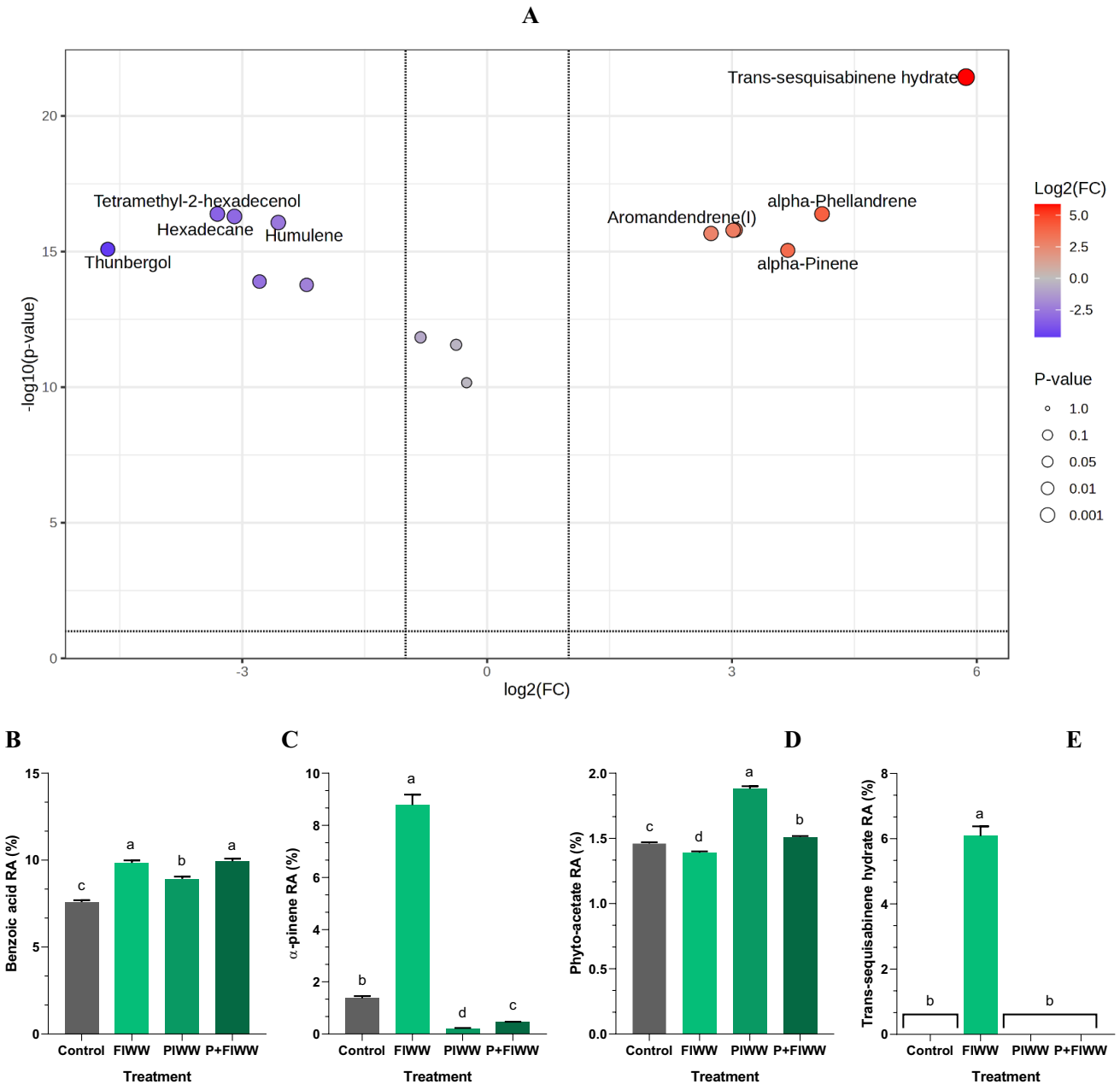


Fig. 2 Effect of endophytes inoculation on some tentatively identified hypoglycemic compounds of *Endostemon obtusifolius* under well-watered regimes. **A** – volcano plot from metabolite fold change (FC) analysis and T-tests (comparing FIWW and control). The FC threshold was 2.0, whereas 1.0 indicated no FC. **B –** benzoic acid, **C** – α-pinene, **D** – phyto-acetate and **E** – *trans*-sequisabinene hydrate. For each treatment, bars (mean±SEM) for the univariate one-way analysis of variance (ANOVA) are significantly different ($P \leq 0.05$) based on Duncan's multiple range Test (DMRT). **Abbreviations**: Control (UI), uninoculated; FIWW, *Fusarium oxysporum* inoculated well-watered; PIWW, *Paenibacillus polymyxa* inoculated well-watered and; P+FIWW, *Paenibacillus polymyxa* and *Fusarium oxysporum* inoculated and well-watered

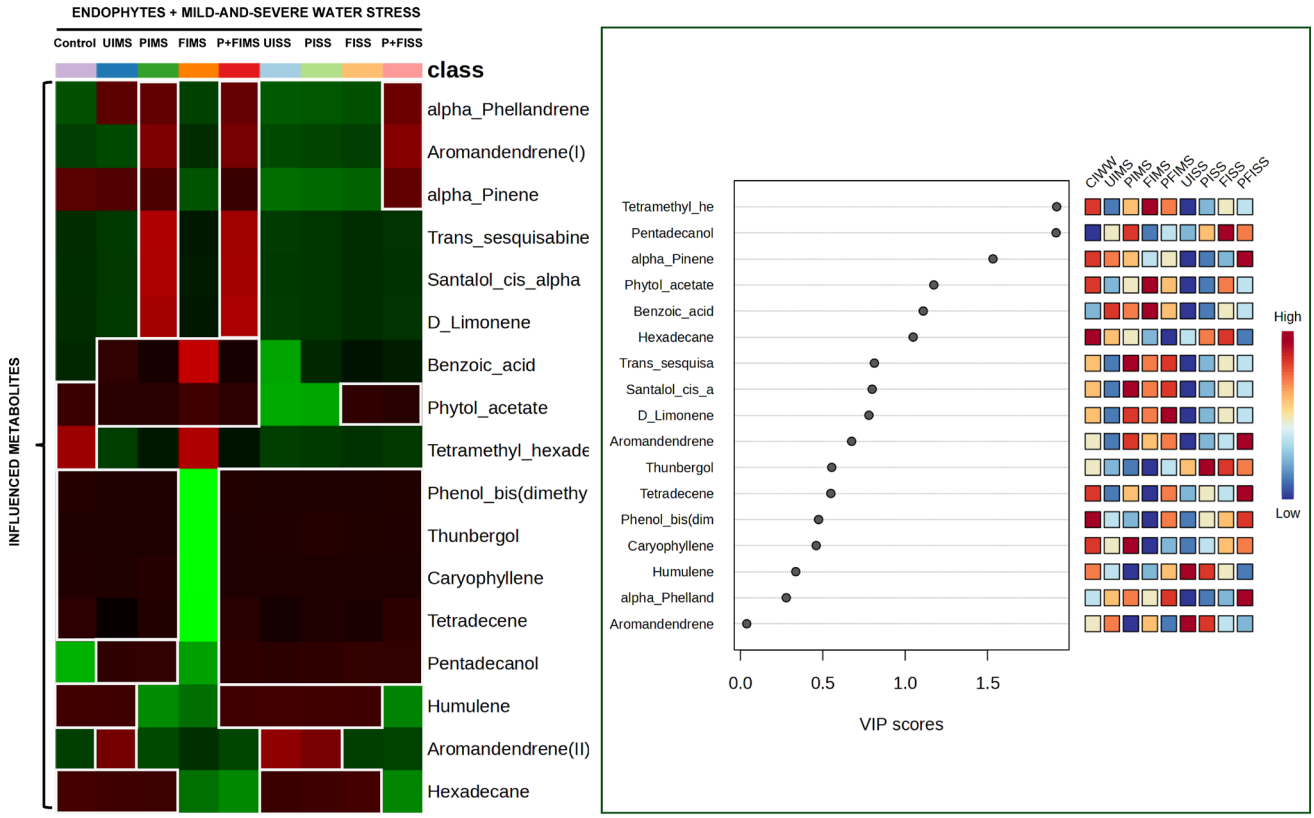


Fig. 3 Partial least squares discriminant analysis on some tentatively identified hypoglycemic compounds of endophytes inoculated *Endostemon obtusifolius* (E. Mey. ex Benth.) N. E. Br. under mild and severe water stress regimes. **A** – heatmap of some tentatively identified hypoglycemic compounds triggered by endophytes and, **B** – PLS-DA variable-importance-in-projection (VIP) features. **Abbreviations**: Control (UI), uninoculated; UIMS, uninoculated mild stress; PIMS, *Paenibacillus polymyxa* inoculated mild stress; FIMS, *Fusarium oxysporum* inoculated mild stress; P+FIMS, *Paenibacillus polymyxa* and *Fusarium oxysporum* inoculated mild stress; UISS, uninoculated severe stress; PISS, *Paenibacillus polymyxa* inoculated severe stress; FISS, *Fusarium oxysporum* inoculated severe stress and; P+FISS, *Paenibacillus polymyxa* and *Fusarium oxysporum* inoculated severe stress

acid, trans-sesquisabine hydrate, and α-pinene (Fig. 2B, C and E). In addition to significantly promoting benzoic acid, P+FIWW also increased the relative abundances of phyto-acetate. Nonetheless, PIWW was the most effective treatment at inducing phyto-acetate biosynthesis.

Our results suggest that *E. obtusifolius* synthesises secondary metabolites differently depending on water availability and endophyte inoculation. A similar outcome was reported for drought-stressed, endophyte-inoculated *Camellia sinensis*, where the flavonoid content was significantly altered (Shen et al. 2025). The production of secondary metabolites by endophytic species is well documented, but a thorough molecular understanding of how plant-endophyte interactions influence the plant metabolome under stress remains limited. Possibly, endophytes modify their host's metabolism to combat stress by quickly activating specific biochemical reactions, leading to the synthesis and build-up of certain secondary metabolites (Grabka et al. 2022).

To tentatively profile some previously identified hypoglycemic compounds found in the leaf extract of *E. obtusifolius* (Table 1), the findings from the multivariate and clustering analysis [e.g., heatmaps, PCA and PLS-DA variable importance in projection (VIP) scores] showed different profiles of terpenoids that were stimulated either by endophytic inoculation (single or co-inoculation) or different watering regimes. For example, whereas P+FIWW treatment upregulated the highest number (9) of hypoglycemic agents in *E. obtusifolius*, more than five hypoglycemic agents were increased in the UIWW and FIWW treatments. D-limonene, caryophyllene, humulene, santalol, thunbergol, α-phellandrene, and phenol, 2,4-bis(1,1-dimethylethyl) were all triggered by FIWW and P+FIWW treatments, whereas humulene, thunbergol, and phenol, 2,4-bis(1,1-dimethylethyl) were significantly downregulated in the FIWW treatment. Under mild water-deficit stress, the hypoglycaemic compounds were generally upregulated compared with WW treatments, and both PIMS and P+FIMS treatments enhanced the accumulation of 15 compounds each. Additionally, benzoic acid and phytol acetate were generally upregulated under mild water-deficit stress, with

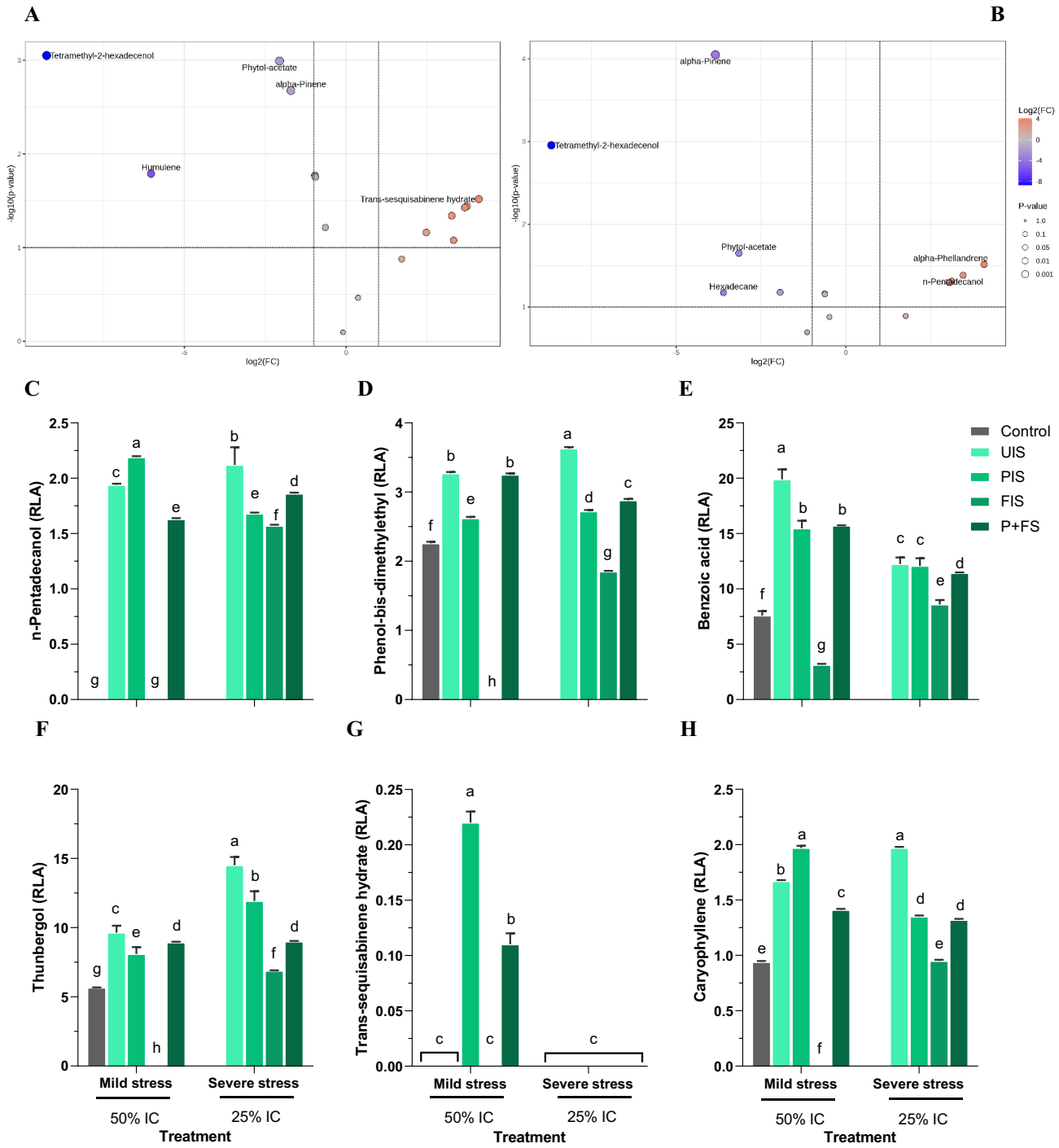


Fig. 4 Effect of endophytes inoculation on hypoglycaemic constituents of *Endostemon obtusifolius* (E. Mey. ex Benth.) N. E. Br. under mild and severe water stress regimes. **A** – mild stress volcano plot of metabolite fold change (FC) analysis and T-tests for the PIMS/control comparison direction. The FC threshold was 2.0, whereas 1.0 indicated no FC. **B** – severe stress volcano plot from metabolite FC analysis and T-tests (comparing P+FISS/control). **C** – n-pentadecanol, **D** – phenol-bis-dimethylethyl, **E** – benzoic acid, **F** – thunbergol, **G** – trans-sequisabinene hydrate and **H** – caryophyllene. For each treatment, bars (mean ± SEM) for the univariate one-way analysis of variance (ANOVA) are significantly different ($P \leq 0.05$) based on Duncan's multiple range Test (DMRT). **Abbreviations**: control (UI), uninoculated; UIMS, uninoculated mild stress; PIMS, *Paenibacillus polymyxa* inoculated mild stress; FIMS, *Fusarium oxysporum* inoculated mild stress; P+FIMS, *Paenibacillus polymyxa* and *Fusarium oxysporum* inoculated mild stress; UISS, uninoculated severe stress; PISS, *Paenibacillus polymyxa* inoculated severe stress; FISS, *Fusarium oxysporum* inoculated severe stress

effects most pronounced under the FIMS treatment. Interestingly, in the severe water-deficit treatments, the fewest and smallest amounts of bioactive compounds were recorded; however, the P+FISS treatment upregulated five hypoglycemic compounds (Fig. 3A). Under severe water-deficit stress conditions, benzoic acid remained unchanged or was downregulated regardless of endophytic inoculation. Moreover, co-inoculated *E. obtusifolius* (P+FISS) accumulated more α-phellandrene, α-pinene, and aromadendrene. Our results align with those of Caser et al. (2019), who reported an increase in the percentage composition of α-pinene in *Salvia dolomitica* as water deficit decreased, and that of García-Caparrós et al. (2019), who demonstrated that endophyte inoculation enhanced the percentage composition of α-pinene in *Mentha piperita*.

The observed chemical changes due to the applied endophytes under various water stresses are better visualised in the heatmap and the PLS-DA VIP scores (Fig. 3A and B). VIP scores greater than 1 for metabolites indicated important variables, and vice versa. Major changes in these important compounds (α-pinene, phytol acetate, benzoic acid, n-pentadecanol and 3,7,11,15-tetramethyl-2-hexadecen-1-ol) were orchestrated by endophytes inoculation, and these compounds significantly altered the chemical composition of *E. obtusifolius* (Fig. 3B). The metabolic response patterns of *E. obtusifolius* were confirmed by results from metabolomics FC analyses and t-tests, which were combined to ascertain significant feature changes in compounds with hypoglycemic properties under the different treatments (Fig. 4A and B). Compared to the control, PISS significantly increased n-pentadecanol, benzoic acid, phenol,2,4-bis(1,1-dimethylethyl), trans-sesquisabinene hydrate, thunbergol and caryophyllene (Fig. 4C-H; Table 1). Apart from trans-sesquisabinene hydrate, P+FISS also triggered other hypoglycaemic compounds, suggesting that most of the synergistic effect was derived from PISS. Evidently, UISS *E. obtusifolius* accrued similar secondary metabolite profiles compared to PISS, FISS and P+FISS treatments. This may be due to epigenetic inheritance, since the endophytic inoculants were initially isolated from a mother *E. obtusifolius*.

Drought stress and endophyte inoculation under varying watering regimes have been reported to influence the accumulation of secondary metabolites of medicinal plants (Zhang et al. 2021; Singh et al. 2021; Abdelhameed et al. 2024). Essentially, the inoculation of indigenous plants with plant growth-promoting microbes improves dry matter contents, water and mineral uptake of plants, which ultimately increases the concentration of certain secondary metabolites (Khalediyan et al. 2021). Accordingly, studies have reported contrasting data on the effects of water shortage on bioactive compound accumulation in Mint plants (García-Caparrós et al. 2019). The variation in the active principle composition of plants subjected to drought could be a consequence of the frequently reported reduction in the biomass production of drought-stressed plants (Ramezani et al. 2020). Alternatively, the increase in the biosynthesis of pharmacologically active compounds under drought stress might be due to an oversupply of $NADPH + H^+$ occasioned by stomata closure and a decline in carbon dioxide concentration in the mesophyll cells. The increase in the biosynthesis of these compounds might also be explained by the upregulation of the corresponding enzyme activity (Ramezani et al. 2020). Several studies have also reported the impacts of mild drought stress and endophyte inoculation on the accumulation of specific active principles of medicinal plants (Qian et al. 2024; Wang et al. 2025). However, the potential positive influence of these two factors on the accumulation of hypoglycemic compounds in medicinal plants has been rarely investigated. Our results suggest that moderate drought stress and appropriate endophyte inoculation led to the accumulation of more hypoglycemic agents in *E. obtusifolius*.

Conclusion

The quantity and quality of medicinal plant products are often influenced by prevailing environmental factors, including drought. This study, for the first time, evaluated the individual and co-inoculation effect of endophytic species (*P. polyxma* and *F. oxysporum*) on the accumulation of hypoglycemic bioactive principles of *E. obtusifolius*. In the absence of endophyte inoculation, water deficit stress enhanced the quantity of the hypoglycemic agents. Moreover, the co-inoculation treatments enhanced the accumulation of the hypoglycemic compounds under varying water regimes. A better understanding of the impact of endophytic species on the accumulation of valuable bioactive compounds in medicinal plants could be a game-changer towards enhancing the cultivation and efficacy of medicinal plants. In our future investigation, we will expand our research question towards gaining genomics and transcriptomics insights on the metabolome differences in medicinal plants influenced by abiotic stress and endophyte inoculation. Field trials are also encouraged to validate our findings under real-world realities.

Acknowledgements We thank the University of KwaZulu-Natal for its support.

Author contributions AAO and SG conceptualised the study. The experiments and data collection were carried out by AAO. AAO, AOG and MPV performed the data analysis. AAO wrote the first manuscript. AAO was supervised by JvS and JFF. All authors read and approved of the final manuscript.

Funding Open access funding provided by University of KwaZulu-Natal. The University of KwaZulu-Natal funded this study.

Data availability All the relevant data is included in the main manuscript. All other data will be available upon request from the corresponding author (JvS).

Declarations

Competing interests The authors declare no competing interests.

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