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Improved Growth, Yield and Essential Oil Content of Basil Grown under Different Levels of Phosphorus Sprays in the Field

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ABSTRACT

Field experiments were conducted during 2008 year in the Experimental Farm of Shiraz University located in the southwest region of Iran (a clay silt loam soil, a semi-arid moderate climatic area), to study the effects of phosphorus fertilization on the essential oil content, fresh and dry weight, plant and flower cluster height and chlorophyll content of basil (*Ocimum basilicum* L.). Phosphorus fertilizer was sprayed at 0, 2, 4, 6, 8 and 10 % at two time of basil growth (20 and 60 % full flowering). The essential oil was obtained by hydro- distillation from the herb of the plant. According to the results, phosphorus spray treatments had marked effects on the essential oil content of basil. Application of phosphorus significantly increased the essential oil content. 2 % treatment produced the highest amount of essential oil (0.93 %) in aerial parts and 10 % treatment produced the maximum amount of plant and flower cluster height (54.33 and 17.73 cm). However, fresh and dry weight was not affected significantly.

Key Words: Basil (*Ocimum b asilicum* L.), chlorophyll content, Hydro-distillation, Essential oil content, Plant growth indices.

INTRODUCTION

Basil (*Ocimum basilicum* L.), one of the major essential oils producing species of Labiatae family (Grayer et al. [11]), is an annual herb commonly used in many kinds of food preparations in Mediterranean countries. Its extracts are also used in the manufacturing of cosmetic and pharmaceutical products or pesticides (Umerie et al. [28]; Keita et al. [15]; Pascual-Villalobos and Ballesta-Acosta [19]). The high economical value of basil oil is due to the presence of phenyl propanoids, like eugenol, chavicol and their derivatives, or terpenoids, like the monoterpene alcohol, linalool, methyl cinnamate, limonene epi-α-cadinol, α–bergamotene, γ-cadinene, methyl chavicol (estragol), Geraniol, Geranial and citral (Bowes and Zheljazkov [2]; Ijaz Hussain et al. [12]; Sifola and Barbieri [24]; Pascual-Villalobos and Ballesta-Acosta [19]; Telci et al. [25]; Chalchat and Ozcan [4]).

Since basil is an important plant in medicine, the investigation on the cultivation aspects of this plant is essential. Previous researches suggest that the content of secondary metabolites in medicinal plants fluctuates with changing of environmental conditions.

Production of essential oil in aromatic plants may be affected positively or negatively by kind and amount of fertilizers (Fonseca et al. [9]; Saharkhiz et al. [20]). Field experiments carried out on many essential oil bearing plants have shown that yield and composition of essential oil varies considerably by application of fertilizers (Tiwari and Banafar [26]; Figueira [8]; Dethier et al. [5]).

Investigations on marjoram (*Origanum majorana* L.) have shown that total yield of volatile oil was increased by 50 %, as phosphorus was increased from zero to 3.0 mM in soil solution (Trivino and Johnson [27]). Also, Saharkhiz et al. [20] reported that application of phosphorus significantly increased the essential oil content of Feverfew as high content of essential oil obtained by adding 150kg/ha P_2O_5. Addition of P-fertilizer in the case of fennel, (*Foeniculum vulgare* Mill.) has also affected the yield and composition of essential oil (Kapoor et al. [14]). These factors control the balance between growth and photosynthesis and determine whether photosynthate is directed towards growth, flowering or synthesis and maturation of secondary metabolites (Loomis [17]; Saharkhiz et al. [20]).

Sangwan et al. [21] reported that there is a direct relationship between photosynthetic carbon products, like glyceraldehyde-3-phosphate or pyruvate and terpenoids biosynthesis. Sucrose and CO_2 are apparently the preferred exogenous precursors for essential oil synthesis. Also, phosphorus is the plant nutrient elements that have an important role in assimilation and essential oil synthesis of medicinal plants. Farnesyl diphosphate will compress to geranyle diphosphate and linalool diphosphate, so these compounds are precursor of essential oils production.

Most labiates accumulate terpenes and a range of other components in the epidermal glands of leaves, stems and reproductive structures (Gershenzon et al. [10]). As oil biosynthesis occurs in the leaves, their growth and photosynthetic capacity are important factors for oil production (Burbott and Loomis [3]; Duriyaprapan and Britten [6]). The rate of biosynthesis is the chief process that determines monoterpene accumulation in peppermint (Gershenzon et al. [10]).

Investigations about Phosphorus effect on medicinal and aromatic plants have shown that most of these experiments carried out as adding Phosphorus to soil. Therefore, study of phosphorus spray effects on growth and essential oil content, is necessary.

In the present study, we investigated the effect of different levels of Phosphorus sprays on above-ground fresh and dry weight, plant height, flower cluster height, essential oil content, yield and leaf chlorophyll content of basil (*Ocimum basilicum* L.) grown in the southwest region of Iran.

Table 1. Geographical situation and weather condition of the field under basil.

Characteristics	Results
Latitude	29º 36'
Altitude	52º 32'
Sea level	1810 m
Min. Tem*. In recent 10-year period	-9 ºC
Max. Tem. In recent 10-year period	38 ºC
Rain fall in recent 10-year period	400 mm
Climate class	Semi arid moderate
* Temp = Temperature	

Table 2. The daily climatic data of the field under basil (*ocimum basilicum* L.).

Date	T(˚C) Max.	T(˚C) Min.	T (˚C) Avg.	RH (%) Max.	RH (%) Min.	RH (%) Avg.	Sunshine H/Day
Sowing (24/Apr/08)	27.5	4.4	15.95	55	13	34	11.4
First spray (18/June/08)	36.5	15	25.75	65	14	39.5	10.4
Second spray (6/July/08)	37	14	25.5	45	14	29.5	9.40
harvest (17/July/08)	36	17	26.5	35	15	25	11.80

MATERIALS AND METHODS

Location and plant material

The experiment was carried out in 2008 year at the Experimental Farm of Agricultural Faculty, Shiraz University in Badjgah, Shiraz (Table 1) located in the southwest region of Iran.

The soil of experimental plots was a clay-silt-loam with pH of 7.6. The daily climatic data (maximum and minimum temperatures, maximum and minimum relative humidity, Sunshine) were obtained from the Agro-meteorological Station of Irrigation Department located in a state farm about 1 km far from the experimental site (Table 2).

Seeds of basil (O*cimum basilicum* L. cv. green) were sown by hand on April 24 (temperature average: 16 ˚C) in each plot (1×2 m) in rows of 30 cm apart and spaced 10 cm distances between every plant in the row. Each plot had six rows.

Application of Spray treatments

The spray treatments were; 0 (control), 2, 4, 6, 8 and 10 % that applied at two stages of plant growth. Stages of sprays were 20 % of flowering (54 days after sowing time) and 60 % flowering (12 days before harvest time) of plots at early morning. Spray solutions were prepared by adding Potassium phosphate (KH_2PO_4, Merck Company, Germany) in water and 1 ml l^{-1} Rica (commercial detergent). Control treatments were sprayed by water and the same detergent.

Five liters of phosphorous solution was applied for each replication. During spray time, blocks were discrete by plastic layer.

All plots were furrow irrigated three times in week and plants were not face to water deficit during the experiment period. Hoeing and mechanical weed control, were done regularly by hand when necessary. All agronomic management practices were performed as needed.

Analysis of plant growth indices

Before harvesting of plants, plant height (include the inflorescence stalk) and flower cluster length were measured on ten plants in each plot. The aerial parts of basil plants were harvested from the central part of each plot in July 17 (80 days after sowing time) at 90% full bloom of plots with a sharp knife and leaving about 5 cm above the ground surface. Fresh and dry weights of aerial part were measured.

Harvested plants were air dried at room temperature (less than 25 °C). Under this experimental condition, 14 days typically was required based on decrease of herb weight (12 % humidity in herb) to complete the drying process.

Chlorophyll content

Chlorophyll readings were taken with a hand-held dual wave length meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot, the 10 youngest fully expanded leaves per plot were used when the plants were at anthesis (full flowering stage of basil). The instrument stored and automatically averaged these readings to generate one reading per plot.

The leaf chlorophyll was measured after extraction with 90% acetone (Jeffrey and Humphrey [13]) using leaf disks of about 2 cm^2 each. After centrifuging the sample at 1500–2000 rpm for 10 min, the absorbance of supernatants was read at 645 and 663 nm using a spectrophotometer Hatch DR-2000 (Hach Company, Loveland, CO, USA).

Oil isolation

100 g of dried herb (aerial parts include foliage+ flower) was subjected to water distillation (hydro-distillation) for 3 h using an all glass Clevenger-type apparatus, to produce oil according to the method recommended by the European Pharmacopoeia [7].

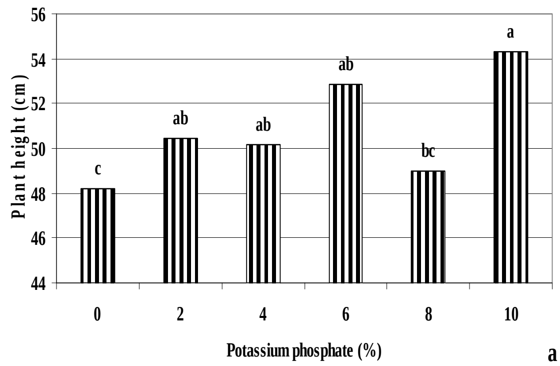
The oil volume was measured directly in the extraction burette. Yield percentage was calculated as volume (ml) of essential oil per 1000 g of plant dry matter. The obtained oil were dried over anhydrous sodium sulphate and stored in sealed vial at low temperature.

Statistical analysis

The experiment was arranged as a completely randomized design (CRD) with three replications for each treatment. Each treatment included 60 plants. An ANOVA was made to determine the effects of each treatment on above-ground fresh and dry biomass, plant height, flower cluster height, leaf essential oil content, yield and leaf chlorophyll content of basil. All analyses were performed with a statistical software package (SPSS version 13) and the means were compared by tukey and Duncan's multiple Range tests at 5% and 1% levels of probability.

RESULTS AND DISCUSSION

Phosphorus increased the plant height and flower cluster height in most concentrations in comparison with control (P = 0.05), so the highest plant and flower cluster obtained in 10 % treatment. No difference at 5 % level at flower cluster height was observed between 2 % and control (Figure 1b). It can be clearly seen that plant height was affected either by P-fertilization (Figure 1a). Also, application of the Phosphorus at 10 % concentration resulted in scorch of leaf margin.



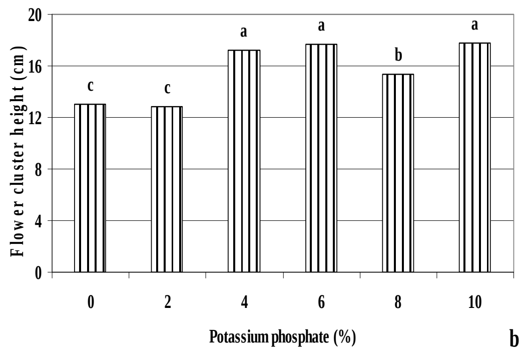


Figure 1. The effect of phosphorus sprays on the plant (a) and flower cluster (b) height of basil (*Ocimum basilicum* L.) in the 2008 year. Means followed by the same letter are not significantly different, as indicated by Duncan's Multiple Range Test at $P > 0.05$.

The positive effect of the fertilization was mostly due to P fertilization, which is in accordance with previous studies in calcareous Mediterranean shrublands (Sardans et al. [22]).

Increasing Phosphorus levels caused a marked positive effect on fresh and dry weights of basil (Figure 2a and b). Alvarez-Castellanos and Pascual-Villalobos [1] reported that application of NPK (52-61-46 kg/ha) before sowing of *Chrysanthemum co ronarium*, increased dry matter.

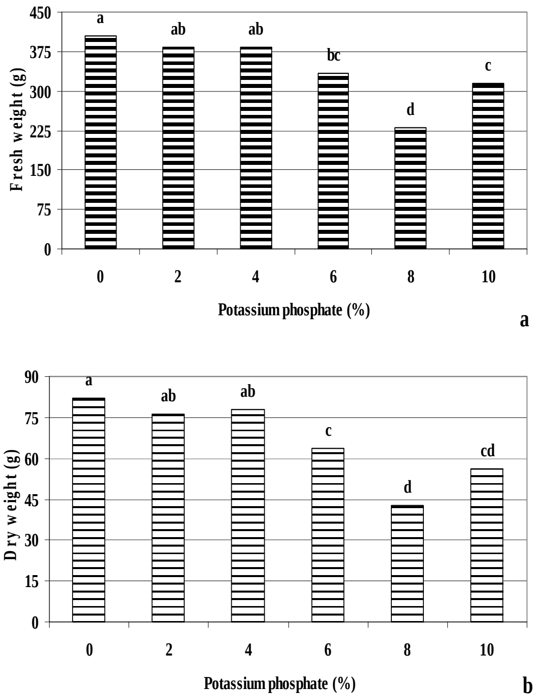
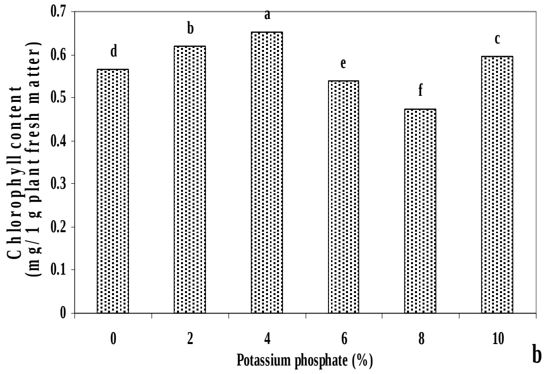


Figure 2. The effect of phosphorus sprays on the fresh (a) and dry (b) weights of basil (*Ocimum basilicum* L.) in the 2008 year. Means followed by the same letter are not significantly different, as indicated by Tukey's Multiple Range Test at $P > 0.01$.

The positive effects of the P fertilization on growth that was still detected with the increases in P concentrations showed the clear limiting role of Phosphorus. In fact, the limiting role of phosphorus in widespread areas of the different Mediterranean zones of the world has been observed in many studies (Zinke [30]; Witkowski et al. [29]; Kruger [16]).

The content of chlorophyll (Figure 3b) ranged from 0.47 to 0.65 mg/ 1 g of plant fresh matter. Significantly higher chlorophyll content was observed with increase of phosphorus concentration up to 4 %, so that Chlorophyll content in P concentrations above of 4 % was decreased. However, chlorophyll content of 10 % treatment again was increased. Chlorophyll content of 2, 4 and 10 % were more than the control. Chlorophyll content in basil can be affected by fertilization especially phosphorus.



Differences in the yield of the essential oils under the influence of the Phosphorus fertilizer, have been reported for several plants (Nikolova et al. [18]; Saharkhiz et al. [20]).Also our results, indicated that various levels of Phosphorus had major effect on the amount of basil essential oils.

The results also, showed that essential oil content was increased significantly by all phosphorus treatments in comparison with control (P = 0.05). These results are similar to the work of (Nikolova et al. 1999; Saharkhiz et al. [20]; Alvarez-Castellanos and Pascual-Villalobos [1]), they reported that P fertilizer significantly increased the essential oil content of chamomile (*Chamomilla recuti ta* L.), Feverfew (*Tanacetum parthenium* cv. Zardband), *Chrysanthemum coronarium*, respectively.

In this study, the essential oil content varied from 0.77-0.93 % dry matter according to the Phosphorus concentration (Figure 3, a), so that, maximum yield of essential oil from herb was obtained in 2 % treatment (93.5 ml/kg dry matter) and lowest yield of essential oil obtained in control treatment (77.3 ml/kg dry matter). These results confirmed those reported by Alvarez-Castellanos and Pascual-Villalobos [1] who indicated that higher yield of essential oil obtained when fertilizer was applied.

Similar the pattern of increasing of the essential oil by application of phosphorus treatments, essential oil content of basil in this study was significantly increased in different Potassium phosphate rates.

Raising the phosphorus rate, increased essential oil content of basil. This may be attributed to the fact that phosphorus is required for production of high quality seed, since it operates in coenzymes involved in energy transfer reactions. Energy is tapped in photosynthesis in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP). This energy is then used in photosynthetic fixation of CO_2 and in the synthesis of lipids and other essential organic compounds (Sawan et al. [23]).

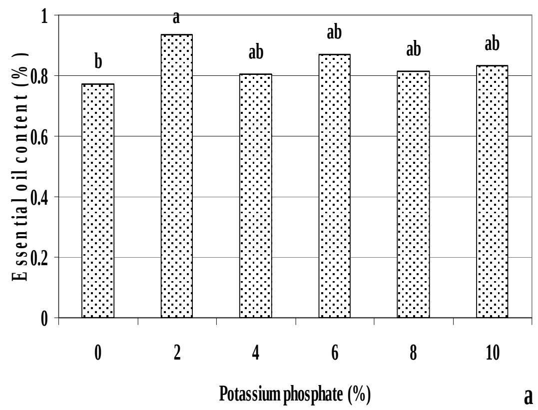


Figure 3. The effect of phosphorus sprays on the essential oil (a) and chlorophyll (b) contents of basil (*Ocimum basilicum* L.) in the 2008 year. Means followed by the same letter are not significantly different, as indicated by Duncan's Multiple Range Test at $P < 0.05$ (left) and Tukey's Multiple Range Test at $P < 0.01$(right).

The present study indicated that phosphorus could be applied as spray when basil in grown in the field. As biomass and oil yield were increased in this study, therefore, this mode of P application (foliar application) could be recommended as a new alternative to farmers. In conclusion, from the practical point of view, the increase in fresh and dry biomass and oil yield induced by P application has positive effects since the commercial value of basil and farmer incomes also depends on the amount of essential oil produced.

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REFERENCES

[1]. Alvarez-Castellanos PP, Pascual-Villalobos MJ. 2003. Effect of fertilizer on yield and composition of flowerhead essential oil of *Chrysanthemum coronarium* (Asteraceae) cultivated in Spain. Industrial Crops Products. 17: 77-81.

[2]. Bowes KM, Zheljazkov VD. 2004. Factors affecting yields and essential oil quality of *Ocimum sanctum* L. and *Ocimum basilicum* L. cultivars. Journal of American Society Horticulture Science. 129: 789-794.

[3]. Burbott AJ, Loomis WD. 1967. Effects of light and temperature on the monoterpenes of peppermint. Plant Physiology. 42: 20-28.

[4]. Chalchat JC, Ozcan MM. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. Food Chemistry. 110: 501-503.

[5]. Dethier M, Sakubu S, Ciza A, Cordier Y, Menut C, Lamaty G. 1997. Aromatic plants of Central Africa XXVIII. Influence of cultural treatment and harvest time on vetiver oil quality in Burundi. Journal of Essential Oil Research. 9: 447-451.

[6]. Duriyaprapan IS, Britten EJ. 1982. The effect of age and location of leaf on quantity and quality of corn mint oil production. Journal of Experimental Botany. 33: 810-814.

[7]. European Pharmacopoeia. 2005. Council of Europe, Strasbourg, 5th ed. 2: 2667-2668.

[8]. Figueira GM. 1996. Mineral nutrition, production and artmisinin content in *Artemisia annua* L. Acta Horticulture. 426: 573-577.

[9]. Fonseca JM, Rushing JW, Rajapakse NC, Thomas RL, Riley MB. 2006. Potential implications of medicinal plant production in controlled environments: the case of feverfew (*Tanacetum parthenium*). Horticulturae Science. 41: 531-535.

[10]. Gershenzon J, McConkey ME, Croteau RB. 2000. Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiology. 122: 205-213.

[11]. Grayer RG, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. 1996. Infraspecific taxonomy and essential oil chemotypes in basil, *Ocimum basilicum*. Phytochemistry. 43: 1033-1039.

[12]. Ijaz Hussain A, Anwar F, Sherazi STH, Przybylski R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry. 108: 986-995.

[13]. Jeffrey SW, Humphrey GF. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemistry Physiology Pflanzen 167: 191-194.

[14]. Kapoor R, Giri B, Mukerji KG. 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill. on mycorrhizal inoculation supplemented with P-fertilizer. Bioresource Technology. 93: 307-311.

[15]. Keita SM, Vincent C, Schmit JP, Arnason JT, Belanger A. 2001. Efficacy of essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobrochus maculatus* (Fab) (*Coleoptera: Bruchidae*). Journal of Stored Products Research. 37: 339-349.

[16]. Kruger, FJ. 1979. South African heathlands. In: Specht, R.L. (Ed.), Ecosystems of the World. Heathlands and Related Shrublands. Elsevier, Amsterdam pp: 19-80.

[17]. Loomis, WD, 1977. Physiology of essential oil production in mint. Proc 28th Annu Mtg Oreg Essential oil growers league, January 13-14.

[18]. Nikolova A, Kozhuharova K, Zheljazkov VD, Craker LE. 1999. Mineral nutrition of chamomile (*Chamomilla re cutita* L.). Acta Horticulture. 502: 203-208.

[19]. Pascual-Villalobos MJ, Ballesta-Acosta MC. 2003. Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on *Callosobruchus maculates* . Biochemical Systematics and Ecology. 31: 673-679.

[20]. Saharkhiz MJ, Omidbaigi R, Sefidkon F. 2007. The Effect of Phosphorus and Irrigation Treatments on the Essential Oil Content and Composition of Feverfew (*Tanacetum parthenium* (L.) cv. Zardband). Journal of essential oil-bearing plants. 10 (5): 391-398.

[21]. Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. 2001. Regulation of essential oil production in plants. Plant Growth Regulation 34: 3-21.

[22]. Sardans J, Roda F, Penuelas J. 2004. Phosphorus limitation and competitive capacities of *Pinus halepensis* and *Quercus ilex* subsp. *rotundifolia* on different soils. Plant Ecology. 174 (2): 307-319.

[23]. Sawan ZM, Hafez SA, Basyony AE. 2001. Effect of phosphorus fertilization and foliar application of chelated zinc and calcium on seed, protein and oil yields and oil properties of cotton. Journal of Agricultural Science Cambridge. 136: 191-198.

[24]. Sifola MI, Barbieri G. 2006. Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Scientia Horticulturae. 108: 408-413.

[25]. Telci I, Bayram E, Yılmaz G, Avcı B. 2006. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochemical Systematics and Ecology. 34: 489-497.

[26]. Tiwari RJ, Banafar RNS. 1995. Application of nitrogen and phosphorus increases seed yield and essential oil of corriander. Indian Cocoa Arecanut and Spices journal. 19: 51-55.

[27]. Trivino MG, Johnson CB. 2000. Season has a major effect on the essential oil yield response to nutrient supply in *Origanum majorana* . Journal Horticulture Science and Biotechnology. 75 (5): 520-527.

[28]. Umerie SC, Anaso HU, Anyasoro JC. 1998. Insecticidal potentials of *Ocimum basilicum* leaf–extract. Bioresource Technology. 64: 237-239.

[29]. Witkowski ETF, Mitchell DT, Stock WD. 1990. Response of Cape fynbos ecosystems to nutrient additions: shoot growth and nutrient contents of a Proteoid (*Leucospermun parile*) and an ericoid (*Phylica cepha lanta*) evergreen shrub. Acta Oncologica. 11: 311-326.

[30]. Zinke, PJ. 1973. Analogies between the soil vegetation types of Italy, Greece, and California. In: Di Castri, F., Mooney, H.A. (Eds.), Mediterranean-Type Ecosystems, Origin and Structures. Chapman & Hall, London, pp. 61-80.