

## Impact of Plant Density and Irrigation on Yield of Hemp (*Cannabis sativa* L.) in a Mediterranean Semi-arid Environment

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### ABSTRACT

Within the context of climate change, water scarcity is the major constraint to the viability of many crops. Thus, it is necessary to develop strategies for sustainable water management, and introduce alternative crops to sustain the viability of agro-ecosystems. The main objective of this work was to assess the performance of hemp (*Cannabis sativa* L.) subjected to different plant densities and irrigation. Two cultivars (Carma and Ermes) were tested at three plant densities i.e. 40,000, 20,000, and 10,000 plants ha<sup>-1</sup>, under two irrigation regimes: i) fully irrigated with total water supply equal to 100% of ET<sub>c</sub>; and ii) deficit irrigation with 80% of ET<sub>c</sub>. The experimental design was a split-split plot with four replications per combination. At harvest, yield and its components (weight, plant height, stem diameter, and the weight of leaves and flowers relative to the stem weight) were evaluated. Also, the production of chemical compounds for medical use (terpenoids, and fatty acids Omega 3 and 6) were analyzed. The results showed that cv. Carma was the most appropriate in agricultural terms, with a yield significantly higher than cv. Ermes. In terms of plant density, 40,000 and 20,000 plants ha<sup>-1</sup> gave the best results for yield, without significant impact by irrigation rates. Regarding the capability of these varieties to produce relevant chemicals, cv. Ermes yielded higher amounts than did cv. Carma. This work offers a preliminary assessment for hemp cultivation in Andalusia (SW Spain), with important potential under local agro-climatic conditions.

**Keywords:** Carma, Ermes, Omega 6, Omega 3, Terpenoids, Water stress.

### INTRODUCTION

Hemp, an annual crop of Asian origin, has long been used for fibre and fibre-oil extraction, and in recent years for the production of cannabinoids, fatty acids, and other nutritional products for biomedical uses (Leizer *et al.*, 2000). Hemp-seed oil is highly valued for its nutraceutical properties, as well as for its health benefits associated because of some of its components such as linoleic acid, linolenic acid,  $\beta$ -caryophyllene or myrcene (Leizer *et al.*, 2000). The

production strategy is key for determining the fibre quality, seed-oil composition, and biomass production as an energy source (Deferne and Pate, 1996). In the field, the crop response is the result of an interaction between genotype, environment, and management (Struik *et al.*, 2000), with plant density, mineral nutrition, and irrigation regime being the main factors involved in the final yield and its quality (Mediavilla *et al.*, 2001; Amaducci *et al.*, 2002). The interaction of these factors affects plant development, with the flowering time being

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one of the most limiting factors involved in fibre, biomass yield, and seed-oil composition. In this sense, flowering period is closely related to photoperiod control. Early flowering can be related to a reduction in final yield because, once flowering occurs, the plant responds by suppressing its vegetative development, thereby ending plant elongation (Amaducci *et al.*, 2005, 2008; Cosentino *et al.*, 2012).

When crops are healthy and under no limiting factors (water or nutrients), biomass production is directly related to the amount of light received by the crop (Monteith, 1997). In the case of hemp, Asgharipour *et al.* (2006) argued that the light received by the canopy can be described as a logistic function of thermal time. In this sense, after flowering, light reception by hemp plants diminishes as a result of a progressive senescence; this starting point not being well defined for hemp, or the relationship between the plant density and the biomass production. This crop is cultivated under a wide range of plant densities, depending on the final product (biomass, fibre, or seeds) or the expected yield level. According to Rosenthal (1987), a plant density of 10 units  $\text{m}^{-2}$  could be good for cannabinoid production, while for seed production a plant density of 30 units  $\text{m}^{-2}$  (van de Werf, 1994) is best, or between 50-75 plants  $\text{m}^{-2}$  if the main goal is the fibre production (Dempsey, 1975).

In relation to limiting factors such as water availability, the effects of water stress in hemp have not been clarified and the irrigation needs of this crop have not been properly defined. In this context, Lisson (1998) contended that irrigation may be successful in compensating deficiencies in the rainfall amount and shortage of available water in soil, especially during months in which crop needs are higher than the soil-water availability. More pointedly, Hackett (1991) defined water stress as the most limiting factor affecting the fibre yield and quality.

Finally, another problem is the appropriate selection of the crop variety. New improved

varieties have been developed in order to lower the levels of psychoactive ingredients such as  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and to achieve better adaptability to the different agro-climatic conditions.

The main objective of this work was to assess the impact of the combined effect of hemp variety, irrigation rate, and plant density on yield (leaves and flowers), and the biosynthesis of substances having biomedical interest under Mediterranean agro-climatic conditions in south-western Spain.

## MATERIALS AND METHODS

### Experimental Site and Treatments

The trial was conducted in 2012, in an experimental orchard (2,880  $\text{m}^2$ ) located in the Guadalquivir river basin, SW Spain (37° 30' 47" Lat N; 05° 58' 02" Long W). The local climate is typically Mediterranean semi-arid, with an approximate mean annual reference evapotranspiration ( $\text{ET}_0$ ) of 1,200 mm and an average annual rainfall of 550 mm, distributed mainly from October to April, generating a potential deficit of about 700 mm.

The soil is a Typical Fluvisol (Soil Survey Staff, 2006) with an effective depth down to 2.5 m, low content in organic matter ( $< 15 \text{ g kg}^{-1}$ ), and an available water-holding capacity of 170  $\text{mm m}^{-1}$ . Soil texture is clay loam, with 300, 310, and 390  $\text{g kg}^{-1}$  of sand, clay, and silt, respectively.

Two new varieties were tested: cv. Carma, owned by Vivacell Biotechnology Spain SL, and Ermes, owned by Phytoplant Research SL, both developed by the CRA-CIN Rovigo (Italy), registered in the European Community Plant Variety Office and with contents of  $\Delta^9\text{THC}$  below to 0.2% (RD, 1729/1999; EU, CE 1177/2000; EU, CE 1122/2009).

The experimental design was a split-split plot, where irrigation was assigned to the main plots, plant density to the sub-plots, and hemp variety to sub-sub plots. The experimental unit (24  $\text{m}^2$ ) had four plant rows spaced at 0.9 m, and varying the distance between plants on the

rows, with the plant density being 40,000 (PD<sub>1</sub>), 20,000 (PD<sub>2</sub>), and 10,000 (PD<sub>3</sub>) plants ha<sup>-1</sup>, depending on the experimental design.

Two irrigation rates were tested: i) full irrigation (IR<sub>1</sub>), with 100% of crop evapotranspiration (ET<sub>c</sub>), and deficit irrigation (IR<sub>2</sub>) with 80% of ET<sub>c</sub>.

The crop was irrigated with a drip system, using one pipe line per row with a flow rate of 5 L m<sup>-1</sup> h<sup>-1</sup> in IR<sub>1</sub> and 3.8 L m<sup>-1</sup> h<sup>-1</sup> in IR<sub>2</sub>. During the first crop growth stage i.e. from sowing (on day 138 of the year; DOY) until the plant was established (173 DOY), the same amount of water was applied in both treatments in order to avoid negative effects at this early stage of plant establishment. On 173 DOY, water deficit was applied in IR<sub>2</sub> according to the experimental design.

The seasonal values of ET<sub>c</sub> were determined by the modified Penman equation (Allen *et al.*, 1998) and the Doorenbos and Pruitt (1977) equation, with average K<sub>c</sub> values ranging as follows: 0.6 from 136 to 166; 0.8 from 167 to 197 DOY; 1.0 from 198 to 228 DOY; and 1.2 from 229 DOY to harvest.

Both treatments received the same amount of irrigation water (143 mm) during crop establishment, whereas, later, 253 mm were applied in IR<sub>1</sub> and 187 mm in IR<sub>2</sub>. Given the ET<sub>c</sub> values during the growing period (445 mm), IR<sub>1</sub> treatment received water amounting to almost 90% ET<sub>c</sub>, while IR<sub>2</sub> was irrigated with 75% ET<sub>c</sub>.

Plant nutrients were applied through the irrigation system at equal amounts for all treatments. During the vegetative stage, higher amounts of N were applied, whereas more P was applied during the flowering stage. Thus, the seasonal average of nutrients applied was 100, 72, and 172 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, respectively.

### Plant Sampling and Statistical Analysis

Ermes plants were harvested on 247-248 DOY, while Carma was harvested 15 days later. The plants located in the two central rows of each experimental unit were hand collected to determine the yield. The

collected sample size varied according to the sowing density, with 6 plants from the smallest density, 9 from the second, and 16 plants from the highest density. Plants fresh weight was measured using a precision balance, recording the plant height prior to being harvested. After harvest, the plants were dried in a greenhouse. When the plants were sufficiently dry, 7-10 days after the harvest, the leaves and flowers were separated from the stems to determine their dry weight. For each sample (flowers and leaves), the main chemical compounds of biomedical interest were analyzed:

-- Omega 3 acids:  $\alpha$ -linolenic and stearidonic.

-- Omega 6 acids: Linoleic, and  $\gamma$ -linolenic.

-- Terpenes:  $\alpha$ -pinene,  $\beta$ -myrcene, and limonene.

These analyses were made by gas chromatography, using a fused silica capillary column Zebron-5MS (30 m x 0.25 mm I.D., 0.25  $\mu$ m), and a flux of 1.3 mL min<sup>-1</sup> (Phenomenex, Torrance, CA, USA) according to the Official Analysis Methods of EU (AOAC, 2000).

For the statistical analysis, the data were subjected to a three-way analysis of variance (ANOVA, SPSS 15.0 statistical package; SPSS, Chicago, IL, USA), comparing irrigation treatments, hemp varieties, and plant densities, while Tukey's test was used for mean separations (P < 0.05).

## RESULTS AND DISCUSSION

### Irrigation, Planting Density, and Yield Response

During the growth period (May to September), the weather conditions were typical of the area, with an average temperature of 26°C and maximum values ranging between 30 and 45°C. Minimum temperatures ranged between 15 and 20°C, averaging 17°C. Daily ET<sub>0</sub> values were close to 6-7 mm from May to July, and fell to 4

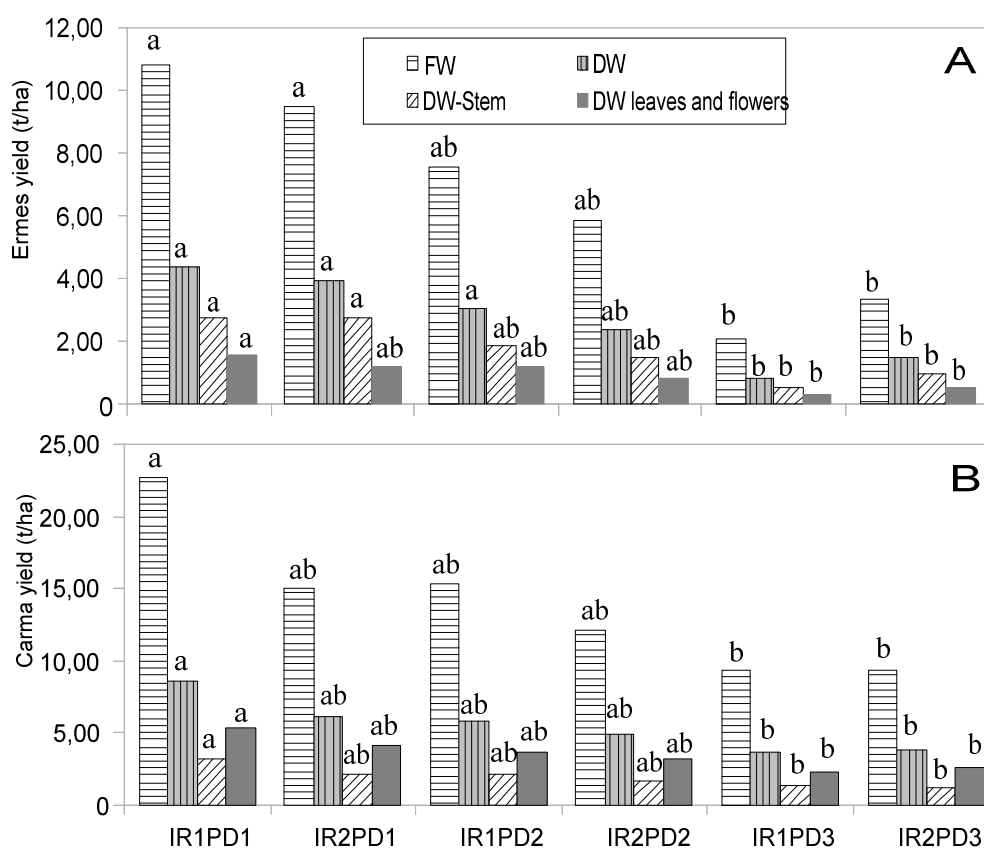


mm day<sup>-1</sup> in September, when the crop was harvested.

In general, the data recorded for both Ermes and Carma fresh and dry weight, leaf and inflorescence weight, and stem weight showed significant differences in the plots with the highest planting densities (PD<sub>1</sub> and PD<sub>2</sub>) compared with the plots with the lowest density (PD<sub>3</sub>). Within each planting density treatment, yield values of IR<sub>1</sub> were slightly higher than those of IR<sub>2</sub>, without significant differences. Only PD<sub>3</sub> registered a minor yield increment in IR<sub>2</sub> vs. IR<sub>1</sub> (Figure 1-A). Similar results were found for Carma, although in the case of yield levels the fresh and dry weights were noticeably higher than for Ermes (Figure 1-B). The statistical analyses concerning the influence of the irrigation treatments on fresh matter,

total dry weight, dry weight of leaves and inflorescence, and stem weight showed significant differences between the fully irrigated plots (IR<sub>1</sub>) and highest planting density (PD<sub>1</sub>) compared with the lowest density plots (PD<sub>3</sub>). Regarding the differences between the two cultivars, the ratio of dry to fresh weight was very similar (close to 0.40). However, considering the dry weight, the relationship between flower and leaf weight with respect to the total dry weight markedly differed between varieties.

Table 1 shows the main differences between varieties according to the parameters studied. Carma registered better results in terms of total yield and dry weight of flowers and leaves. Furthermore, 60% of the total dry weight corresponded to the leaves and flowers, with no significant



**Figure 1.** Yield values in Ermes (A) and Carma (B). Bars with different letters significantly differ ( $p < 0.05$ ) by Tukey's test. IR<sub>1</sub>, irrigated at 100% of crop evapotranspiration (ET<sub>c</sub>); IR<sub>2</sub>, irrigated at 80% of ET<sub>c</sub>; PD, plant density; FW, fresh weight; DW, dry weight.

**Table 1.** Yield components for each hemp variety studied.

Treatments	Fresh weight		Dry weight		Dry weight of flowers and leaves	
	Ermes	Carma	Ermes	Carma	Ermes	Carma
			(t ha <sup>-1</sup> )			
IR <sub>1</sub> PD <sub>1</sub>	10.8 ± 6.0c	22.7 ± 7.4a	4.4 ± 2.4ab	8.5 ± 2.7a	1.6 ± 0.8ab	5.3 ± 1.0a
IR <sub>2</sub> PD <sub>1</sub>	9.5 ± 3.1c	15.1 ± 2.1b	4.0 ± 1.3b	6.1 ± 0.9a	1.2 ± 0.5b	4.1 ± 0.6ab
IR <sub>1</sub> PD <sub>2</sub>	7.6 ± 5.6c	15.3 ± 5.6b	3.1 ± 2.5b	5.8 ± 2.0ab	1.2 ± 0.9b	3.7 ± 0.9ab
IR <sub>2</sub> PD <sub>2</sub>	5.8 ± 2.4c	12.1 ± 5.6c	2.4 ± 1.0bc	4.9 ± 2.1ab	0.9 ± 0.4b	3.2 ± 0.8ab
IR <sub>1</sub> PD <sub>3</sub>	2.1 ± 0.2c	9.3 ± 3.6c	0.8 ± 0.1c	3.6 ± 1.4b	0.3 ± 0.1c	2.3 ± 0.5b
IR <sub>2</sub> PD <sub>3</sub>	3.4 ± 0.6c	9.3 ± 2.9c	1.5 ± 0.5c	3.8 ± 1.2b	0.5 ± 0.1c	2.5 ± 0.4b

IR<sub>1</sub>, irrigated at 100% of crop evapotranspiration (ETc); IR<sub>2</sub>, irrigated with 80% of ETc; PD, plant density ± standard deviation. Different letters within the same column statistically differ at  $p < 0.05$  Tukey's test.

variations due to irrigation or plant density. The percentage distribution of stem, flowers, and leaves in cv. Ermes was completely the opposite of Carma, with 60% of the total weight belonging to the stem. Carma showed not only higher yield values but this ratio was significantly better than for Ermes, which had higher stem weight. Given that the main goal was to grow hemp to produce different substances of biomedical interest from leaves and flowers, Ermes would not be a recommendable variety.

Regarding the yield response of these varieties, the fresh and the dry weight were significantly higher in Carma, with the largest differences in the lowest planting density. In Carma, the highest weight was found in PD<sub>1</sub> in both irrigation rates and in PD<sub>2</sub> only in fully irrigated plants (Table 1). In Ermes, the average fresh weight and dry weight of leaves and flowers were higher in PD<sub>1</sub> and IR<sub>1</sub>PD<sub>2</sub>.

The irrigation regimes showed no significant differences for the same planting density and variety, although IR<sub>2</sub> showed higher values in PD<sub>2</sub> and PD<sub>3</sub> for Carma in comparison to IR<sub>1</sub> at these same densities. Given the comparison between varieties, Carma registered significantly higher results than did Ermes, this being linked to the differences found in yield, values for Carma being six-fold higher than that for Ermes.

These findings suggest that plant density is the main factor governing yield under

Mediterranean conditions. Moreover, van der Werf *et al.* (1995) and Struik *et al.* (2000) reported that plant density and harvesting time were the most determining factors in hemp yield, exerting a major impact on fibre production (in terms of quantity and quality). Along this line, van der Werf *et al.* (1995) held that there was a direct relationship between the sowing density and flowering date. Finally, Amaducci *et al.* (2008) argued that plant density was a decisive point closely related to the balance between stem or flower and leaf production. According to this, the stem biomass decreases with increasing the plant density, and, in our case, this would be more suitable than attaining greater stem biomass.

In terms of the effects of irrigation rates on yield, Lisson and Mendham (1998) showed that irrigation was a determining factor on yield, although, in their case, the water stress applied was higher (irrigated at 30 and 60% of the fully irrigated treatment) in contrast to our findings.

However, the total biomass yield in the present study proved lower than reported by other authors such as Tofani (2006), Cosentino *et al.* (2012) or Struik *et al.* (2000). These differences are presumably related to the crop variety and its management, making it necessary to study the suitability of Ermes and Carma in other crop-management situations.

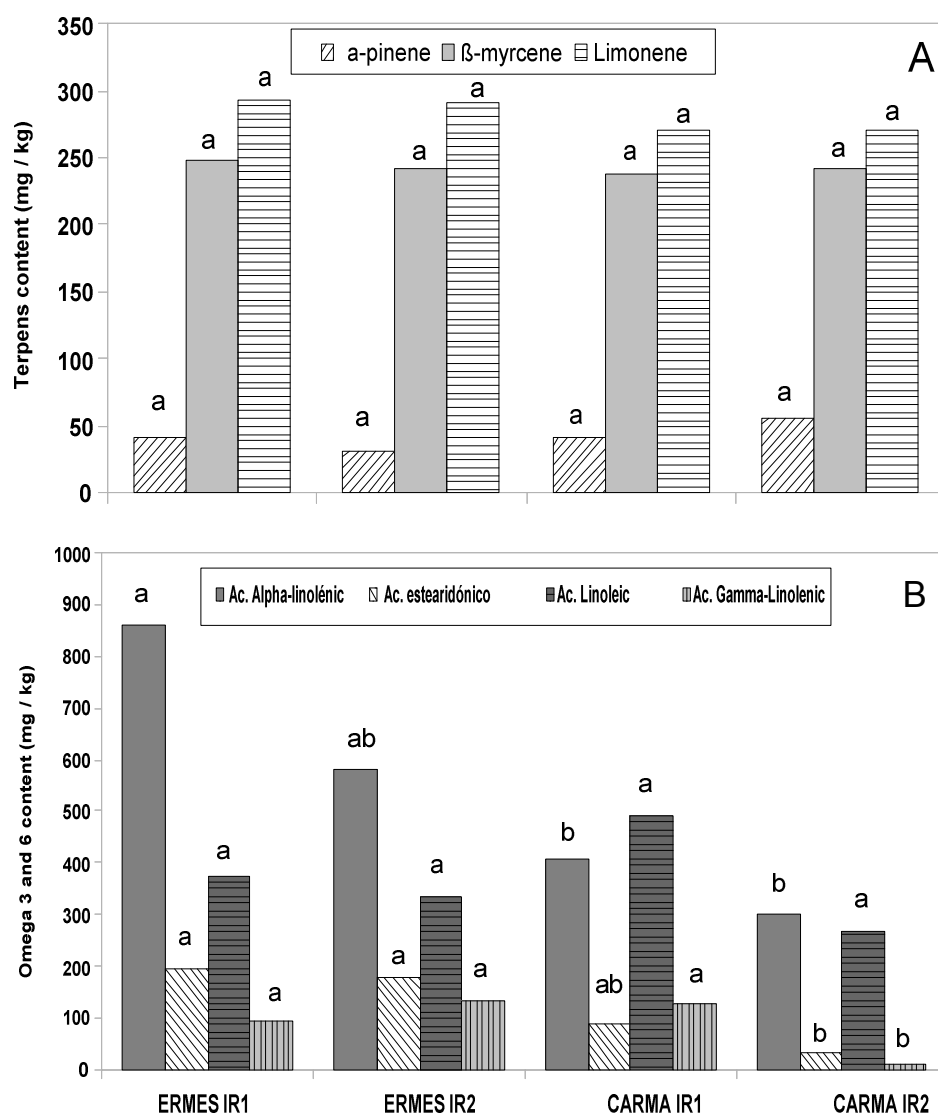


## Chemical Compounds Contents

In both cultivars, no significant differences were found in terpenes and fatty acids due to the irrigation rate or plant density, whereas some significant differences were detected between the cultivars. The overall analysis showed higher contents of  $\beta$ -myrcene and limonene in comparison to  $\alpha$ -pinene. The contents of these compounds were not determined by the crop variety, and were very similar in the studied cultivars (Figure

2-A).

With regard to fatty acids, the content of  $\alpha$ -linolenic was higher than that of stearidonic acid, and these contents were higher in Ermes than in Carma (Figure 2-B). Regarding Omega-6 acids, linoleic acid levels were significantly higher than  $\gamma$ -linolenic, with no differences in plant density or irrigation treatments, whereas significant differences were observed between the two crop varieties, and in Carma, a significant reduction was found in



**Figure 2.** Terpenes (A) and Omega 3 and 6 contents (B) for each variety and irrigation treatment. Each sample corresponds to a mixture of leaves and flowers of the three studied densities. Within each component, bars with different letters significantly differ ( $p < 0.05$ ). IR<sub>1</sub>, irrigated at 100% of crop evapotranspiration (ET<sub>C</sub>); IR<sub>2</sub>, irrigated at 80% of ET<sub>C</sub>.

$\gamma$ -linolenic under deficit irrigation (Figure 2-B).

Ermes showed higher contents of Omega-3 acids than did Carma, the  $\alpha$ -linolenic content being more strongly affected by the irrigation rate. However, in Carma, irrigation affected both  $\gamma$ -linolenic acid and stearidonic acid. These results agree with those of Casano *et al.* (2010), who reported that the presence of some of these compounds, specifically terpenes, is defined by genetic factors and not by environmental ones.

A major question is not only the amount of these specific fatty acids but the ratio between them. This ratio can be altered by the field conditions, crop management, and the variety studied (Leizer *et al.*, 2000). However, the results found did not agree with these assumptions, as the ratio sharply differed for each variety, and was affected by the irrigation rate and plant density.

Thus, on the basis of our experimental results, we conclude that plant density and hemp variety were the most determinant factors affecting yield and quality, with cv. Carma registering the best results in terms of yield and quality parameters. As to the effect of plant density, PD<sub>1</sub> (40,000 plants ha<sup>-1</sup>) gave the highest yield. Certain agricultural practices need to be defined when an alternative crop is developed to achieve the best yield, especially in relation to the amount and quality of irrigation water. However, further research could be focused on developing a strategic planning to identify the potential areas for hemp cultivation in order to increase the yield of biomass as well as its biochemical compounds.

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### اثر تراکم کشت و آبیاری روی عملکرد شاهدانه (*Cannabis sativa* L.) در منطقه نیمه خشک مدیترانه ای

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#### چکیده

در بحث های مربوط به تغییرات آب و هوایی، کمبود آب به عنوان عامل عمده محدودیت رشد گیاهان مطرح می شود. بنا بر این لازم است که استراتژی های مناسبی برای مدیریت پایدار منابع آب تهیه شود و گیاهان جایگزین برای حفظ و تداوم زیست بوم های کشاورزی معرفی شوند. هدف اصلی

پژوهش حاضر برآورد اثر تراکم کشت و آبیاری روی رشد شاهدانه (*Cannabis sativa L.*) بود. دو کلتیوار این گیاه به نام های کارما (Carma) و ارمس (Ermes) در سه تراکم کشت (۴۰۰۰۰، ۲۰۰۰۰ و ۱۰۰۰۰ بوته در هکتار) با دو تیمار آبیاری (آبیاری به مقدار ۱۰۰٪ تبخیر و تعرق گیاه ( $ET_c$ ) و کم آبیاری در حد ۸۰٪ تبخیر و تعرق) مورد آزمون قرار گرفتند. طرح آماری به صورت کرت های دو بارخرد شده (اسپلیت-اسپلیت) در چهار تکرار اجرا شد. در هنگام برداشت، عملکرد و اجزای آن (وزن عملکرد، ارتفاع گیاه، قطر ساقه و وزن برگ ها و گل ها نسبت به وزن ساقه) اندازه گیری شد. همچنین، تولید مواد شیمیایی مورد مصرف در پزشکی (ترپنoidز و اسیدهای چرب امگا ۳ و ۶) بررسی شد. نتایج نشان داد که کالتیوار کارما از نظر کشاورزی بهتر بود چرا که عملکرد آن به طور معنی داری از ارمس بیشتر بود. از نظر تاثیر تراکم کشت، تراکم های ۴۰۰۰۰ و ۲۰۰۰۰ بوته در هکتار بهترین عملکرد را به دست دادند ولی تیمارهای آبیاری تفاوتی نداشتند. از نظر توانایی این کالتیوارها در تولید مواد شیمیایی مربوط، کالتیوار ارمس از کارما تولید بیشتری داشت. نتایج پژوهش حاضر برآوردی از مناسب بودن کشت شاهدانه در اندولس (جنوب غربی اسپانیا) و استعداد خوب آن در شرایط آب و هوایی و کشاورزی محلی به دست میدهد.