TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2013) 37: 153-159
© TÜBİTAK
doi:10.3906/bot-1202-1

Stimulation of the production of hypericins in in vitro seedlings of *Hypericum adenotrichum* by some biotic elicitors

Ömer YAMANER[1], Bengi ERDAĞ[1,*], Cengiz GÖKBULUT[2]

[1]Department of Biology, Faculty of Arts & Sciences, Adnan Menderes University, 09010 Aydın, Turkey

[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Adnan Menderes University, Işıklı Köyü, 09100 Aydın, Turkey

Received: 28.02.2012 • **Accepted:** 23.07.2012 • **Published Online:** 26.12.2012 • **Printed:** 22.01.2013

Abstract: The goal of this work was to study the stimulating effects of mannan and pectin on the biosynthesis of hypericins in in vitro seedlings of *Hypericum adenotrichum* Spach. Seedlings of *H. adenotrichum* were grown on a modified MS medium containing mannan (10, 50, and 100 mg/L) or pectin (10, 50, and 100 mg/L) for 15 and 30 days. Seedlings of *H. adenotrichum* were extracted with methanol. The methanol extracts of the seedlings were analysed by HPLC to investigate the changes in hypericin and pseudohypericin levels. The best results were obtained by exposure to 50 mg/L mannan or pectin for 15 days. Mannan stimulated pseudohypericin production up to 2.8-fold and hypericin production up to 1.7-fold. Pectin stimulated pseudohypericin production up to 4.8-fold and hypericin production up to 2.7-fold. The increases in hypericins in response to mannan and pectin demonstrate that these elicitors can be evaluated for production of secondary metabolites in *Hypericum* species.

Key words: *Hypericum adenotrichum*, elicitor, hypericins, mannan, pectin, in vitro

1. Introduction

The genus *Hypericum* L. belongs to the family *Guttiferae* and includes about 450 species (Robson, 2003). *Hypericum* species have been used as medicinal plants for centuries and have an important place among medicinal plants. The economic importance of the genus *Hypericum* resides largely in a wide range of medicinal effects exhibited by *Hypericum perforatum* L. such as anti-inflammatory, antiviral, antimicrobial, antifungal, cytotoxic, and antidepressant activity (Pilepić et al., 2010). *Hypericum* species contain a number of biologically active compounds such as hypericin, pseudohypericin, hyperforin, adhyperforin, xanthones, flavonoids, biflavonoids, tannins, and phenolic acids (Greeson et al., 2001). Among them, hypericins (hypericin and pseudohypericin) belong to a group of compounds known as naphthodianthrones (Hölzl & Petersen, 2003). Hypericin and pseudohypericin, naturally occurring red pigments, have been very intensively studied (Kitanov, 2001). Hypericins have antidepressive, antimicrobial, antiviral, antitumor, and anti-inflammatory properties (Karioti & Bilia, 2010). *H. perforatum* is the most important and commercially recognised representative species among *Hypericum* species (Karioti & Bilia, 2010). *H. perforatum* was among the top 10 best-selling herbal dietary supplements sold in the food, drug, and mass market channel in the United States for 2008, with sales of about $8.2 million (Cavaliere et al., 2009). Anti-depressant applications of *H. perforatum* medicinal products (e.g., Psychotonin®, Neuroplant®, and Hyperforat®) have become increasingly popular in Europe, particularly in Germany (Gioti et al., 2005).

Hypericum adenotrichum Spach is a perennial, herbaceous medicinal plant that grows in Turkey. This plant has great potential value as a pharmaceutical because it contains hypericin, pseudohypericin, chlorogenic acid, hyperoside, quercetin, quercitrin, rutin, amentoflavone, apigenin-7-*O*-glucoside, and kaempferol (Çırak et al., 2009). Additionally, it has been shown that *H. adenotrichum* has a potent p53-independent antineoplastic property (Özmen et al., 2009). *H. adenotrichum* can be used for studies related to the genus *Hypericum* in view of the above information.

The production, consumption, and international trade in medicinal plants and phytomedicine (herbal medicine) have grown and are expected to grow further in the future. To satisfy growing market demands, surveys are being conducted to unearth new plant sources of herbal remedies and medicines and at the same time develop new strategies for better yield and quality (Gokhale & Bansal, 2010). Plant tissue culture has an important role in the production

* Correspondence: berdag@adu.edu.tr

of plant material for secondary metabolite studies, germplasm conservation, and genetic improvement studies in medicinally important species (Hasançebi et al., 2011; Verma et al., 2011). The deliberate stimulation of defined chemical products within carefully regulated in vitro cultures provides an excellent form for in-depth investigation of biochemical and metabolic pathways, under highly controlled micro-environmental regimes (Karuppusamy, 2009). Elicitors are signal molecules that activate the signal transduction network and lead to activation or de novo biosynthesis of transcription factors, which regulate the expression of biosynthetic genes involved in plant secondary metabolism (Zhao et al., 2005). Elicitors are useful tools for improving the production of valuable compounds as enhancers of plant secondary metabolism (Vasconsuelo & Boland, 2007). In vitro culture systems of *H. perforatum* have been tested for their ability to produce naphthodianthrones upon treatment with sucrose, polyethylene glycol, mannan, β-1,3-glucan, pectin, yeast extract, jasmonic acid, methyl jasmonate, salicylic acid, and fungal elicitors from *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Phytophthora cinnamomi* Rands (Kirakosyan et al., 2000; Sirvent & Gibson, 2002; Gadzovska et al., 2007; Pavlik et al., 2007).

Elicitors are classified as biotic or abiotic depending on their origin. The biotic elicitors have biological origin, derived from the pathogen or from the plant itself (Vasconsuelo & Boland, 2007). Pectin is a biotic and composition-defined elicitor (Radman et al., 2003; Vasconsuelo & Boland, 2007) and is a family of complex polysaccharides present in all plant primary cell walls (Ridley et al., 2001). Mannan is an important structural component of the yeast cell wall (Jones & Ballou, 1969) and a biotic elicitor (Radman et al., 2003). Oligosaccharides derived from fungal and plant cell wall polysaccharides are well-defined elicitors that, in some cases, can induce defence responses at a very low concentration (Shibuya & Minami, 2001). Polysaccharides or oligosaccharides are the most studied signal molecules in elicitation pathways and these compounds can substitute for fungal elicitors during a pathogen attack (Zhao et al., 2005).

The aim of the present paper was to study the stimulating effects of pectin and mannan on production of hypericins (hypericin and pseudohypericin) in *H. adenotrichum* under in vitro conditions.

2. Materials and methods

2.1. Chemicals

Hypericin and pseudohypericin were obtained from Carl-Roth. Mannan (from *Saccharomyces cerevisiae*) and pectin (from *Citrus* L.) was obtained from Sigma. All solvents and chemicals for the HPLC analysis such as ammonium acetate (Fluka), acetonitrile (Fluka), glacial acetic acid (Carl-Roth), and methanol (Sigma–Aldrich) were of HPLC grade. Ultrapure water was used to prepare some solutions for the HPLC method.

2.2. Plant material

Seeds of *H. adenotrichum* were collected from the natural habitat of the plant at about 1400 m on Mount Karıncalı in Aydın Province, Turkey, in July 2008 and July 2009. The seeds were stored in paper bags at room temperature (20–25 °C in the dark) until used.

2.3. In vitro culture and elicitation

Stratified seeds (at 4 °C for 2 months) of *H. adenotrichum* were surface sterilised by immersion in 70% (V/V) ethanol for 1 min and subsequently in a 2.25% NaOCl solution containing 2 drops of Tween 20 for 5 min. Finally, the seeds were rinsed 3 times in sterile distilled water for 10 min each. The sterilised seeds were cultured in glass jars containing 30–35 mL of culture medium containing quarter-strength macro and full-strength micro MS salts (Murashige & Skoog, 1962), Galzy vitamins (Galzy, 1964), and 30 g/L sucrose. The media were solidified with 8 g/L agar (Sigma). The pH of media was adjusted to 5.8 before solidifying agent (agar) was added. The media were autoclaved at 121 °C for 15 min. This medium used for culture of the seeds was defined as ¼ MS/Galzy medium. The cultures were maintained at 18 ± 1 °C with 16 h light/8 h dark photoperiod under cool white fluorescent lamps (40 μE m^{-2} s^{-1}). Two weeks after incubation, seedlings obtained from the germinated seeds were transferred to ¼ MS/Galzy control medium and ¼ MS/Galzy medium containing varying levels of mannan (10, 50, and 100 mg/L) or pectin (10, 50, and 100 mg/L) for elicitation. The seedlings were cultured on the elicitation and control media at 22 ± 1 °C under a 16 h light/8 h dark photoperiod. The seedlings were harvested after 15 or 30 days. After root sections of the seedlings were removed, shoots were weighed immediately to obtain fresh weight and inserted into plastic test tubes, frozen in liquid nitrogen, and stored in a freeze box at –80 °C until analysis.

2.4. Extraction

The frozen plant materials were lyophilised using a Labconco freeze-drier system. Dry samples were later crushed to powder for the analysis. Finely powdered plant material of *H. adenotrichum* (100 mg) was weighed into a 10-mL borosilicate amber glass tube with a polypropylene screw cap and extracted twice with 4 mL of methanol using a direct sonicator under strict exclusion of light in iced water for 30 min. Direct sonication was performed with ultrasonic power of 60 W at 20 kHz. The mixture was centrifuged at 3000 rpm for 10 min. The extracts were combined in a 10-mL borosilicate amber glass tube and then concentrated in a vacuum centrifuge. The final

volume of the concentrated extracts was adjusted to 4 mL with the same extraction solvent (methanol), and 1.5-mL aliquots were filtered through PTFE syringe filters (0.45 µm, ValuPrep 25 mm, Pall) into HPLC amber borosilicate sample vials before injection into the HPLC system.

2.5. HPLC analysis of hypericins

HPLC analysis for the determination of hypericin and pseudohypericin of *H. adenotrichum* seedlings was performed according to the method described by Ganzera et al. (2002) with small changes. RP-HPLC-DAD analysis was performed on an Agilent-1100 HPLC system (Agilent Technologies, GmbH, Germany), equipped with a photodiode array detector (G1315B), a degasser, a quaternary pump (G1354A), an autosampler (G1313), and a column oven (G1316A). A Synergi MAX-RP 80 Å column (150×4.6 mm, 4-µm particle size) from Phenomenex (Torrance, CA, USA) was used for all separations. The mobile phase consisted of 10 mM ammonium acetate buffer adjusted to pH 5.0 with glacial acetic acid (A) and a 9:1 mixture of acetonitrile and methanol (B). Gradient elution was performed using the following solvent gradient: from 87A/13B in 10 min to 83A/17B and then in 25 min to 100 B; each run was followed by an equilibration period of 10 min. The flow rate of the mobile phase was 1 mL/min. An aliquot of 50 µL of extract per sample was injected onto the column and detection was performed at 270 and 590 nm wavelengths. All separations were performed with a constant column temperature of 40 °C. Comparison of the UV spectra and retention times of standard injection peaks against sample peaks was used to assign peak identity. Standard curves were obtained by plotting the peak areas of standard concentrations of hypericin (0.02, 0.2, 0.5, 1.0, 2.5, 5.0, and 10 µg/mL), pseudohypericin (0.05, 0.1, 0.5, 2.5, 5.0, and 10 µg/mL). Two linear regression equations ($R^2 > 0.99$) were obtained. Quantification of pseudohypericin and hypericin was based on peak area (RT, retention time of 22.4 and 25.0 min, respectively) in comparison with the standard curves.

2.6. Statistical analysis

All experiments were of complete randomised design and treatments consisted of 5 replications. All the experiments were performed 3 times. Treatments were compared to controls by one-way ANOVA using the Tukey test ($P < 0.05$). The statistical analyses were performed with SPSS (version 15.0).

3. Results and discussion

Total hypericin content can be increased by elicitation (biotic and abiotic) and this may lead to affirmation that these metabolites are part of the inducible defensive system of *Hypericum* species (Bruni & Sacchetti, 2009). Various biotic and abiotic stress factors can cause the upregulation of the biosynthesis of secondary metabolites in both intact plants and in cell cultures (Kirakosyan et al., 2008). Previous studies have reported the upregulation of hypericin and pseudohypericin in in vitro seedling (Pavlik et al., 2007), shoot (Kirakosyan et al., 2000), callus (Vardapetyan et al., 2006), and cell (Gadzovska et al., 2007) cultures of *H. perforatum*. The hypericin/pseudohypericin-containing black glands are located along the margins of the leaves (Kornfeld et al., 2007). We observed similar black glands on the newly forming leaves of our in vitro seedlings (Figure 1A, B), and hypericin and pseudohypericin were detected in the extracts of these seedlings by HPLC analysis.

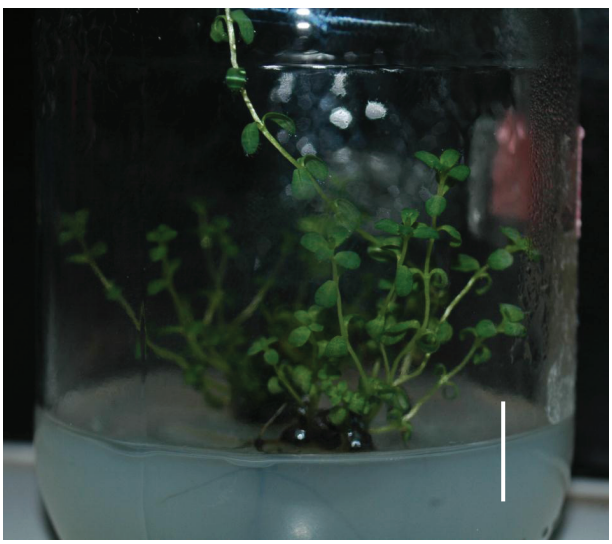

Figure 1A. In vitro seedling of *Hypericum adenotrichum* with dark glands on the leaves. Scale bar: 1 cm.

Figure 1B. The dark glands located on leaf margin of in vitro *Hypericum adenotrichum* seedling. Scale bar: 0.5 mm.

In our study, the quantities of pseudohypericin and hypericin in the seedlings of *H. adenotrichum* exposed to mannan (10, 50, and 100 mg/L) or pectin (10, 50, and 100 mg/L) for 15 and 30 days varied depending on the concentrations and applying time of the elicitors.

The level of pseudohypericin increased proportionally with the amount of mannan up to 50 mg/L mannan treatment and decreased slightly at 100 mg/L mannan after 15 days. The amount of pseudohypericin (15.9 mg/100 g dry wt.) at 50 mg/L of mannan was 2.8-fold higher than that of control seedlings (5.6 mg/100 g dry wt.). The production of pseudohypericin was stimulated less after 30 days' mannan treatment as compared to the mannan treatment for 15 days. The best result from treatment for 30 days was observed at the highest concentration of mannan. Pseudohypericin production (13.3 mg/100 g dry wt.) at the 100 mg/L concentration of mannan was 1.8 times higher than that of the control (7.3 mg/100 g dry wt.) (Figure 2).

Although the content of hypericin and pseudohypericin in the control seedlings was almost the same concentration, the mannan treatment affected the production of pseudohypericin more than that of hypericin. The mode of increase and decrease of hypericin in seedlings of *H. adenotrichum* according to mannan concentrations was similar to that of pseudohypericin. The amount of hypericin (9.7 mg/100 g dry wt.) at 50 mg/L of mannan was 1.7-fold higher than that of the control seedlings (5.5 mg/100 g dry wt.) for 15 days' treatment, while hypericin production (8.9 mg/100 g dry wt.) at the 100 mg/L concentration of mannan was 1.2 times higher than that of the control seedlings (7.2 mg/100 g dry wt.) for 30 days' treatment (Figure 3).

The best result from mannan treatment was obtained by exposure to 50 mg/L mannan for 15 days because 50 mg/L mannan stimulated simultaneously pseudohypericin production up to 2.8-fold and hypericin production up to 1.7-fold. These results suggest that mannan (from yeast) is an effective elicitor of the production of especially pseudohypericin in in vitro *H. adenotrichum* seedlings.

Mannan has been reported to enhance the production of secondary metabolites in some plant cultures. It has been reported that mannan stimulated both hypericin and pseudohypericin production at 0.05–0.1 mg/mL in *H. perforatum* shoot organ cultures (Kirakosyan et al., 2000). Pseudohypericin production at 0.1 mg/mL of mannan was almost 4 times higher than that of control shoot cultures and hypericin production at the same concentration of mannan was 2 times higher than that of the control. Similar results were also reported for callus cultures of *H. perforatum* by Vardapetyan et al. (2006). Yamamoto (1995) reported that mannans stimulated prenylated flavanone production in callus culture of *Sophora flavescens* Ait. It has been reported that mannan promoted tyrosine ammonia-lyase activity by 22% and phenylalanine ammonia-lyase, coumarate 3-hydroxylase, and polyphenoloxidase activities by 10% as compared to the control treatment in callus cultures of *Linum austriacum* L. (Vardapetyan et al., 2003).

Pectin treatments significantly affected production of hypericins in the seedlings of *H. adenotrichum* after 15 days' treatment. Pectin stimulated pseudohypericin production especially at 10 and 50 mg/L. The amount of pseudohypericin increased proportionally with the amount of pectin up to 50 mg/L pectin treatment and then decreased dramatically at 100 mg/L pectin for 15 days. Pseudohypericin production (27.2 mg/100 g dry wt.) at 50 mg/L of pectin was 4.8-fold higher than that of control seedlings (5.6 mg/100 g dry wt.) and pseudohypericin production (12.8 mg/100 g dry wt.) at 10 mg/L pectin

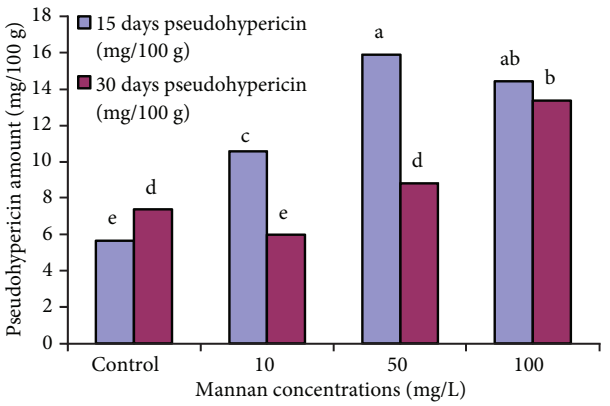

Figure 2. Fluctuation of pseudohypericin levels depending on mannan concentrations. Different letters indicate significant differences between the treatments at $P < 0.05$ determined by Tukey's test.

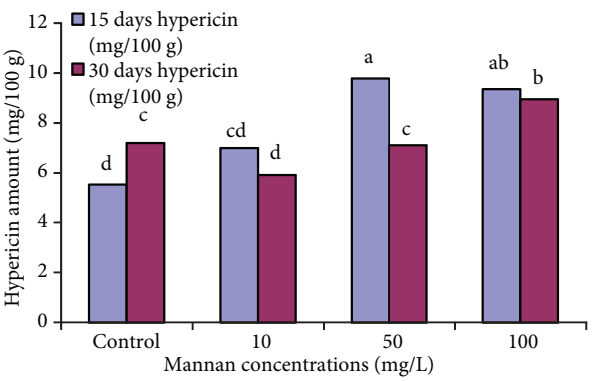

Figure 3. Fluctuation of hypericin levels depending on mannan concentrations. Different letters indicate significant differences between the treatments at $P < 0.05$ determined by Tukey's test.

concentration was 2.2-fold higher than that of control seedlings. After 30 days' pectin treatment, the levels of pseudohypericin remained significantly unchanged and only the level of pseudohypericin at 50 mg/L pectin concentration (12.8 mg/100 g dry wt.) was 1.7-fold higher than that of control seedlings (7.3 mg/100 g dry wt.) (Figure 4).

Although pectin stimulated hypericin as well as pseudohypericin production especially at 10 and 50 mg/L after 15 days' treatment, the stimulation of hypericin was less than that of pseudohypericin. The highest hypericin level was obtained with 50 mg/L pectin treatment. The level of hypericin (15.3 mg/100 g dry wt.) showed a 2.8-fold increase as compared to control seedlings (7.3 mg/100 g dry wt.) after 50 mg/L pectin treatment. After 30 days' pectin treatment, the level of hypericin was significantly unchanged or slightly reduced as compared to control seedlings (Figure 5).

The highest levels of hypericins after pectin treatment were obtained when 50 mg/L pectin was applied for 15 days, because 50 mg/L pectin stimulated simultaneously pseudohypericin production up to 4.8-fold and hypericin production up to 2.8-fold. These results show that pectin (from *Citrus*) is a more effective elicitor in the elicitation of hypericin and pseudohypericin as compared to mannan in in vitro *H. adenotrichum* seedlings.

Pectin and pectic substances have been reported to enhance the production of secondary metabolites in various plant cultures. Kirakosyan et al. (2000) reported that pectin stimulated pseudohypericin production up to 1.8-fold, while not affecting hypericin production in *H. perforatum* shoot organ cultures. In our study, pectin stimulated better production of hypericins in in vitro *H. adenotrichum* seedlings compared to *H. perforatum* shoot organ cultures. It has been reported that pectin increased the production of triterpene acids (ursolic and oleanolic acid) in *Uncaria tomentosa* (Willd.) DC. cell suspension cultures (Flores-Sánchez et al., 2002). That study also showed that pectin treatment caused a rapid 3-fold increase in the activities of enzymes such as isopentenyl diphosphate isomerase and squalene synthase in cell cultures. Pectin was previously used successfully to stimulate the production of oleanolic acid in suspension cultures of *Calendula officinalis* L. (Wiktorowska et al., 2010) and of anthraquinones in *Morinda citrifolia* L. cultures (Dörnenburg & Knorr, 1994).

Hypericin and pseudohypericin are thought to be products of polyketide biosynthesis (Kirakosyan et al., 2004). Pectin and mannan may have activated key enzymes of this metabolic pathway directly or indirectly. It has been suggested that hypericins have a defensive role against herbivores and plant pathogens (Sirvent & Gibson, 2002). It has been reported that mannan elicits the shikimate and phenylpropanoid pathways, and that mannan treatment, similar to the effect of pathogens, promotes H_2O_2 accumulation in the cytoplasm (Vardapetyan et al., 2003). Pathogens enter plant tissues by various ways. Pectins are one of the first targets of digestion by invading pathogens (Ridley, 2001). Oligogalacturonides (OGs) are endogenous elicitors of defence responses released after partial degradation of pectin in the plant cell wall during pathogen infection (Galletti et al., 2008). The OGs released are a carbon source for the pathogens, but can also be detected by plants as signals to initiate defence responses (Ridley, 2001). Flores-Sánchez (2002) reported that a variety of oligogalacturonic fragments must have formed after sterilisation at 121 °C and pH 4.63 from *Citrus* pectin. Highly methylated *Citrus* pectin (HM-pectin) is stable at room temperature only, at neat-to-neutral pH (5–6). As the temperature increases, so-called elimination starts, which

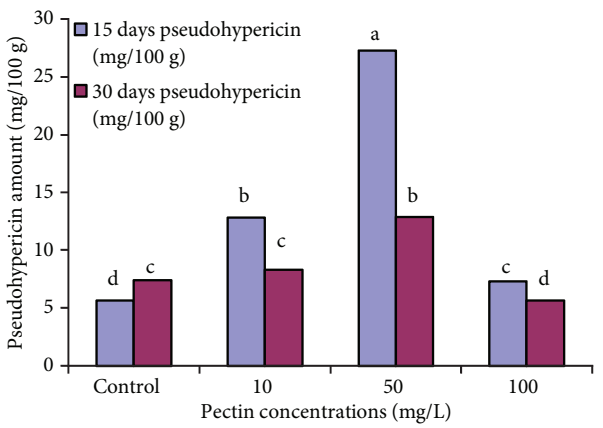

Figure 4. Fluctuation of pseudohypericin levels depending on pectin concentrations. Different letters indicate significant differences between the treatments at $P < 0.05$ determined by Tukey's test.

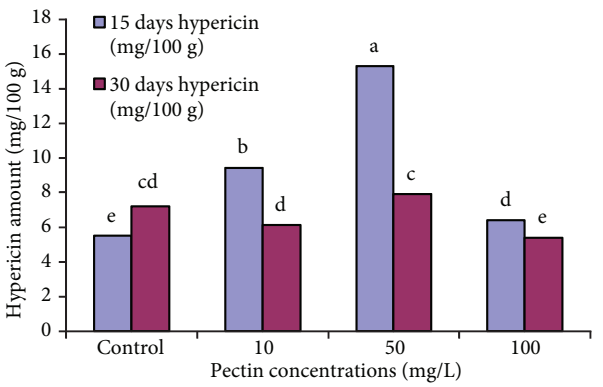

Figure 5. Fluctuation of hypericin levels depending on pectin concentrations. Different letters indicate significant differences between the treatments at $P < 0.05$ determined by Tukey's test.

results in chain cleavage and very rapid loss of viscosity and gelling properties (Srivastava & Malviya, 2011). In our study, a variety of oligogalacturonic fragments may have also formed from *Citrus* pectin.

This study is the first report showing the stimulatory effects of elicitors on the production of hypericins in *H. adenotrichum*. Our results show that production of hypericins in seedlings of *H. adenotrichum* can be modified by elicitors such as mannan and pectin. Increases in hypericins in response to mannan and pectin demonstrate that these elicitors can be evaluated in experimental botany and the pharmaceutical industry intended for biosynthesis of hypericins in *Hypericum* species.

Acknowledgement
This work was supported by the Scientific Research Projects Unit of Adnan Menderes University (project no: FBE-09013).

References

Bruni R & Sacchetti G (2009). Factors affecting polyphenol biosynthesis in wild and field grown St. John's wort (*Hypericum perforatum* L. *Hypericaceae/Guttiferae*). *Molecules* 14: 682–725.

Cavaliere C, Rea P, Lynch ME & Blumenthal M (2009). Herbal supplement sales experience slight increase in 2008. *HerbalGram* 82: 58–61.

Çırak C, Ivanauskasb L, Janulisb V & Radusiene J (2009). Chemical constituents of *Hypericum adenotrichum* Spach, an endemic Turkish species. *Natural Product Research* 23: 1189–1195.

Dörnenburg H & Knorr D (1994). Effectiveness of plant-derived and microbial polysaccharides as elicitors for antraquinone synthesis in *Morinda citrifolia* cultures. *Journal of Agricultural and Food Chemistry* 42: 1048–1052.

Flores-Sánchez IJ, Ortega-López J, Montes-Horcasitas MC & Ramos-Valdivia AC (2002). Biosynthesis of sterols and triterpenes in cell suspension cultures of *Uncaria tomentosa*. *Plant Cell Physiology* 43: 1502–1509.

Gadzovska S, Maury S, Delaunay A, Spasenoski M, Joseph C & Hagege D (2007). Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphthodianthrones. *Plant Cell Tissue Organ Culture* 89: 1–13.

Galletti R, Denoux C, Gambetta S, Dewdney J, Ausubel FM, De Lorenzo G & Ferrari S (2008). The AtrbohD-mediated oxidative burst elicited by oligogalacturonides in Arabidopsis is dispensable for the activation of defense responses effective against *Botrytis cinerea*. *Plant Physiology* 148: 1695–1706.

Gokhale M & Bansal YK (2010). Assessment of secondary metabolites in in vitro regenerated plantlets of *Oroxylum indicum* (L.) Vent. *Plant Tissue Culture and Biotechnology* 20: 21–28.

Galzy R (1964). Technique de thermothrrapie des viroses de la vigne. *Annales der Epiphyties* 15: 245–256.

Ganzera M, Zhao J & Khan IA (2002). *Hypericum perforatum*-chemical profiling and quantitative results of St. John's Wort products by an improved high-performance liquid chromatography method. *Journal of Pharmaceutical Sciences* 91: 623–630.

Gioti EM, Skalkos DC, Fiamegos YC & Stalikas CD (2005). Single-drop liquid-phase microextraction for the determination of hypericin, pseudohypericin and hyperforin in biological fluids by high-performance liquid chromatography. *Journal of Chromatography A* 1093: 1–10.

Greeson J, Sanford B & Monti DA (2001). St. John's wort (*Hypericum perforatum*): a review of the current pharmacological, toxicological and clinical literature. *Psychopharmacology* 153: 402–414.

Hasançebi S, Kara NT, Çakır Ö & Arı Ş (2011). Micropropagation and root culture of Turkish endemic *Astragalus chrysochlorus* (Leguminosae). *Turkish Journal of Botany* 35: 203–210.

Hölzl J & Petersen M (2003). Chemical constituents of *Hypericum* ssp. In: Ernst E (ed.) *Hypericum, The Genus Hypericum*. London, Taylor & Francis Group, pp. 77–93.

Jones GH & Ballou CE (1969). Studies on the structure of yeast mannan. I. Purification and some properties of an α-mannosidase from an *Arthrobacter* species. *The Journal of Biological Chemistry* 244: 1043–1051.

Karioti A & Bilia AR (2010). Hypericins as potential leads for new therapeutics. *International Journal of Molecular Science* 11: 562–594.

Karuppusamy S (2009). A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *Journal of Medicinal Plants Research* 3: 1222–1239.

Kirakosyan A, Gibson DM & Kaufman PB (2008). The production of dianthrones and philroglucinol derivatives in St. John's wort. In: Ramawat KG & Mérillon JM (ed.) *Bioactive Molecules and Medicinal Plants,* pp. 151–164. Berlin and Heidelberg: Springer.

Kirakosyan A, Hayashi H, Inoue K, Charchoglyan A & Vardapetyan H (2000). Stimulation of the production of hypericins by mannan in *Hypericum perforatum* shoot cultures. *Phytochemistry* 53: 345–348.

Kirakosyan A, Sirvent T, Gibson D & Kaufman P (2004). The production of hypericins and hyperforin by in vitro cultures of *Hypericum perforatum*. *Biotechnology and Applied Biochemistry* 39: 71–81.

Kitanov GM (2001). Hypericin and pseudohypericin in some *Hypericum* species. *Biochemical Systematics and Ecology* 29: 171–178.

Kornfeld A, Kaufman PB, Lu CR, Gibson DM, Bolling SF, Warber, SL, Chang SC & Kirakosyan A (2007). The production of hypericins in two selected *Hypericum perforatum* shoot cultures is related to differences in black gland structure. *Plant Physiology and Biochemistry* 45: 24–32.

Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Özmen A, Bauer S, Gridling M, Singhuber J, Krasteva S, Madlener S, Vo TP, Stark N, Saiko P, Fritzer-Szekeres M, Szekeres T, Askin-Celik T, Krenn L & Krupitza G (2009). In vitro anti-neoplastic activity of the ethno-pharmaceutical plant *Hypericum adenotrichum* Spach endemic to Western Turkey. *Oncology Reports* 22: 845–852.

Pavlik M, Vacek J, Klejdus B & Kuban V (2007). Hypericin and hyperforin production in St. John's wort in vitro culture: influence of saccharose, polyethylene glycol, methyl jasmonate, and *Agrobacterium tumefaciens*. *Journal of Agricultural and Food Chemistry* 55: 6147–6153.

Pilepić KH, Morović MF, Orač Šantor M & Vejnović V (2010). RFLP analysis of cpDNA in the genus *Hypericum*. *Biologia* 65: 805–812.

Radman R, Saez T, Bucke C & Keshavarz T (2003). Elicitation of plants and microbial cell systems. *Biotechnology and Applied Biochemistry* 37: 91–102.

Ridley BL, O'Neill MA & Mohnen D (2001). Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry* 57: 929–967.

Robson NKB (2003). *Hypericum* botany. In: Ernst E (ed.) *Hypericum: the Genus Hypericum*, pp. 196–241. New York: Taylor and Francis.

Shibuya N & Minami E (2001). Oligosaccharide signalling for defense responses in plant. *Physiological and Molecular Plant Pathology* 59: 223–233.

Sirvent T & Gibson D (2002). Induction of hypericins and hyperforin in *Hypericum perforatum* L. in response to biotic and chemical elicitors. *Physiological and Molecular Plant Pathology* 60: 311–320.

Srivastava P & Malviya R (2011). Sources of pectin, extraction and its applications in pharmaceutical industry – An overview. *Indian Journal of Natural Products and Resources* 2: 10–18.

Vardapetyan H, Kirakosyan A & Hovhannisyan A (2003). Effect of various elicitors on lignan biosynthesis in callus cultures of *Linum austriacum*. *Russian Journal of Plant Physiology* 50: 297–301.

Vardapetyan HR, Oganesyan AA, Kabasakalyan EE & Tiratsuyan SG (2006). The influence of some elicitors on growth and morphogenesis of *Hypericum perforatum* L. callus cultures. *Russian Journal of Developmental Biology* 37: 350–353.

Vasconsuelo A & Boland R (2007). Molecular aspects of the early stages of elicitation of secondary metabolites in plants. *Plant Science* 172: 861–875.

Verma SK, Yücesan BB, Şahin G, Gürel S & Gürel E (2011). Direct shoot regeneration from leaf explants of *Digitalis lamarckii*, an endemic medicinal species. *Turkish Journal of Botany* 35: 689–695.

Wiktorowska E, Długosz M & Janiszowska W (2010). Significant enhancement of oleanolic acid accumulation by biotic elicitors in cell suspension cultures of *Calendula officinalis* L. *Enzyme and Microbial Technology* 46: 14–20.

Yamamoto H, Ichimura M & Inoue K (1995). Stimulation of prenylated flavanone production by mannans and acidic polysaccharides in callus culture of *Sophora flavescens*. *Phytochemistry* 40: 77–81.

Zhao J, Davis LC & Verpoorte R (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances* 23: 283–333.