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Original Article

Fertilization-Induced Changes in Growth Parameters and Antioxidant Activity of Medicinal Plants Used in Traditional Arab Medicine

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In response to increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation systems. We collected four medicinal herbs *Cichorium pumilum, Eryngium creticum*, *Pistacia palaestina* and *Teucrium polium* used in traditional Arab medicine for greenhouse cultivation to assess the effects of different fertilization regimes on their growth and antioxidant activity. Wild seedlings were collected and fertilized with either 100% Hoagland solution, 50% Hoagland solution, 20% Hoagland solution or irrigated with tap water. Plant height was measured and the number of green leaves and branches counted weekly. Thereafter, the aboveground parts of plants were harvested for preparing a water-soluble powder extracts of which antioxidant activity was measured by their ability to suppress the oxidation of β-carotene. Of the fertilization regimes, we found either 20 or 50% Hoagland solution produced the most consistent response of the plant growth parameters. All powders prepared from the four wild growing plants inhibited oxidation of β-carotene. Increasing the amount of fertilizer caused a significant concentration-dependent increase in antioxidant activity of the cultivated *T. polium* compared with the wild type. In contrast, increasing the amount of fertilizer caused a significant concentration-dependent reduction in the antioxidant activity of powders prepared from the cultivated *E. creticum* when compared with wild plants. Our results showed that cultivation success should not rely solely on parameters of growth but should incorporate assessment related to indices of therapeutic potential.

Keywords: antioxidant activity – medicinal plants – plant conservation – plant cultivation

Introduction

In recent years, the use of herbal therapies has gained in popularity (1–4). This has lead to an increased demand for medicinal plants and there is now increasing alarm about the potential extinction of some medicinal plants (5). In response, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation systems (6). Numerous ethnopharmacological surveys have attested to the use of indigenous plants among practitioners of traditional Arab medicine in Israel, the Palestinian territories and Jordan (7–11). Against this background, we decided to collect several indigenous medicinal plants for cultivation under greenhouse conditions and to assess the effects of different fertilization regimes on their growth.

Plants have an almost limitless ability to synthesize aromatic substances that have been evaluated for their therapeutic potential. These include alkaloids, coumarins, saponins and flavonoids (12,13). Flavonoids are probably the best known of these substances due to their antioxidant properties

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(14,15). Accordingly, we decided to use antioxidant activity as a surrogate index of their therapeutic potential to compare the antioxidant potential of extracts prepared from wild growing medicinal plants with those cultivated in the greenhouse.

Materials and Methods

Plant Species

The four plants were *Cichorium pumilum* Jacq. (RDC 1029), *Eryngium creticum* Lam. (RDC 1046), *Pistacia palaestina* L. (RDC 1130) and *Teucrium polium* L. (RDC 1117). Following their field identification by botanists, seedlings were collected for transplanting and wild specimens for preparation of plant powders (Table 1).

Collection of Wild Plants for Extraction

Leaves, stems and fruits of the four plants were collected separately from the hills of the Galilee region of Israel during the spring and summer (Table 1). After collection, the plants were dried for 7–10 days at room temperature. The dried plant parts were then ground. The ground product was stored in cloth bags at 5°C until their transfer to the laboratory for preparation of the plant powder.

Plant Cultivation

Wild seedlings were cultivated in a greenhouse (relative humidity 50–70%; temperature 25–35°C) during the spring and the summer. Each seedling was grown in a pot (25 cm height and 20 cm diameter) filled with sandy-loamy soil. Treatments consisted of four different fertilization regimes: (i) full (100%) Hoagland solution (16); (ii) 50% Hoagland solution; (iii) 20% Hoagland solution; and (iv) control (irrigation with tap water). For each treatment, 10 uniform seedlings were grown for 9–11 weeks before being harvested. Each week, plant height was measured and the number of green leaves and branches counted. At the end of the period, aboveground parts of the plants were harvested and weighed.

Table 1. Plant species studied, time of seedling collection for replanting, time of harvest of cultivated or wild plant specimens, plant parts and the extraction yield of wild species

Plant species	Time of seedling collection	Harvest time	Plant parts used for extraction	% Extraction yield (w/w)
Cichorium pumilum	February	April–May	Leaves and stems	15.4
Eryngium creticum	January	March–April	Leaves and stems	13.2
Pistacia palaestina	March	July–August	Leaves	14.8
			Fruits	6.5
Teucrium polium	March	May–July	Leaves	8.0
			Stems	8.1

The plants were then dried and ground for preparing the plant powder, as previously described.

Preparation of Plant Powders

The method for preparing water-soluble plant powders has been previously described (17). Briefly, dried plant material (31.5 g) was stirred in 350 ml of distilled water for 15 min at 100°C, followed by rapid filtration through a crude cellulose filter and then by a more delicate filtration through Whatman filter paper no. 1. The resulting filtrate was frozen and freeze-dried. The powder was stored at −18°C in a desiccant until required.

β-Carotene Oxidation Levels of Plant Powders

The antioxidant potential of different aqueous plant extracts was evaluated by measuring their ability to suppress the oxidation of β-carotene as described by Vaya *et al.* (18), with two minor modifications: the plant powder was dissolved in water instead of alcohol to a final concentration of 100 μg ml^{-1} and the spectrophotometric readings were taken at 454 nm. The assay was performed at 50°C. The OD_{454} readings of the rate of β-carotene bleaching were recorded at 20 min intervals for 2 h against the water blank. This method allowed evaluation of the antioxidative activity of the preparations along a time scale. The antioxidant butylated hydroxyanisole (BHA) (100 μg ml^{-1}) (Sigma Chemical Co., St Louis, MO, USA) served as a positive control. The assay was repeated three times for each powder.

Statistical Analysis

The data were analyzed by either a student's *t*-test or a repeated measures of the one-way analysis of variance (ANOVA) with Dunnett's post-test using Instat™ version 3 (GraphPad Software Inc., San Diego, CA, USA). All data are presented as mean ± standard deviation (SD). Significance was set at 5%.

Results

Fertilization Regimes Affect Parameters of Plant Growth under Greenhouse Conditions

In the greenhouse experiment, we succeeded in cultivating the four plant species. In general, all plants responded positively to the different fertilization regimes (Figs 1–4). Although we found individual differences among the plants, the overall picture indicated that fertilization with either 20 or 50% Hoagland solution produced the most consistent response when measuring the number of leaves, plant height and fresh weight at harvest as indices of growth. Full (100%) Hoagland solution caused some reduction in the fresh weights of *P. palaestina* and *T. polium* (Figs 3C and 4C). We did not observe any effect of the different fertilization regimes on numbers of branches of the plants (data not shown).

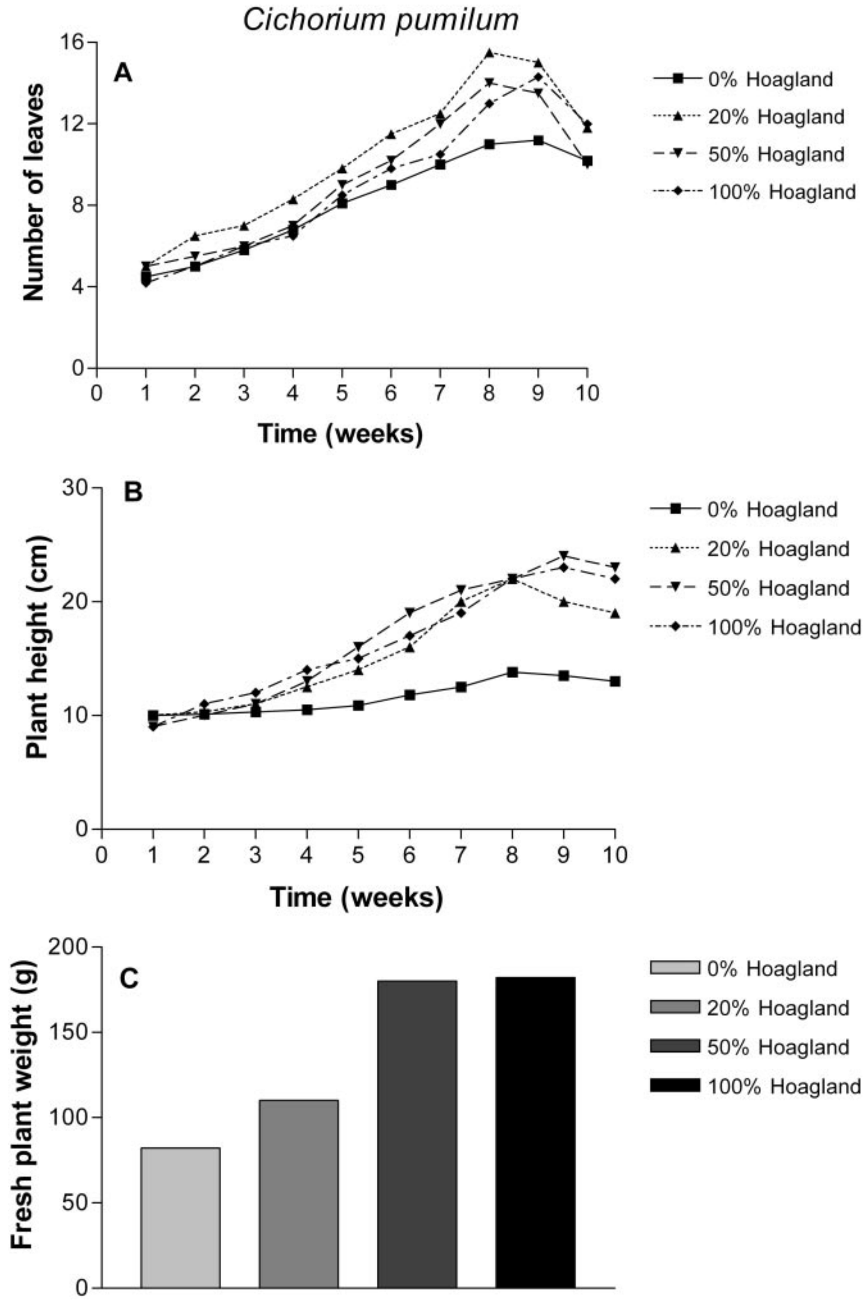

Figure 1. Effect of different fertilization regimes on the average number of green leaves (**A**), plant height (**B**) and fresh weight (**C**) of *C. pumilum* cultivated for 10 weeks under greenhouse conditions. Values presented are mean of 10 plants.

Antioxidant Activity of Powders Prepared from Wild and Cultivated Plants

All powders prepared from the aerial parts of the four wild growing plants inhibited oxidation of β-carotene (Fig. 5). The extent of inhibition was dependent on the species and the plant parts. The extent of inhibition caused by powders prepared from *C. pumilum, E. creticum* and *P. palaestina* was greater than 84%, whereas the powders prepared from either the leaves or fruits of *T. polium* were less effective (60–65%) (Fig. 5). Fig. 6 describes the effect of different fertilization regimes on antioxidant potential of powders prepared from *E. creticum* and *T. polium*. Increasing the amount of fertilizer caused a significant concentration-dependent reduction in antioxidant activity of powders prepared from the cultivated *E. creticum* when compared with the wild plant (Fig. 6, top). In contrast, we observed a significant concentration-dependent increase in antioxidant activity of the cultivated *T. polium* compared with the wild type (Fig. 6, bottom).

Discussion

The increased demand for medicinal plants has created an ecological crisis for medicinal herbs growing in the wild raising

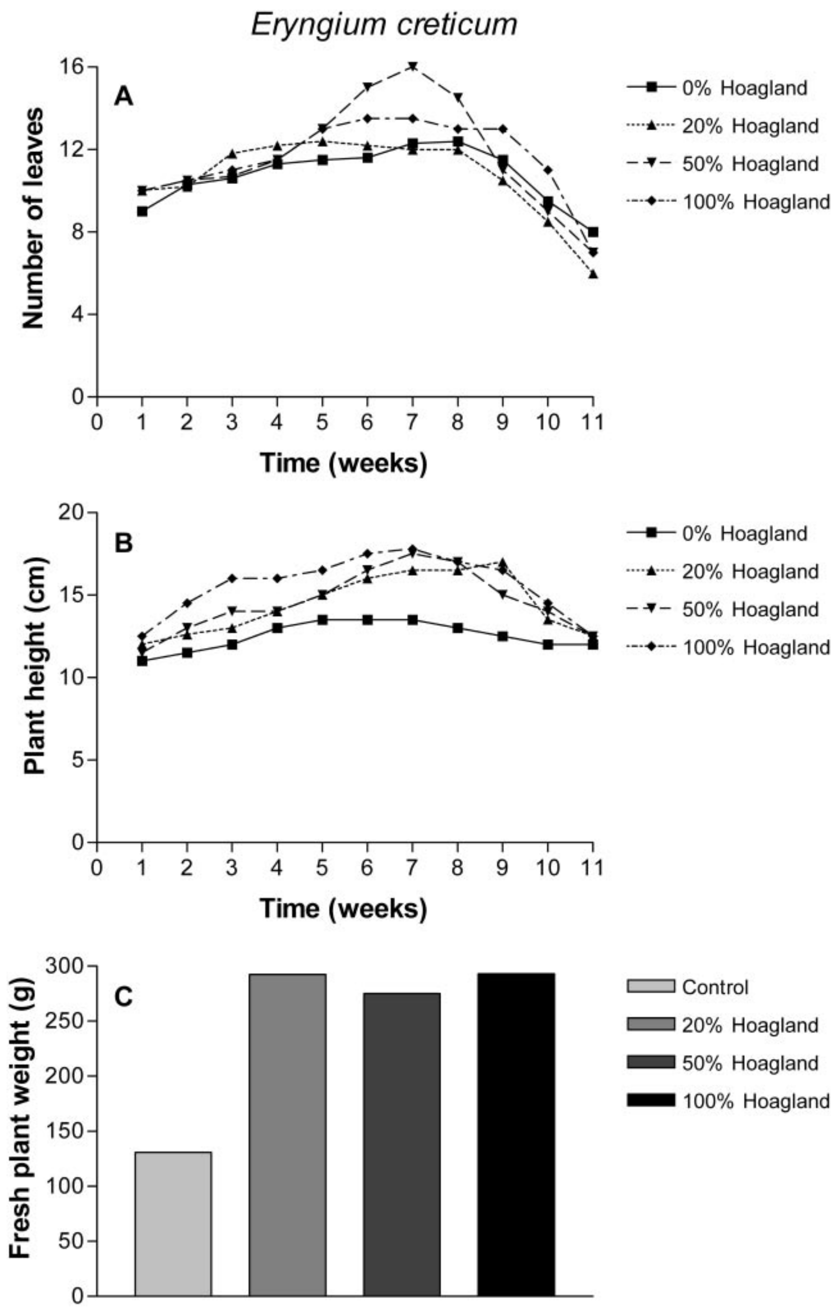

Figure 2. Effect of different fertilization regimes on the average number of green leaves (**A**), plant height (**B**) and fresh weight (**C**) of *E. creticum* cultivated for 11 weeks under greenhouse conditions. Values presented are mean of 10 plants.

alarm about their rate of extinction (5,19,20). Accordingly, conservation agencies are now recommending that wild species be brought into cultivation systems (6). The four plant species analyzed in this study are well-known medicinal herbs used to treat various diseases including liver, diabetes, rheumatism and microbial inflammation in traditional Arab medicine (8,11,21–23). Against this background, the first aim was to try to cultivate these plants under greenhouse conditions. While these four plants are not threatened or endangered species, our results indicate that it is possible to cultivate wild medicinal plants under greenhouse conditions because each plant grew following transplantation. Natural-product chemists are expressing concern that potential therapeutically useful phytopharmaceuticals are at risk of being lost irretrievably due to overexploitation of wild-growing medicinal plants (24,25). Therefore, the second aim was to assess the contribution of different fertilization regimes to antioxidant activity of powders prepared from cultivated medicinal plants as a surrogate marker of potential therapeutic usefulness. In this regard, we found that fertilization regimes influenced the antioxidant activity.

The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties due to the presence of flavonoids, a class of natural polyphenols found in green plant

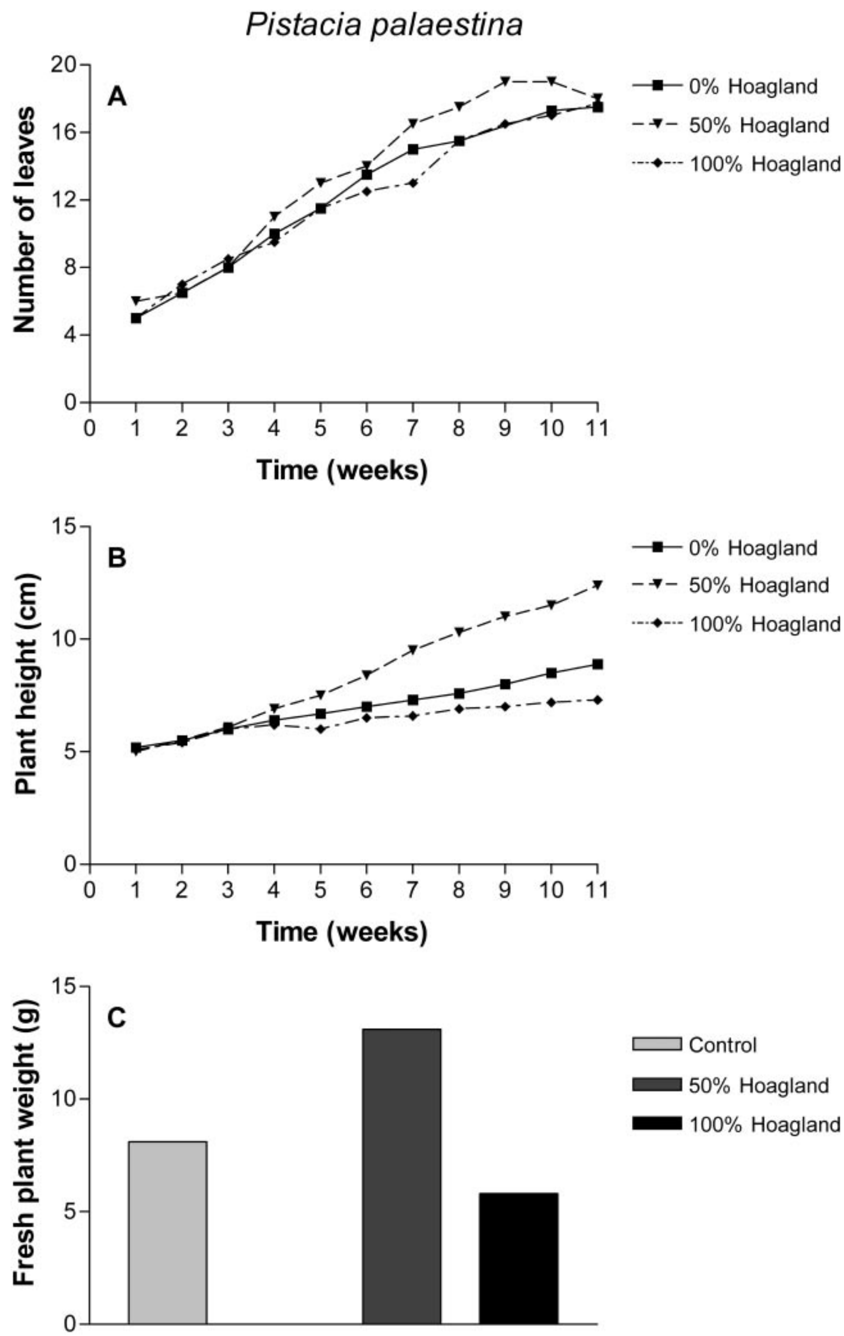

Figure 3. Effect of different fertilization regimes on the average number of green leaves (**A**), plant height (**B**) and fresh weight (**C**) of *P. palaestina* cultivated for 11 weeks under greenhouse conditions. Values presented are mean of 10 plants.

cells (13–15). Wild plants tend to exhibit great variation in their content of aromatic compounds due to environmental and genetic differences (19,26). We showed that fertilizations regimes may alter antioxidant activity of cultivated medicinal plants when compared with the antioxidant activity of wild medicinal plants. More specifically, increasing the amount of fertilizer increased the antioxidant activity of the powders prepared from cultivated *T. polium*. In contrast, increasing the amount of fertilizer decreased the antioxidant activity of powders prepared from cultivated *E. creticum.* Although we demonstrated that fertilization regimes influence the antioxidant potential, this finding should not be interpreted to suggest that other therapeutically beneficial plant ingredients may not be affected in the same manner. Accordingly, we conclude that the indices of plant growth cannot be solely used as parameters of successful growth of cultivated plants.

Leaf senescence is a crucial developmental state in the life of plants. It is the time during which compounds synthesized by the plant during its growth phase are mobilized into younger tissues. Leaf senescence also indicates the beginning of the

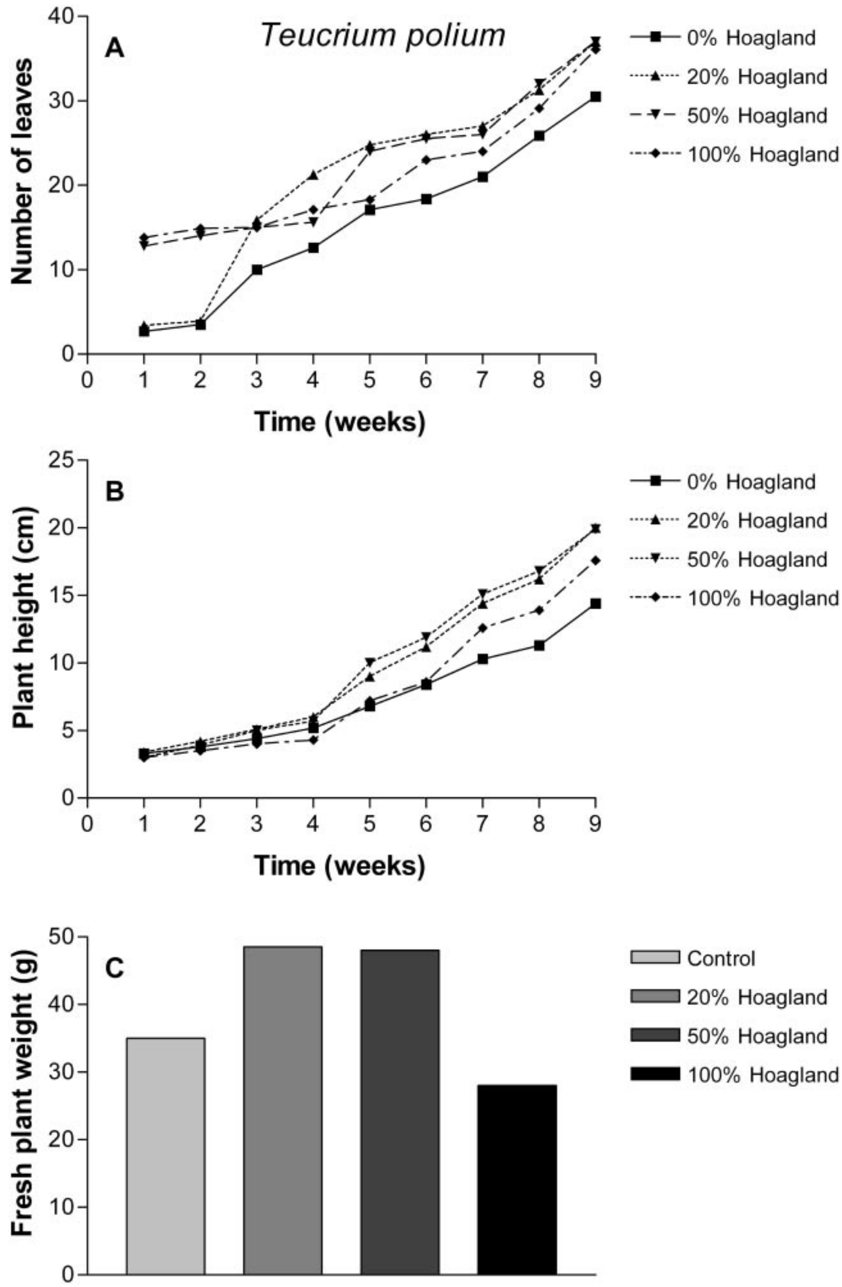

Figure 4. Effect of different fertilization regimes on the average number of green leaves (**A**), plant height (**B**) and fresh weight (**C**) of *T. polium* cultivated for 9 weeks under greenhouse conditions. Values presented are mean of 10 plants.

harvest period (27) and has been shown to affect antioxidant activity (28). In our current work, the antioxidant activity of *T. polium* was measured in powders prepared while the plant was still growing or before the onset of leaf senescence. In contrast, the antioxidant activity of *E. creticum* was determined when the plant was no longer growing or during leaf senescence because some leaves started wilting. We believe leaf senescence of the two cultivated plants may account for differences in antioxidant activity in response to different fertilization regimes. Wild plants and cultivated species exhibit great variation in content of secondary metabolites as well as their biological activities due to the growth environment (i.e. soil type, nutrients, topographic, salinity, drought, allelochemicals) and genetic differences among species (19,26,29,30). These factors should be considered by ethnopharmacologists before prescribing remedies from plants collected from various regions as well as the pharmaceutical companies dealing with medicinal herbs. This fact is crucial nowadays since traditional medicine and alternative systems of medicine (CAM) is practiced in more than 70% of the developing world's population and there is increase demand in the modern world (1,2,31). Evidence-based CAM therapies have shown remarkable success in healing acute as well as chronic diseases. In addition, the growing popularity of

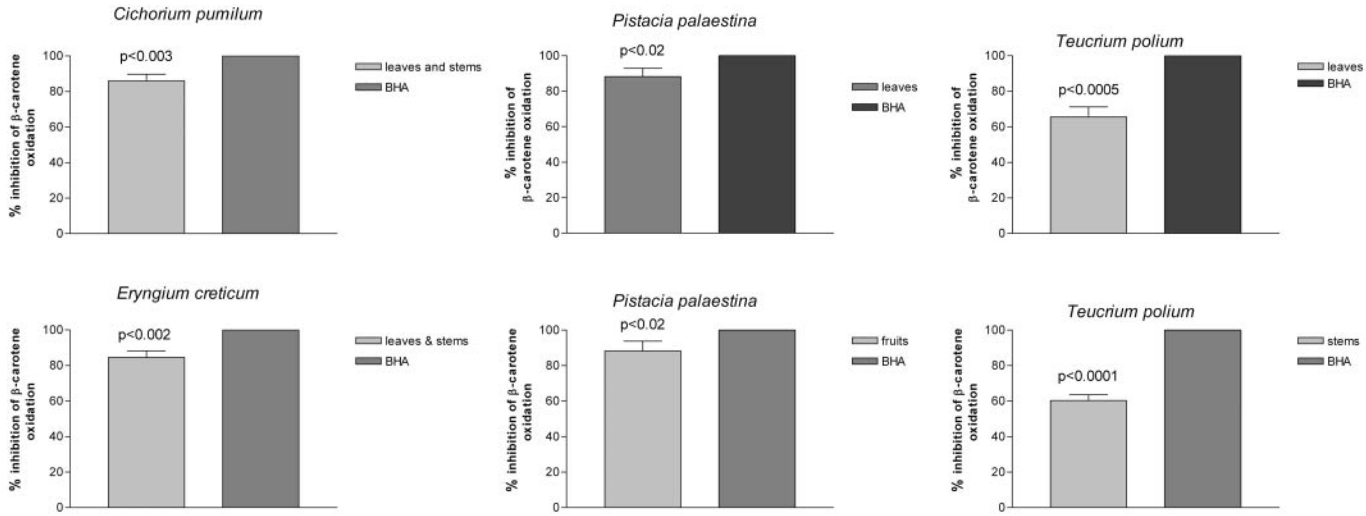

Figure 5. The effect of powders (100 μg ml^{-1}) prepared from various aerial parts of *C. pumilum* (top left), *E. creticum* (bottom left), *P. palaestina* (top and bottom middle) and *T. polium* (top and bottom right) on the inhibition of β-carotene oxidation. The *P*-values represent the significance of the difference from the extent of inhibition obtained by plant powders (100 μg ml^{-1}) and 100 μg ml^{-1} of BHA control. Values presented are mean of three replicates ± SD.

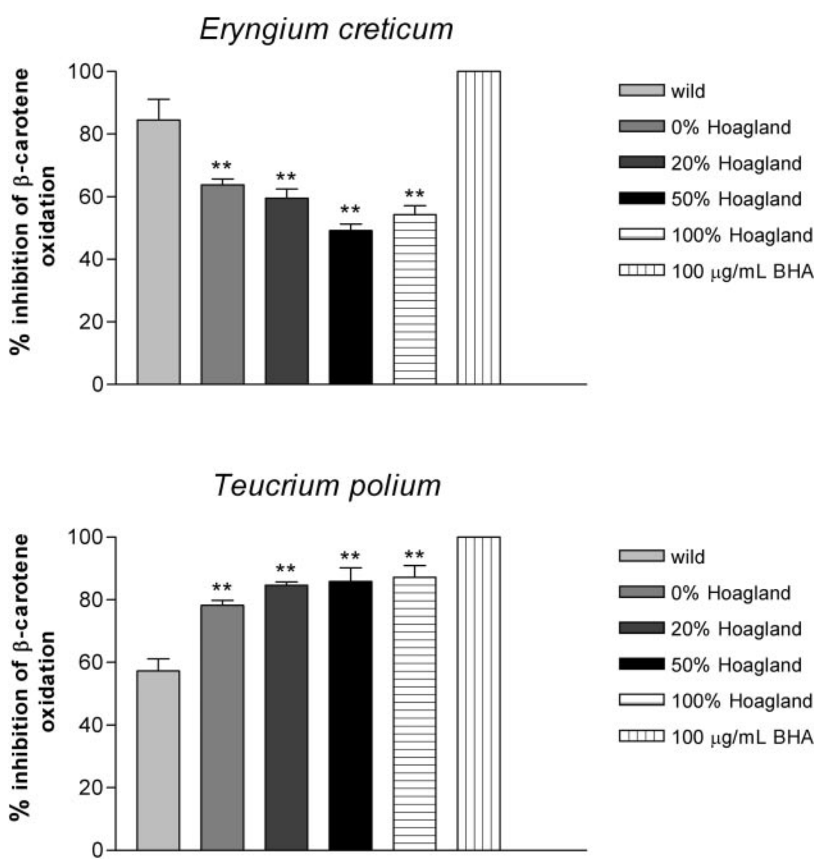

Figure 6. The effect of different fertilization regimes on powders (100 μg ml^{-1}) prepared from cultivated *E. creticum* (top) and *T. polium* (bottom) on the inhibition of β-carotene oxidation. *$P < 0.05$ and represent the significance of the difference from the extent of inhibition obtained by powders (100 μg ml^{-1}) prepared from the wild growing species and 100 μg ml^{-1} BHA control. Values presented are mean of three replicates ± SD.

botanical dietary supplements has been accompanied by concern regarding the quality of commercial products and their sources; therefore, health care providers should keep themselves informed regarding product quality in order to be able to appropriately advise patients utilizing both conventional and herbal medicines (3,4,32).

To conclude, we were able to successfully cultivate wild growing medicinal plants. Cultivation *per se* has several advantages. First, cultivation reduces the possibility of incorrect identification and adulteration. Second, cultivated plants can be irrigated and fertilized to increase their growth rate thereby improving yield. Third, cultivated plants can be grown in areas of similar climate and soil. Our results also showed that cultivation success of medicinal plants should incorporate indices of therapeutic potential. Such an approach can reduce or even eliminate the variation in content of the desired aromatic compounds as well as providing information on the best time to harvest so that the content of the desired aromatic compound(s) is maximal.

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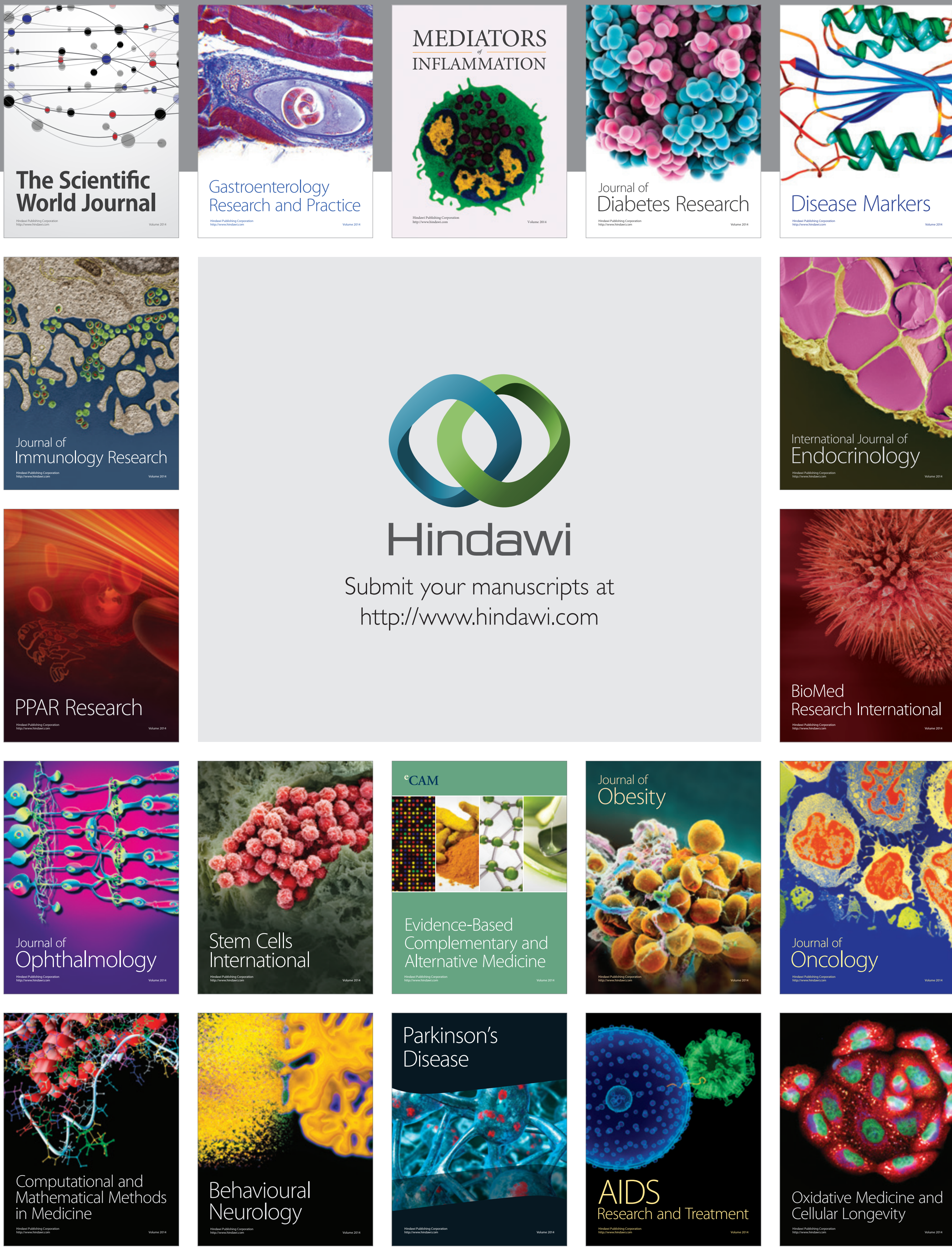
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