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Phenolics and parthenolide levels in feverfew (*Tanacetum parthenium*) are inversely affected by environmental factors

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Abstract

Feverfew (*Tanacetum parthenium* [L.] Schultz-Bip., Asteraceae) products have shown high variability in the market. The objective of this study was to determine whether environmental factors affect the composition of key phytochemicals in feverfew. Plants of feverfew were exposed to water stress in greenhouse and commercial field conditions. The highest yield of parthenolide (PRT) was found in plants that received reduced-water regimes. Phenolics concentration was higher in plants grown under adequate-water conditions. The effect of time of harvest on PRT concentration and phenolics content was also investigated. Increased PRT was found during afternoon hours whereas total phenolic compounds decreased during the photoperiod and increased at night. When plants were exposed to artificial light during night hours, the phenolics content remained low. Our results revealed that manipulating the environment to favour increased accumulation of PRT resulted in a decline of phenolics content in feverfew. These findings have implications on standardization of herbal products.

Key words: Asteraceae, feverfew, *Tanacetum parthenium*, time of harvest, water stress, parthenolide, phenolics

Introduction

Products of feverfew (*Tanacetum parthenium* [L.] Schultz-Bip., Asteraceae), an herb regarded as an effective prophylactic treatment of migraine, rheumatoid arthritis and menstrual cramps (Grauds *et al.*, 1995), have shown large variability of parthenolide (PRT) content, a sesquiterpene lactone commonly associated with the medicinal effects (Awang *et al.*, 1991).

In addition to PRT, certain phenolics, notably tanetin, a lipophilic flavonoid, contribute to the medicinal value of feverfew (Williams *et al.*, 1995). The major flavonols and flavone methyl ethers found in feverfew have been shown to inhibit the arachidonic acid pathway (Williams *et al.*, 1999). Phenolic content may be another criterion for assessment of commercial quality of feverfew.

Evidence indicating that pre-harvest factors influence final quality of herbs has been reported. In various plants, environmental stress has increased the accumulation of phenolics (Keinanen, 1999). Secondary metabolites are prone to diurnal fluctuation (Veit *et al.*, 1996), a response likely due to the effects of light intensity on carbon partition (Stoker *et al.*, 1998; Middleton *et al.*, 1994). It is uncertain whether PRT or any other secondary metabolite in feverfew fluctuates during the day. Water stress increases the production of jasmonic acid followed by increase of abscisic acid (ABA), which induces stomata closure (Wasttenack and Parthier, 1997) and sesquiterpene accumulation (Singh *et al.*, 1998), and may interfere with tannin yields (Horner, 1998). Our early work showed that ABA and PRT biosynthesis are connected and both increase during or after a wilt event (Fonseca *et al.*, 2005) prompted us to determine whether PRT and other secondary metabolites of interest fluctuate with environmental conditions in a similar fashion.

It is possible that pre-harvest conditions influence concentration of key metabolites in feverfew, however, research on PRT and phenolics dynamics as a response of changing environments is lacking. Thus, the objective of this study was to evaluate the effect of water stress and time of day of harvest on PRT and total phenolics content in feverfew.

Materials and Methods

Effect of water stress: Feverfew seeds (Richter's Seed Co. Ontario, Canada) were germinated in 60 mL-cell trays under an intermittent mist and were transferred to 4 L pots six weeks after germination. Four months after germination, plants were divided in two groups, one continuing with daily watering, and the other group receiving water only after a wilt event. Typically, plants were allowed to dry for 4-6 days until they wilted (-4.47 to –8.65 MPa). After wilt, plants were watered daily to run off as the controls (-1.94 to -2.07 MPa) for five days before exposing the plants to water withdrawal again. The plants were subjected to a total of three wilt events and harvested before the onset of the fourth water stress event. The experiment was repeated once and included treatments consisting of twelve plants, harvested during summer months. Maximum irradiance of visible light, measured with a LI-250 Quantum meter (Licor Inc. Lincoln, NE, USA), was 461.4 $\mu mol\ s^{-1}\ m^{-2}$.

The effect of water stress on PRT and total phenolics content in feverfew was also evaluated in a grower's field. Feverfew plants

Table 1. Conditions at 11 am in a commercial trial that evaluated the effect of water stress on parthenolide concentration in feverfew

Factors	Non-Irrigated	Irrigated
Temperature	26.00	26.00
Sunlight	1645.80	1645.80
Water potential soil	-70.46	-0.04
Water potential plant	-3.63	-1.11

Units are °C for temperature, μmol s^{-1} m^{-2} for sunlight intensity and P Ma for water potential.

were grown for 5 months with and without drip irrigation at a commercial production site near Kingsburg, SC, USA, from November to April. Plants were harvested when approximately 5 percent of the plants had begun to bloom. Batches of 8 non-flowering plants from each field (non-irrigated and irrigated) were randomly selected and harvested four times during daytime. Water potential in soil and leaves, photosynthetic radiation (PAR) and temperature at the harvest site are provided in Table 1.

Effect of time of day of harvest: Plants were grown in greenhouse following cultural practices described earlier. Five months after planting, plants were harvested at four different times of the day (5 AM, 9 AM, 1 PM, 5 PM) during January and February. To evaluate the effect of light during night hours plants harvested at 5 AM were exposed to light (8 μmol s^{-1} m^{-2}) for 8 hours (starting at 9 PM) and compared with non-irradiated plants. The trial included harvest of three plants per treatment per each of four different days. The experiment was arranged in a randomized complete block design and it was conducted twice.

Analysis of parthenolide and total phenolics: In preparation for PRT analysis, all plant tissue above ground were harvested and dried in a conventional oven at 50 °C until moisture content reached 4-6% and then ground with a coffee grinder. The powder was sieved and particles of <500 μm size were used for immediate analysis. Samples of 150 mg were combined with 10 mL 90% acetonitrile for 10 min and extracted using the bottle stirring method. Aliquots of each extraction solution were taken from supernatant, filtered through 0.45 PTFE μm membranes and 10 μL injected onto a RP-HPLC system (Waters ™ 1525 pump), equipped with a C-18, 5 μm column (Waters Symmetry ®) of 150 x 4.6 mm dimensions. The injections were performed in duplicate. Mobile phase was an isocratic 55% acetonitrile: 45% water per 8 min at 1.5 mL/min. The peaks were analyzed at 210 nm using an ultraviolet detector.

Total phenolic content was measured using the Folin Ciocalteau procedure (Singleton and Rossi, 1965) modified by Kähkhönen *et al.* (1999). The extraction was performed by combining 50 mg feverfew powder samples with 10 ml 80% methanol with stirring for 10 min. Samples were vortexed for 1 min before a 200 μL aliquot was taken from the supernatant**.** One mL of Folin-Ciocalteu reagent (2N, Sigma) was added to the 200 μL sample. After 3 minutes, 0.8 mL of sodium carbonate (7.5%) was added and the mixture was allowed to stand for 30 min. Absorption was measured at 765 nm using a Spec 20® spectrophotometer (Thermo Spectronic, Rochester, NY, USA). Total phenolics were expressed as gallic acid units. A multipoint linear curve was obtained with gallic acid standard (Sigma) ranging from 20 to 400 μg/mL. Two standards (20 and 100 μg) were included for comparison with each set of samples analyzed.

Statistical analysis: Experiments were arranged in a completely randomized design. Data were subjected to analysis of variance (ANOVA) at $P \leq 0.05$ to determine statistical significance. Mean comparisons were conducted using Fisher's Protected LSD Method at $P \leq 0.05$ (SAS Institute, Cary, NC).

Results and discussion

Plants under water stress had higher PRT levels than plants receiving water daily regardless of the growing environment, greenhouse or commercial field. Unlike the pattern observed with PRT, plants in greenhouse conditions under reduced-water conditions had lower phenolic content than plants that received continuous irrigation, but no difference was observed in plants grown in the field (Table 2).

Unlike our previous work (Fonseca *et al.*, 2005), in this study the plants were harvested after subjecting the plants for extended time to water stress, and the leaves were not fully turgid at the moment of the harvest. The results from this study revealed that water stress in commercial field conditions enhance PRT content in feverfew, however, the plants showed 20-35% reduced dry weight (data not shown), discouraging field production without irrigation systems. The dry weight of plants grown in pots, receiving only three wilt events, was reduced by less than 5% (data not shown) which encourages studies involving the evaluation of mild stress conditions. It is possible that regulated water stress programs increase metabolites concentration without changing quantities of phytochemicals per area. The total phenolics levels found in feverfew are similar to those reported for other herbs and medicinal plants (Kähkönen *et al.*, 1999), however, to our knowledge this is the first time that phenolics content variability in a medicinal plant is associated with a single agricultural practice.

Plants harvested during the afternoon contained significantly more PRT than plants harvested in the morning. Plants harvested at 5 PM had the highest PRT. The 5 AM harvest had the lowest PRT

Table 2. Effect of water stress on parthenolide and phenolics content in feverfew grown in greenhouse and field conditions

Environment	Irrigation regime	Parthenolide content (g kg^{-1} dry weight)	Phenolics content (g GAC kg^{-1} dry weight)
Greenhouse	Daily watering	0.47 a	64.77 a
	Reduced watering	1.89 b	33.45 b
	LSD ($P \leq 0.05$)	1.36	24.15
Field – commercial production	Drip irrigation	5.38 a	110.50
	No irrigation	6.76 b	93.85
	LSD ($P \leq 0.05$)	1.31	49.33

Values are the averages of 16 samples taken during the day of harvest. Different letters within the same column and environment indicate significant differences between treatments.

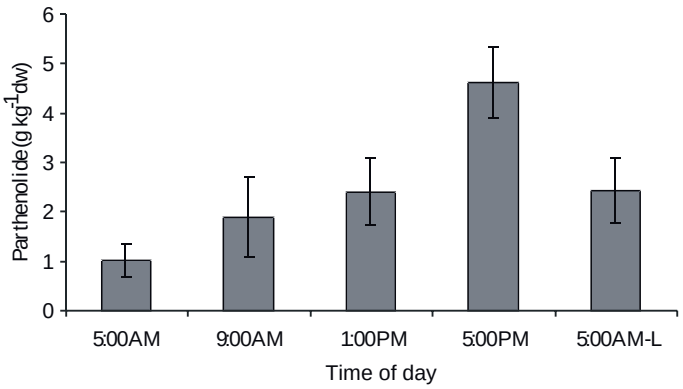

Fig. 1. Effect of time of day of harvest on parthenolide content in feverfew. "L" means plants exposed to artificial light during night hours (5 PM to 5 AM). Values are the average of 12 samples. Error bars indicate S.E.

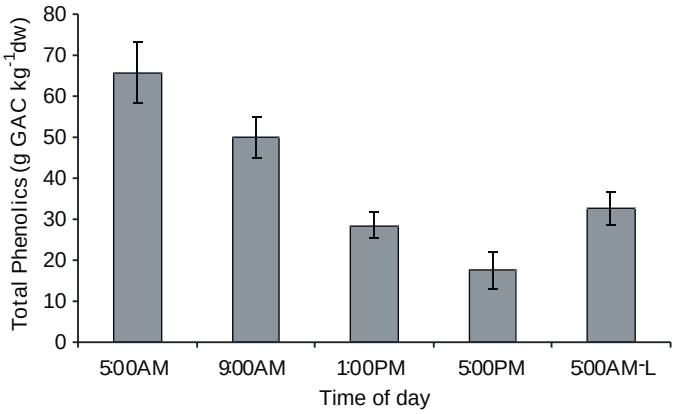

Fig. 2. The effect of time of day of harvest on total phenolics content in feverfew. "L" means plants exposed to artificial light during night hours (5 PM to 5 AM). Values are the averages of 12 samples. Error bars indicate S.E.

concentration. Although no significant difference was detected, when 5 AM plants were exposed to light PRT levels averaged two fold higher than those in non-irradiated plants (Fig. 1). In contrast, the phenolic concentration was higher during night time, decreasing during daylight. At 5 AM harvest, the plants exposed to light had significantly less concentration of phenolics (Fig. 2). These results show that light prior to harvest influences the levels of PRT and total phenolics in feverfew. The pattern observed with phenolic levels, increasing in the dark and decreasing during daylight or with artificial light irradiance, has been observed in other plants (Veit *et al.*, 1996; Burns *et al.*, 2002).

It is interesting that the concentration of PRT in plants grown during the winter (the field experiment and the study of time of day), which are normally shorter and bushy, was markedly higher than in plants grown in the summer (experiment of water stress in greenhouse conditions). Moreover, it has been observed that vegetative plants accumulate higher PRT levels than reproductive plants (Hendriks *et al.*, 1997), which normally bloom during the summer. There may be a synergistic interaction between long day conditions and environmental factors.

Overall results in this study showed that environmental factors may produce opposite effects among secondary metabolites in medicinal plants and this was clearly observed between PRT and phenolic content in feverfew. The accumulation of secondary metabolites is driven by the availability of excess carbon. When growth is reduced due to stress, more carbon becomes available for secondary metabolism and, as the plant adjusts to the specific stress the accumulation of some metabolites is favored over others (Tuomi *et al.*, 1998). Phenolics fluctuate daily in part because the plant produces them in preparation to periods of high UV sunlight irradiance (Middleton and Teramura, 1994). Moreover, our results with artificial light revealed that visible light functions as an external signal that turns on and off the phenolic production mechanism in feverfew, regardless of UV light incidence.

The results obtained in this study demonstrate that medicinal or "commercial" quality of feverfew is affected by environmental factors. The visible light prior to harvest and the water regime during the plant growth are crucial in altering the content of PRT and phenolics in feverfew. Our results revealed however, that practices that increased PRT result in lower total phenolic content. The many interactions among phytochemicals, and the rapid adjustment of the plant to changing environments, involve a major decision of which key metabolites to target, knowing that this will potentially carry a "trade off" including a decrease in the content of other metabolites. Clearly, manipulation of production and handling protocols can alter secondary metabolism of feverfew but studies are needed to verify if this holds true for other medicinal plants as well. Such studies are crucial for the development of appropriate regulations for herbal product quality.

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