Nikule H *et al.* (2023)
Notulae Scientia Biologicae
Volume 15, Issue 3, Article number 11644
DOI:10.15835/nsb15311644
Research Article

Production of an important antidiabetic compound mangiferin through elicitation in *Salacia chinensis* under *in vivo* condition

Harichandra A. NIKULE[1,2], Tukaram D. NIKAM[1], Harshad A. SHIRKE[1], Vikas A. SULE[3], Archana A. NAIK[1], Mahesh Y. BORDE[1*]

[1]*Savitribai Phule Pune University, Department of Botany, Pune 411 007, Maharashtra, India; hanikule@gmail.com; tdnikam@unipune.ac.in; harshadshirke28@gmail.com; archanaleorodrigues@gmail.com; maheshmyco@gmail.com (*corresponding author)*

[2]*PG Research Center, Department of Botany, Shardabai Pawar Mahila Arts, Commerce and Science College, Baramati 413115, Maharashtra, India; hanikule@gmail.com*

[3]*Annasaheb Awate Arts, Commerce & Hutatma Babu Genu Science College Manchar, 410 503, India; vikassule328@gmail.com*

Abstract

Salacia chinensis L. (Celastraceae) is an important antidiabetic and anticancer plant. Mangiferin is a principal bioactive component of this plant and is well known for important antidiabetic and anticancer properties. The objectives of the present study were to examine the accumulation of mangiferin in *S. chinensis* grown under *in vivo* conditions, upon application of abiotic (NaCl and salicylic acid) and biotic elicitors (mycorrhiza, *Pseudomonas aeruginosa*) and fungal endophytes (*Cladosporium tenuissimum* and *Trichoderma atroviride*). The present study shows that bioactive metabolite accumulation was recorded in all tested plant parts. Significantly, mangiferin content was more elevated in treated plant parts as compared to non-treated ones. NaCl treated plant had higher production of mangiferin than other treatments. Mangiferin content was higher at 50 mM NaCl (368.8 ±5.6 µg/g DW), which is 2.08 times higher than the control (160.05± 2.5 µg/g DW). Upon foliar spray of salicylic acid (100 µM), root mangiferin content (263.80±5.14 µg/g DW) was 1.04 times higher than the control. Among the biotic elicitors, plants treated with *P. aeruginosa* produced more mangiferin than mycorrhiza-treated ones in tested plant parts. Overall, the root (368.8 5.6 µg/g DW) produced a higher quantity of mangiferin than the stem (297.91±4.05 µg/g DW) and leaves (168.36±5.25 µg/g DW) in *S. chinensis*. This is the first report on the exogenous application of endophytes *in vivo* to elicit mangiferin in different parts of *S. chinensis*. The current investigation revealed that isolated fungal endophytes can be used to produce industrially important bioactive metabolites at a large scale.

Keywords: antidiabetic; elicitors; endophytes; mangiferin; *Salacia chinensis*

Received: 04 Aug 2023. Received in revised form: 06 Sep 2023. Accepted: 00 Xxx 2023. Published online: 00 Xxx 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Salacia chinensis L. (family- Celastraceae) is a climbing shrub or small tree that grows up to 2-4 meters in height and has hooked and glabrous branches. The plant is distributed in Peninsular India, including Andaman and Nicobar Islands, West Bengal and Odisha. It also occurs in Southeast Asia, including Sri Lanka (Nandikar, 2021). According to the literature, *S. chinensis* is an essential anti-diabetic plant and numerous pharmacological properties of this plant have been reported. Traditionally, the plant species have been used to treat diseases like diabetes, liver disorders, obesity and inflammation. Pharmacological studies have revealed that *S. chinensis* could be used as an astringent, blood purifier, blood tonic, carminative, and cardio-tonic in amenorrhea and dysmenorrhea disorders (Yoshikawa *et al*., 2001; Matsuda *et al*., 2005). Different medicinal properties viz. antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, antitumor, anti-mutagenic, antihyperglycemic, immunomodulatory and gastroprotective activities have been well reported from this plant (Yoshikawa *et al*., 2001; Minh *et al*., 2010; Chavan *et al*., 2013; 2021). The plant parts possess various secondary metabolites, viz. terpenoids, flavonoids, quinones, flavonols, lignans, xanthones, proanthocyanidins, and sterols. Major bioactive compounds, mangiferin, salacinol, kotalanol, salaprinol, and ponkoranol are also present in *S. chinensis*. Primarily, the pharmacological and medicinal properties are associated with specific bioactive molecules.

Plants have a response system against environmental factors like abiotic (temperature, light, saline condition, drought) and biotic factors (viruses, fungal attack, nematodes, insects). Plants recognize these hazardous signals through their cell receptors and activate their defense responses by accumulating different types of secondary metabolites against these stresses. Stress-responsive secondary metabolites, including alkaloids, glycosides, flavonoids, tannins, volatile oils and resins, etc., can be isolated and utilized commercially (Thakur *et al*., 2019; Jan *et al*., 2021).

Mangiferin is a principal component of *S. chinensis* with important antidiabetic properties. It belongs to the C-glucosyl xanthone group and possesses anticancer (e.g., breast, lung, colon, neuronal cancer), antidiabetic, antiallergic, antimicrobial, antioxidant, immunomodulatory and hypocholesterolemia effects. In addition, it possesses anti-infection, cardiovascular and neuroprotective properties. It also blocks lipid peroxidation (Imran *et al*., 2017; Morozkina *et al*., 2021). In plant parts, higher secondary metabolite content accumulates in response to various stress conditions. Various physical, chemical and biological factors could elicit the secondary metabolite production or lead to synthesis of novel metabolites in plants (Jan *et al*., 2021; Guru *et al*., 2022). Bioprospecting of fungal endophytes is a field that is still being researched. Fungal endophytic strains are particularly appealing as they can biosynthesize novel bioactive compounds with significant industrial applications (Gakuubi *et al*., 2022). Other than promoting secondary metabolite accumulation, endophytic fungi can also promote the enhanced production of plant metabolites through elicitation in host plants (Gupta and Chaturvedi, 2019; Chen *et al*., 2021).

The literature survey revealed the scope to research the application of elicitors and isolated fungal endophytes for enhanced production of bioactive secondary metabolites in *S. chinensis*. Therefore, the present research was conducted to evaluate the potential of different elicitors and fungal endophytes in augmenting the production of bioactive metabolites in the root, stem and leaves of *S. chinensis*.

Materials and Methods

Plant material

Plantlets of *S. chinensis* were procured and collected from Ganesh Nursery, Purandar, Pune, India and conserved at the Botanical Garden at Department of Botany, Savitribai Phule Pune University, Pune, India. The herbarium was prepared and submitted to the Botanical Survey of India, Western Regional Centre, Pune,

India. The plantlets were planted in pots filled with garden soil and the experiment was conducted in the same pots. Well-maintained plants were used for the experiments as shown in Figure 1 (Treated and non-treated experimental plants of *S. chinensis*: A. Non-treated; B. treated with 50 mM NaCl; C. 100 µM SA; D. Mycorrhiza; E. *P. aeruginosa*; F. *C. tenuissimum* and G. *T. atroviride*). Different treatments were applied to the plantlets in growing pots.

Figure 1. Treated and non-treated experimental plants of *S. chinensis*: A. Non-treated; B. treated with 50 mM NaCl; C. 100 µM SA; D. Mycorrhiza; E. *P. aeruginosa*; F. *C. tenuissimum* and G. *T. atroviride*.

Elicitor preparation and application

Salt treatment

In the experiment, various treatments were given to the plantlets, with one set of plantlets treated with sterile distilled water considered as a control. In each treatment, the experimental plantlets were treated with 50 ml of different concentrations (50 mM, 100 mM and 200 mM) of NaCl. A similar treatment of 50 ml was given to each treated plantlet after the subsequent 3rd, 5th, and 7th day from the 1st day of treatment. In total, 200 mL solutions of different concentrations of NaCl (0, 50, 100 and 200 mM) were applied to each plantlet and after ten days of the treatment; plant parts, root, stem and leaves were harvested for bioactive metabolites extraction and analysis.

Mycorrhiza application

In order to induce the production of bioactive metabolites, Vesicular Arbuscular Mycorrhizae (VAM) were procured from BIOSAR, CNG Agrocare Pvt. Ltd., Kolkata, India. The mixture of spores, mycelia, infected root fragments, and sand was treated as a mycorrhizal inoculant. The soil application method was used to inoculate VAM by inoculating 15 g of inoculum per pot. Mycorrhizal inoculum was applied near the root

of the plant in the soil. The plantlet used as the control in the experiment was not given any VAM treatment. After 28 days of treatment, the plant parts were harvested for further analysis.

Bacterial cultures and treatment

Plant growth-promoting rhizobacterium (PGPR) strain *Pseudomonas aeruginosa* was isolated and cultured as described by Rikame and Borde (2022) and cultures were maintained on Pikovskayas (PVK) media and incubated at 28-30 °C. PVK media, containing 15% (v/v) glycerol, was used to maintain *P. aeruginosa* culture stocks, which were stored at -80 °C. 3 ml/L liquid culture was applied to the roots of plantlets, and plantlets not applied with the bacterial culture were used as a control. After 48 hours of treatment, plant parts like root, stem and leaves were harvested for metabolite extraction and analysis.

Salicylic acid

A working salicylic acid (SA) solution was used to prepare the desired concentration. The foliar spray method was used to apply SA with different concentrations (50, 100, 150 µM). SA was applied to individual experimental plantlets and plantlets sprayed with distilled water were considered as control. In the early morning, the foliar spraying was done manually with sufficient volume till it covered individual whole plantlets (Figure 1C). After four weeks, the plant parts were harvested to extract and analyze the targeted metabolite.

Source and application of endophytes

Endophytes were isolated from the roots of *S. chinensis* and identified using molecular characterization. The cultures were maintained on potato dextrose agar (PDA) medium. Two fungal isolates (*Cladosporium tenuissimum*, *Trichoderma atroviride*) of *S. chinensis* were selected and applied for higher production of mangiferin. The fungal cultures were cultured on potato dextrose broth (PDB) before use. 3 ml of 14-day-old cultures of each species were applied to the root of the experimental plant and after 28 days, plant parts were harvested.

Preparation of extract

Plant parts (root, stem and leaves) were harvested and separated using scissors. Plant parts were washed using tap water, gently cleaned using sterile distilled water, and after drying with blotting paper, were kept in a hot air oven for 72 hrs at 40 °C for drying. Dried plant material was crushed into a powder using mortar and pestle. 1 gram of fine powder of each part (root, stem and leaves) was accurately weighed and extracted in 100 ml methanol using an ultrasonic bath at 45 °C for 48 min. All the samples were filtered using Whatman filter paper (No. 1). The methanolic extracts were evaporated to dryness under a nitrogen flow in a rotary evaporator. The residue was reconstituted in MS grade methanol. Finally, the samples were filtered through a 0.22 µm filter before being used for LC-MS analysis, and the remaining sample was stored at -20 °C.

Quantification of mangiferin using LC-MS/MS

From the samples, mangiferin was quantified using LC-MS/MS-8045 (Shimadzu) instrument. In the experiment, a shim-pack velox, C18 column (1.8 µm pore size, 2.1×50 mm diameter) was used. The gradient flow of the mobile phase (A: 0.1% Formic Acid in Water, B: 100% Acetonitrile) was applied with initial 0-2 min A 90% and B 10%, for 3-4 min 15% A and 85% B and last gradient applied was 90% A and 10% B upto 5 min with the flow rate 0.3 ml/min. 10 µL volume was injected, and the column temperature was maintained at 40 °C. The mass spectrometer was run with parameters: ESI (-ve) mode for the ionization with nebulizing gas flow rate at 3L/min, 10 L/min rate of heating gas flow and 10 L/min drying gas flow. 300 °C interface temperature was maintained with a desolvation temperature of 526 °C and heat block temperature of 400 °C. After the chromatographic separation, mangiferin specificity was determined by comparing the retention time

(Rt) and molecular ions (m/z) of the external reference standard compound. The mangiferin quantity was quantified from the linear regression equation plotted on a standard calibration curve.

Experimental design and data analysis

All the experiments were planned in a Completely Randomized Design (CRD) triplicate. Non-treated plants were considered as a control in the experiment. Data was produced in three replications, and the significance of data was tested by the analysis of variance ANOVA with SPSS (16.0) software. Mangiferin was identified and quantified in different parts (root, stem, leaves) by an external standard. The different means of treatments were compared with the control and represented by DMRT (Duncan's Multiple Range Test) at probability level ($p < 0.05$). Data variability has been expressed as mean ± standard error from the experiment.

Results

Effect of elicitors on mangiferin production

In the present investigation, different elicitors, including isolated fungal endophytes, were tested for the enhanced accumulation of mangiferin in the root, stem and leaves of *S. chinensis*. LC-MS analyzed single ion monitoring (SIM) chromatogram of mangiferin shows that, mangiferin was detected among tested samples (Figure 2). The effect of different elicitors and endophytes on the accumulation of mangiferin is summarized in Table 1. The present study shows that NaCl treatment (50 mM) exhibited higher mangiferin content accumulation than other treatments of elicitors, including fungal endophytes.

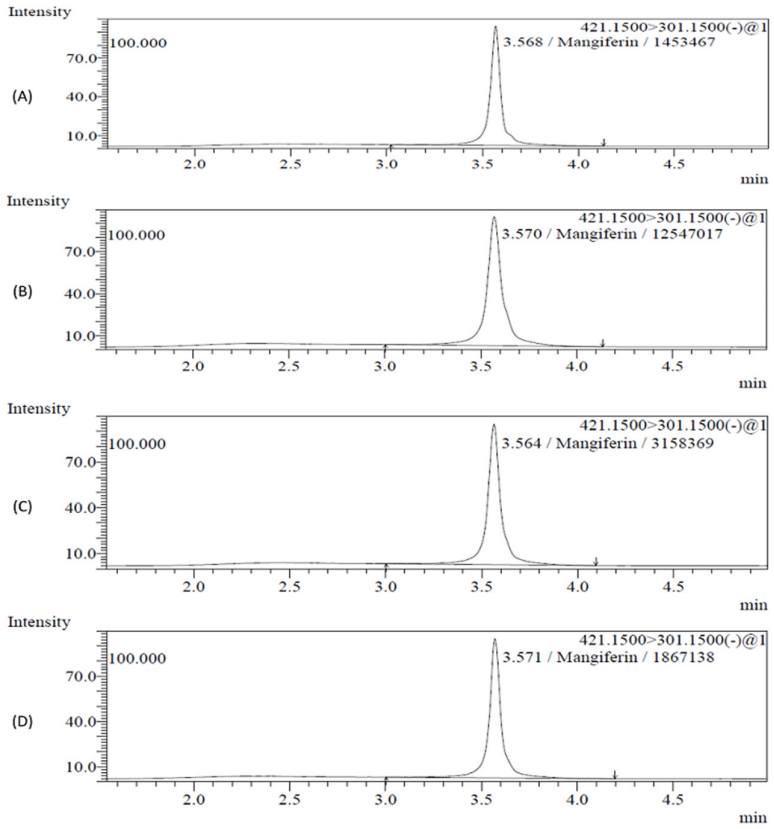

Figure 2. LC-MS single ion monitoring (SIM) chromatogram of mangiferin: (A) Reference standard; (B) 50 mM NaCl treated root; (C) 50 mM NaCl treated stem and (D) 50 mM NaCl treated leaves

Effect of abiotic elicitors

It was observed that salinity influenced the accumulation of mangiferin in all the tested extracts (Figure 3). It was found that 50 mM NaCl produces higher mangiferin content (368.8±5.6 µg/g DW) in root extract, which is 2.08 times higher than the control (160.05±2.57 µg/g DW). In the stem, the highest mangiferin content (297.9±4.0 µg/g DW) was observed upon treatment with 50 mM NaCl. It was observed that the content decreased with increasing salinity concentration in the root and stem. At the same time, the higher mangiferin accumulation (118.1±4.5 µg/g DW) was recorded when the plants were treated with 100 mM concentration of NaCl in leaves. Among the three plant parts, viz. root, stem and leaves, maximum mangiferin was accumulated in the root, followed by the stem and leaves. The mangiferin accumulation was higher in abiotic elicitor treatment than in biotic elicitors.

Table 1. Effect of elicitors and fungal endophytes on the production of mangiferin in the root, stem and leaves of *S. chinensis*

Type of treatment		Conc.	Mangiferin content (µg/g DW)		
			Roots	Stem	Leaves
Control	Without treatment	0.0	160.05±2.57^{h}	121.65±5.49^{f}	73.82±3.42^{d}
Elicitor (Abiotic)	NaCl	50 mM	368.82±5.68^{a}	297.91±4.05^{a}	118.13±4.53^{bc}
		100 mM	341.98±4.70^{b}	253.62±6.47^{b}	168.36±5.25^{a}
		150 mM	247.62±5.54^{d}	212.25±5.08^{c}	106.04±5.37^{c}
	Salicylic acid	50 µM	172.81±5.91^{gh}	150.04±4.91^{e}	76.69±5.79^{d}
		100 µM	263.80±5.14^{c}	219.87±5.09^{c}	127.46±6.71^{b}
		150 µM	182.77±1.05^{fg}	151.51±5.18^{e}	84.08±6.14^{d}
Elicitor (Biotic)	Mycorrhiza	15 g/ pot	188.21±3.59^{f}	149.56±4.25^{e}	127.62±6.74^{d}
	Pseudomonas aeruginosa	3.0 ml/L	192.80±4.50^{f}	168.48±5.34^{d}	129.46±5.56^{b}
Fungal Endophytes	*C. tenuissimum*	3.0 ml/L	188.21±3.59^{f}	149.56±4.25^{e}	118.13±4.53^{bc}
	T. atroviride	3.0 ml/L	227.62±3.42^{e}	168.48±5.34^{d}	129.46±5.56^{b}

The values represent means of triplicates with ± standard error (SE). Average mean values in the same column with different alphabet/s are statistically different from each other at $p < 0.05$ significant differences according to DMRT.

In the current study, salicylic acid (SA) was applied through the foliar spray. SA treated plants showed variation in the accumulation of mangiferin. Among the SA treatments, maximum mangiferin content (263.80±5.14 µg/g DW) which was 1.04 times higher than the control (160.05±2.57 µg/g DW) was observed when plants were treated with 100 µM SA in the root.

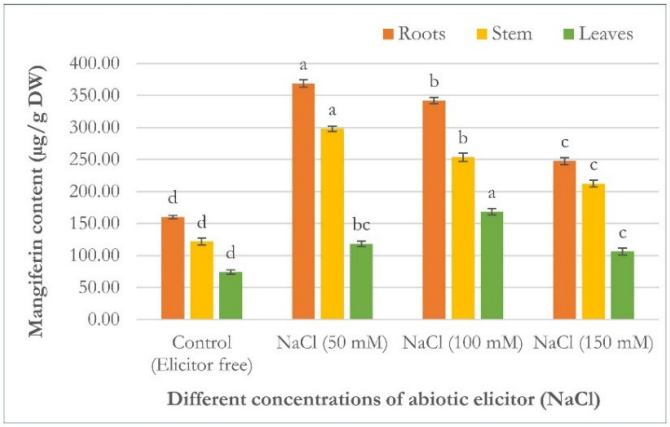

Figure 3. Effect of abiotic elicitor (NaCl) on the production of mangiferin in the root, stem and leaves of *S. chinensis*.

At the two lower concentrations (50 & 100 µM), mangiferin content increased with increasing concentration of SA. At the higher concentration of SA (150 µM), mangiferin content was decreased (Figure 4). However, the SA treated plants accumulated higher mangiferin than the control. Maximum content was observed in roots viz. 263.80±5.14 µg/g DW (1.04 times higher) followed by the stem 219.87±5.09 µg/g DW (0.98 times higher) and leaves 127.46±6.71 µg/g DW (0.54 times higher); which were higher than that of control (73.82±3.42 µg/g DW). The content was organ-specific, and a similar ratio was found in accumulation concerning different parts. The result showed that SA addition influences the accumulation of mangiferin content in the root, stem and leaves of *S. chinensis*.

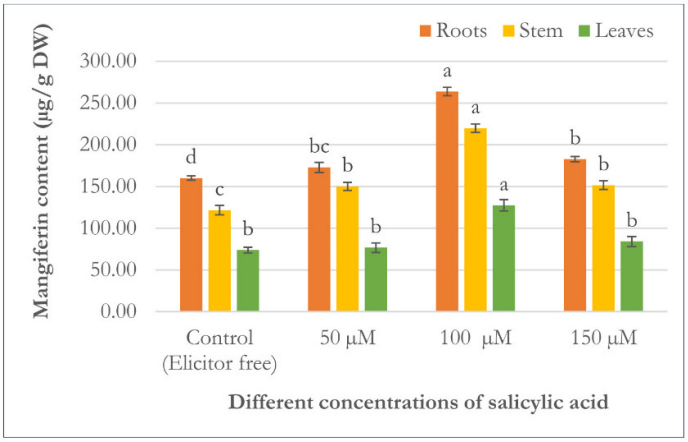

Figure 4. Effect of salicylic acid on mangiferin production in root, stem and leaves of *S. chinensis*

Effect of biotic elicitors

Biotic elicitors, mycorrhiza and *P. aeruginosa* were tested for the accumulation of mangiferin. Among the biotic elicitors, *P. aeruginosa* treatment produced higher mangiferin content than mycorrhiza in all three plant parts viz. root, stem and leaves (Figure 5). The root produces higher mangiferin than the stem and leaves (Table 1). During elicitation, *P. aeruginosa* treatment produced mangiferin in the root with (192.8±4.5 µg/g DW) 0.33 times more, in the stem (168.48±5.34 µg/g DW) 0.47 times more and in the leaves (129.46±5.56 µg/g DW) 0.56 times more than that of produced in control of each (Table 1). The content produced during biotic elicitation is not significantly different; however, compared with control, biotic elicitors produce significant amounts of mangiferin in root, stem and leaves.

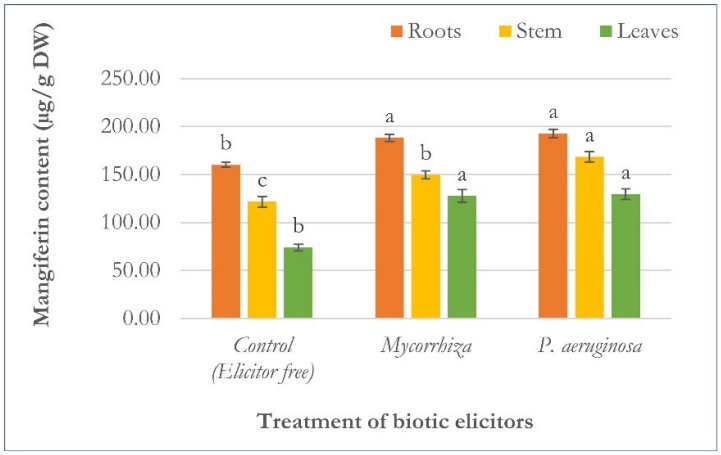

Figure 5. Effect of biotic elicitors on mangiferin production in root, stem and leaves of *S. chinensis*

Effect of endophytes on mangiferin production

Two endophytes isolated from the roots of *S. chinensis* viz. *Cladosporium tenuissimum and Trichoderma atroviride* were applied to the experimental plants to test the enhanced production of mangiferin in *S. chinensis*. The variation was observed in the mangiferin content among different plant parts (Table 1). Exogenous endophyte application influenced contents in the root, stem and leaves of *S. chinensis*. The higher content was found upon the treatment of *T. atroviride* in root (227.6±3.4 µg/g DW) followed by stem (168.4±5.3 µg/g DW) and leaves (129.4±5.5 µg/g DW). The root produces maximum mangiferin content (0.68 times more) followed by leaves (0.56 times more) and stem (0.47 times more) than control of each plant part (Figure 6). The plant treated with *C. tenuissimum* also produced considerable amounts of mangiferin than that of control.

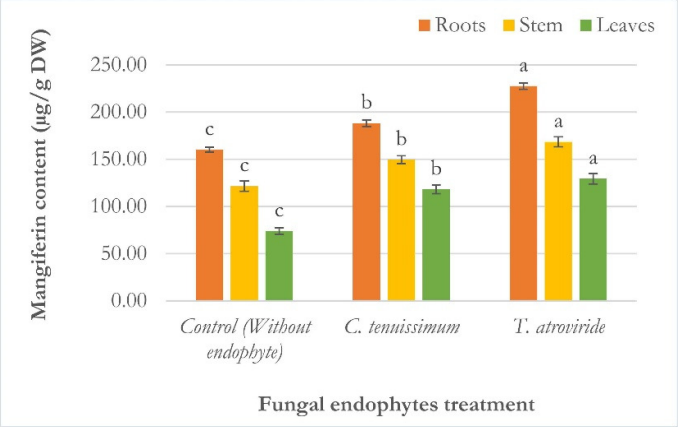

Figure 6. Effect of exogenous application of endophytes on the production of mangiferin in the root, stem and leaves of *S. chinensis*

Discussion

In the present study, bioactive metabolite mangiferin was evaluated for augmented production in root, stem and leaves of *S. chinensis*. Mangiferin is a principal bioactive component of *S. chinensis* and is well known for important pharmacological properties like antidiabetic and anticancer properties. The present experiment showed that tested plant parts viz. root, stem and leaves produced an elevated quantity of mangiferin and it was significantly more than control; when they were treated with elicitors and fungal endophytes.

Elicited production and accumulation of the mangiferin is determined in different parts of the plant. The quantitative analysis showed that all the treatments influence the mangiferin content. However, the variation was observed in the contents according to the elicitor treatment (Figures 3-6). Elicitor-mediated production of bioactive metabolite production has been reported by several researchers (Ahire *et al*., 2014; Chavan *et al*., 2021; Otari *et al*., 2023). The present results revealed that a lower NaCl concentration (50 mM) supported elevated mangiferin content in root, stem and leaves. The current findings correspond to earlier studies, which show that bioactive metabolites could be produced by treating with lower NaCl concentrations. Many compounds have higher accumulation at lower concentrations of NaCl, including reserpine in *Rauvolfia tetraphylla* (Anitha and Kumari, 2006), alpha-tocopherol and anthocyanins in *Ipomoea aquatica* (Kitayama *et al*., 2019), athujene and b-myrcene in *Trachyspermum ammi* (Niazian et al., 2021) and hevcogenin glycoside in *Agave salmiana* (Puente-Garza *et al*., 2021).

The application of mycorrhiza and *P. aeruginosa* influences the mangiferin content in all three tested parts. It was observed that the content accumulation was tissue-specific. Though the content produced by both

the elicitors (mycorrhiza and *P. aeruginosa*) was not significantly different, the content accumulated was higher than that of the control. This is the first report on the effect of the application of mycorrhiza on the production of mangiferin in *S. chinensis*. *P. aeruginosa* also induces a higher accumulation of this active component. Generally, it is suggested that *P. aeruginosa* triggered the stress conditions in the plant (Bedoya *et al*., 2010). This condition might lead to enhanced production of mangiferin in *S. chinensis*. Mycorrhiza-induced higher accumulation was observed in the root, followed by the stem and leaves. The results showed that mycorrhiza can induce a higher accumulation of secondary metabolites. Similarly, mycorrhiza-mediated secondary metabolites accumulation like phenolic acids, flavonoids in *Viola tricolor* (Zubek *et al*., 2015) and phenolic acids in *Salvia miltiorrhiza* have been reported (Wu *et al*., 2021).

SA upregulates the expression of defense-related genes, through which secondary metabolite quantity can be enhanced (Obinata *et al*., 2003; Rai *et al*., 2016). In this study, exogenous salicylic acid treatment benefited the higher accumulation of mangiferin content. 100 µM concentration of SA resulted in elevated production of mangiferin in tested samples. It is clearly seen that SA application influences the yield of active components. A similar effect of SA was reported to produce secoiridoid and xanthone glycoside in *Swertia paniculata* (Kaur *et al*., 2020), total phenolics and flavonoids in callus cultures of *Givotia moluccana* (Woch *et al*., 2023) and polyphenols in callus of *Theobroma cacao* (Rosabal *et al*., 2022). In contrast, it was reported that the Jasmonic Acid-treated callus produces elevated mangiferin content in the callus culture of *S. chinensis* (Chavan *et al*., 2021).

Fungal genetic factors are involved in stress-related responses, particularly against oxidative stress. During this, the response gets activated by regulating stress-responsive metabolites and, subsequently, higher accumulation of secondary metabolites (Pusztahelyi *et al*., 2015). Hence, endophytes are becoming important sources of bioactive molecules with diversity in chemical skeletons. According to earlier studies, the genus *Cladosporium* is a well-known source of metabolomic diversity that contains novel and beneficial bioactive compounds (Salvatore *et al*., 2021). Genus *Trichoderma* is also a prolific producer of secondary metabolites as it has plant-protecting abilities and unique abilities to synthesize a variety of secondary metabolites with important medical properties (Zhang *et al*., 2021). Therefore, in this study, isolated endophytic fungus viz. *C. tenuissimum* and *T. atroviride* from the root of *S. chinensis* were applied for the elevated production of mangiferin. The mangiferin accumulation was observed in all the treatments, and was higher than the control. Among three parts, maximum content was recorded in roots treated with *T. atroviride*. The content was found to be higher when the plants were treated with *T. atroviride* in root (227.6±3.4 µg/g DW), stem (168.4±5.3 µg/g DW) and leaves (129.4±5.5 µg/g DW) compared to plants treated with *C. tenuissimum* in *S. chinensis*. Though the selected endophytes in present study have not been used as an elicitor in the previous studies, various other fungal species have been used for the higher production of secondary metabolites. *Aspergillus sp*. was reported as the most effective fungus involved in the promotive accumulation of bioactive components viz. chrysophaein, resveratrol, chrysophanol, emodin and physcion in *Rumex gmelina* (Ding *et al*., 2018). Our results agree with the previous research in which *Alternaria alternata* were used for elevated production of vincristine and vinblastine in *Catharanthus roseus* (Paul *et al*., 2022).

Conclusions

The experimental data of the present investigation revealed that all the elicitors and endophytes used in the experiment showed a promotive effect on enhanced production of mangiferin in *S. chinensis*. In elicited production, the maximum content of metabolites was observed in the roots of the plant, followed by the stem and leaves. It was observed that different elicitors influence the metabolite content. Higher production of metabolites was observed in the treatment of NaCl as an elicitor. The addition of endophytes in *S. chinensis*

also influenced metabolite content. Mycorrhizal inoculum also has the ability to enhance bioactive secondary metabolites. In addition, isolated endophytes from the roots of *S. chinensis* were also tested for their elicitor activity. Therefore, the elicitors tested in the current study can be used to boost the defense responses in relation to secondary metabolite production in other plant species in the future. The developed protocol could be used to produce mangiferin at an industrial scale throughout the year.

Authors' Contributions

MYB and TDN put forth the initial work idea and HAN, HAS, VAS, AAN performed the laboratory experiments like treatment of elicitors, endophytes and data analysis. HAN, MYB and AAN prepared the first draft of the manuscript, and the corrections were made and finalized by MYB and TDN and approved the final manuscript. All authors read and approved the manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

All the authors gratefully acknowledge Department of Botany, Savitribai Phule Pune University, Pune, for providing the necessary facilities for the research and for permission to avail the Botanical Garden to conduct the experiments. Authors acknowledge Agricultural Development Trust, Baramati, India for availing LC-MS facility. TDN is grateful to the University Grants Commission (UGC), Government of India, India for the Basic Scientific Research (BSR) faculty fellowship.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ahire ML, Laxmi S, Walunj PR, Kavi Kishor PB, Nikam TD (2014). Effect of potassium chloride and calcium chloride induces stress on *in vitro* cultures of *Bacopa monnieri* (L.) Pennell and accumulation of medicinally important bacoside A. Journal of Plant Biochemistry and Biotechnology 23:366-378. *https://doi.org/10.1007/s13562-013-0220-z*

Anitha S, Kumari BR (2006). Reserpine accumulation in NaCl treated calli of *Rauvolfia tetraphylla* L. Science Asia 32:417-419. *https://doi.org/10.2306/scienceasia1513-1874.2006.32.417*

Bedoya JC, Dealis ML, Silva CS, Niekawa ET, Navarro MO, Simionato AS, Andrade G (2019). Enhanced production of target bioactive metabolites produced by *Pseudomonas aeruginosa* LV strain. Biocatalysis and Agricultural Biotechnology 17:545-556. *https://doi.org/10.1016/j.bcab.2018.12.024*

Chavan JJ, Jagtap UB, Gaikwad NB, Dixit GB, Bapat VA (2013). Total phenolics, flavonoids and antioxidant activity of Saptarangi (*Salacia chinensis* L.) fruit pulp. Journal of Plant Biochemistry and Biotechnology 22:409-413. *https://doi.org/10.1007/s13562-012-0169-3*

Chavan JJ, Kshirsagar PR, Jadhav SG, Nalavade VM, Gurme ST, Pai SR (2021). Elicitor-mediated enhancement of biomass, polyphenols, mangiferin production and antioxidant activities in callus cultures of *Salacia chinensis* L. 3 Biotech 11:285. *https://doi.org/10.1007/s13205-021-02836-2*

Chen H, Qi Y, He X, Xu L, Zhang W, Lv X, Liang Z (2021). Endophytic fungus *Mucor circinelloides* DF20 promote tanshinone biosynthesis and accumulation in *Salvia miltiorrhiza* root. Plant Science 307:110898. *https://doi.org/10.1016/j.plantsci.2021.110898*

Ding CH, Wang QB, Guo S, Wang ZY (2018). The improvement of bioactive secondary metabolites accumulation in *Rumex gmelini* Turcz through co-culture with endophytic fungi. Brazilian Journal of Microbiology 49:362-369. *https://doi.org/10.1016/j.bjm.2017.04.013*

Gakuubi MM, Ching KC, Munusamy M, Wibowo M, Liang ZX, Kanagasundaram Y, Ng SB (2022). Enhancing the discovery of bioactive secondary metabolites from fungal endophytes using chemical elicitation and variation of fermentation media. Frontiers in Microbiology 13:898976. *https://doi.org/10.3389/fmicb.2022.898976*

Gupta S, Chaturvedi P (2019). Enhancing secondary metabolite production in medicinal plants using endophytic elicitors: a case study of *Centella asiatica* (Apiaceae) and asiaticoside. Endophytes for a growing world pp 310-323.

Guru A, Dwivedi P, Kaur P, Pandey DK (2022). Exploring the role of elicitors in enhancing medicinal values of plants under *in vitro* condition. South African Journal of Botany 149:1029-1043. *https://doi.org/10.1016/j.sajb.2021.10.014*

Imran M, Arshad MS, Butt MS, Kwon JH, Arshad MU, Sultan MT (2017). Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. Lipids in health and disease 16:1-7. *https://doi.org/10.1186/s12944-017-0449-y*

Jan R, Asaf S, Numan M, Kim KM (2021). Plant Secondary Metabolite Biosynthesis and Transcriptional Regulation in Response to Biotic and Abiotic Stress Conditions. Agronomy 11:968. *https://doi.org/10.3390/agronomy11050968*

Kaur P, Gupta RC, Dey A, Malik T, Pandey DK (2020). Optimization of salicylic acid and chitosan treatment for bitter secoiridoid and xanthone glycosides production in shoot cultures of *Swertia paniculata* using response surface methodology and artificial neural network. BMC Plant Biology 20:225. *https://doi.org/10.1186/s12870-020-02410-7*

Kitayama M, Tisarum R, Theerawitaya C, Samphumphung T, Takagaki M, Kirdmanee C, Cha-um S (2019). Regulation on anthocyanins, a-tocopherol, and calcium in two water spinach (*Ipomoea aquatica*) cultivars by NaCl salt elicitor. Scientia Horticulturae 249:390-400. *https://doi.org/10.1016/j.scienta.2019.02.021*

Matsuda H, Yoshikawa M, Morikawa T (2005). Antidiabetogenic constituents from *Salacia* species. Journal of Traditional Medicines 22:145-153.

Minh TT, Hoang Anh NT, Thang VD, Van Sung T (2010). Study on chemical constituents and cytotoxic activities of *Salacia chinensis* growing in Vietnam. Zeitschrift für Naturforschung B 65:1284-1288. *https://doi.org/10.1515/znb-2010-1017*

Morozkina SN, Nhung Vu TH, Generalova YE, Snetkov PP, Uspenskaya MV (2021). Mangiferin as new potential anti-cancer agent and mangiferin-integrated polymer systems - A novel research direction. Biomolecules 11:79. *https://doi.org/10.3390/biom11010079*

Nandikar MD (2021). The genus *Salacia* (Celastraceae: Salaciodeae) in peninsular India. Web-bia. Journal of Plant Taxonomy and Geography 76:167-193. *https://doi.org/10.36253/jopt-10390*

Niazian M, Howyzeh MS, Sadat-Noori SA (2021). Integrative effects of stress-and stress tolerance-inducing elicitors on *in vitro* bioactive compounds of ajowan [*Trachyspermum ammi* (L.) Sprague] medicinal plant. Plant Cell Tissue and Organ Culture 146:589-604 *https://doi.org/10.1007/s11240-021-02096-1*

Obinata N, Yamakawa T, Takamiya M, Tanaka N, Ishimaru K, Kodama T (2003). Effects of salicylic acid on the production of procyanidin and anthocyanin in cultured grape cells. Plant Biotechnology 20:105-111. *https://doi.org/10.5511/plantbiotechnology.20.105*

Otari SS, Devkar SP, Patel SB, Ghane SG (2023). Micropropagation and elicited production of triterpenoid saponin glycosides and stigmasterol via precursor and elicitor feeding in *Bacopa floribunda* (R. Br.) Wettst.-A potential nootropic herb. Frontiers in Plant Science 14:1096842. *https://doi.org/10.3389/fpls.2023.1096842*

Paul A, Shamim N, Acharya K, Chakraborty N (2022). Twin effects of fungal elicitor produced from *Alternaria alternata* on induced systemic resistance and secondary metabolites production in *Catharanthus roseus* (L.) G. Don. Plant Stress 6:100124. *https://doi.org/10.1016/j.stress.2022.100124*

Puente-Garza CA, Espinosa-Leal CA, Garcı ′a-Lara S (2021) Effects of saline elicitors on saponin production in *Agave salmiana* plants grown *in vitro*. Plant Physiology and Biochemistry 162:476-482. *https://doi.org/10.1016/j.plaphy.2021.03.017*

Pusztahelyi T, Holb IJ, Pocsi I (2015). Secondary metabolites in fungus-plant interactions. Frontiers in plant science 6:573. *https://doi.org/10.3389/fpls.2015.00573*

Rai A, Nakamura M, Takahashi H, Suzuki H, Saito K, Yamazaki M (2016). High throughput sequencing and de novo transcriptome assembly of *Swertia japonica* to identify genes involved in the biosynthesis of therapeutic metabolites. Plant Cell Reports 35:2091-2111. *https://doi.org/10.1007/s00299-016-2021-z*

Rikame T, Borde M (2022). Whole genome, functional annotation and comparative genomics of plant growth-promoting bacteria *Pseudomonas aeruginosa* (NG61) with potential application in agro-industry. Current Microbiology 79:169. *https://doi.org/10.1007/s00284-022-02845-1*

Rosabal LF, Puebla YG, Perez SR, Cordovi UM, Pupo JJ, Werbrouck SP (2022). Effects of salicylic acid on the production of polyphenols and the reducing power of *Theobroma cacao* calli. Current Research in Biotechnology 4:47-57. *https://doi.org/10.1016/j.crbiot.2021.12.005*

Salvatore MM, Andolfi A, Nicoletti R (2021). The genus *Cladosporium*: A rich source of diverse and bioactive natural compounds. Molecules 26:3959. *https://doi.org/10.3390/molecules26133959*

Thakur M, Bhattacharya S, Khosla PK, Puri S (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation. Journal of Applied Research on Medicinal and Aromatic Plants 12:1-12. *https://doi.org/10.1016/j.jarmap.2018.11.004*

Woch N, Laha S, Gudipalli P (2023). Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem. In Vitro Cellular & Developmental Biology-Plant 2:1-22. *https://doi.org/10.1007/s11627-023-10335-7*

Wu YH, Wang H, Liu M, Li B, Chen X, Ma YT, Yan ZY (2021). Effects of native arbuscular mycorrhizae isolated on root biomass and secondary metabolites of *Salvia miltiorrhiza* Bge. Frontiers in Plant Science 12:617892. *https://doi.org/10.3389/fpls.2021.617892*

Yoshikawa M, Nishida N, Shimoda H, Takada M, Kawahara Y, Matsuda H (2001). Polyphenol constituents from *Salacia* species: quantitative analysis of mangiferin with alpha-glucosidase and aldose reductase inhibitory activities. Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan 121(5):371-378. *https://doi.org/10.1248/yakushi.121.371*

Zhang JL, Tang WL, Huang QR, Li YZ, Wei ML, Jiang LL, Zhang XX (2021). *Trichoderma*: a treasure house of structurally diverse secondary metabolites with medicinal importance. Frontiers in microbiology 12:723828. *https://doi.org/10.3389/fmicb.2021.723828*

Zubek S, Rola K, Szewczyk A, Majewska ML, Turnau K (2015). Enhanced concentrations of elements and secondary metabolites in *Viola tricolor* L. induced by arbuscular mycorrhizal fungi. Plant and Soil 390:129-142. *https://doi.org/10.1007/s11104-015-2388-6*

Notes: