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Original article

Effects of foliar application of a byproduct of the two-step olive oil mill process on maize yield

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Abstract – The main objective of this work was to study the effect of foliar fertilization at different doses with a byproduct of the two-step olive oil mill process on the productivity and quality of maize crops (*Zea mays*, L. cv. Tundra) located in Lora del Río, near Sevilla (Andalusia, Spain). Foliar fertilizer was applied four times during the season and three different concentrations were tested. Foliar fertilization increased leaf soluble carbohydrate contents, chlorophyll A and B and carotenoids, and increased the leaf concentrations of N, K, Fe, Mn and Zn. Yield was significantly increased by 24% to 14.9 $t \cdot ha^{-1}$ by the highest dosage of the byproduct, which also caused a 22% increase in kernel number and a 19% increase in grain protein content.

oil mill byproduct / olive oil / foliar fertilizer / maize

Résumé – **Effet sur le maïs de l'application foliaire d'un sous-produit du processus d'élaboration de l'huile d'olive en deux étapes.** L'objectif principal de ce travail était d'étudier l'effet de la fertilisation foliaire à doses différentes avec un sous-produit du processus d'élaboration de l'huile d'olive en deux étapes sur la production et qualité de la récolte du maïs (*Zea mays*, L. cv. Tundra) dans la région de Lora del Río, près de Séville (Andalousie, Espagne). L'engrais foliaire a été appliqué quatre fois pendant la saison et trois concentrations différentes ont été testées. Cet engrais a augmenté les niveaux d'hydrates de carbone, de chlorophylle A et B et de caroténoïdes et des éléments nutritifs N, K, Fe, Mn et Zn dans la feuille. Le rendement a été augmenté de 24 % et a atteint 14,9 $t \cdot ha^{-1}$ avec la dose la plus élevée de sous-produit et s'est accompagné d'une augmentation de 22 % du nombre de grains et de 19 % du contenu en protéines du grain.

sous-produit d'huilerie / huile d'olive / engrais foliaire / maïs

1. INTRODUCTION

The dynamics of plant nutrient uptake are quite complex and dependent on crop growth stages. There is a time lag between fertilizer application and the uptake of applied nutrient by plant roots. For this reason, the use of foliar fertilizing in agriculture has been a popular practice with farmers for supplying the nutrients that the plant requires in the early stages of development. In this respect, recent studies have shown that a small amount of nutrient (nitrogen, potash or phosphate) applied by foliar spraying can increase the yield of crops significantly [1, 2, 4, 8, 11, 19, 30, 37].

Foliar fertilization does not totally replace soil-applied fertilizer, but it does increase the uptake and hence the efficiency of the soil-applied material [1, 8, 11]. This application technique is especially useful for micronutrients, but can also be used for major nutrients such as N, P and K. The amount that can be applied at any one time is small and thus it requires several applications to meet the needs of a crop for these nutrients. However, plant response is dependent on species, fertilizer form, concentration, and frequency of application, as well as the stage of plant growth. The increased efficiency can reduce the need for soil-applied fertilizer and reduce leaching and runoff of fertilizer nutrients, reducing the environmental impact of fertilizer salts [14, 36, 44].

Recently, the application of industrial byproducts to soil (beet vinasse, byproducts of the two-step olive oil mill plants, etc.) has been considered a good environmental and agricultural practice for maintaining soil organic matter, reclaiming degraded soils and supplying plant nutrients [14, 16, 40, 41]. According to recent studies, the addition of byproducts of the two-step olive oil mill plants (rich in humic acids), especially

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those obtained after the second centrifugation in the two-step process, is a product of great agricultural interest due mainly to its organic matter [32, 40, 41]. In this byproduct, these humic acids arise during the process of obtaining the olive oil. In the original plant tissues there are polyphenols and these are oxidized during the process of production of the olive oil, giving rise to these humic substances.

Foliar application of humic acids positively affects the plant growth. In this respect, it affects the uptake of macro- and micro-elements [2, 13, 30] and biochemical effects such as respiration and photosynthesis [23, 25, 46], protein and nucleic acid synthesis [5, 17, 43], and modulates the activity of H^+-ATPase of both plasmalema and tonoplast H^+-ATPases [18, 24, 27].

However, we have not found studies that make reference to the application of these byproducts by foliar fertilization and their impact on maize yield. For this reason, the main objective of this work was to study the effect of foliar fertilization at different doses with a byproduct of the two-step olive oil mill process on the productivity and quality of maize crops, and to evaluate the utility of this byproduct for this crop.

2. MATERIALS AND METHODS

2.1. Properties of the byproduct

The general properties of the byproduct obtained after the second centrifugation in the two-step olive oil mill process are shown in Table I. The methodology for obtaining this byproduct is described in detail elsewhere [9]. In a summary, the byproduct obtained from the first centrifugation in the two-step process is subjected to a second centrifugation to extract residual oil. The results suggest that the byproduct of the second centrifugation is the most suitable in regard to soil permeability, seed germination and phosphorus contents [9].

Table I. Average properties of the byproduct obtained after the second centrifugation in the two-step olive oil extraction process (oven dry basis)[a]. The analysis was performed by MAPA [20].

	Mean ± standard error
pH	3.9 ± 0.4
Density ($g \cdot cm^{-3}$)	1.18 ± 0.07
Dry matter ($g \cdot kg^{-1}$)	285 ± 24
Organic matter ($g \cdot kg^{-1}$)	150 ± 18
Humic acid-C ($g \cdot kg^{-1}$)	87 ± 6
Fulvic acid-C ($g \cdot kg^{-1}$)	0.64 ± 0.10
Kjeldahl-N ($g \cdot kg^{-1}$)	10 ± 2
P ($g \cdot kg^{-1}$)	8 ± 1
K ($g \cdot kg^{-1}$)	40 ± 6
Ca ($mg \cdot kg^{-1}$)	910 ± 27
Mg ($mg \cdot kg^{-1}$)	440 ± 31
Fe ($mg \cdot kg^{-1}$)	470 ± 66
Cu ($mg \cdot kg^{-1}$)	4.9 ± 0.8
Mn ($mg \cdot kg^{-1}$)	5.4 ± 1.1
Zn ($mg \cdot kg^{-1}$)	14 ± 3
Polyphenols ($g \cdot kg^{-1}$)	23 ± 7
Sugars ($g \cdot kg^{-1}$)	0.48 ± 0.11
Lipids ($g \cdot kg^{-1}$)	0.32 ± 0.09

[a] Data are the means of 7 samples.

2.2. Site

The study was conducted from March to September 2001 in Lora del Río, near Sevilla (Andalusia, Spain). The soil of the field experiment is a calcaric Fluvisol [6]. The main soil (0–25 cm) characteristics are the following: pH 7.5 determined in distilled water with a glass electrode (soil:H_2O ratio 1:1), 0.3 $g \cdot kg^{-1}$ total N [15], 1.2 $g \cdot kg^{-1}$ total C (dry wt. basis) as determined by the dry combustion method [15], 2.3 $mg \cdot kg^{-1}$ available P [8], 17.2 $cmol \cdot kg^{-1}$ cationic exchange capacity [10], and clay 122 $g \cdot kg^{-1}$ soil, silt 143 $g \cdot kg^{-1}$ soil and sand 735 $g \cdot kg^{-1}$ soil (determined by the Robinson's pipette method; [34]).

2.3. Experimental layout and treatments

The experimental layout was a split-plot in a randomized complete block with a total amount of 12 plots, with each plot measuring 8 m × 6 m. Three treatments were used (3 replicates per treatment): (1) treatment A0, plots without foliar fertilizer; (2) treatment A1, plots foliar-fertilized with byproduct at a dose of 15 cm^3/100 L; (3) treatment A2, plots foliar-fertilized with byproduct at a dose of 30 cm^3/100 L, and (4) treatment A3, plots foliar-fertilized with byproduct at a dose of 50 cm^3/100 L. The foliar fertilizer (byproduct) was applied using a hand-held CO_2 powered sprayer adjusted to a constant pressure of 0.017 MPa (no other additives were used). The plots were sprayed during late afternoon or evening hours when wind speed was less than 7 $km \cdot h^{-1}$ and air temperature was less than 23 °C.

Table II shows the irrigation plan carried out during the experiment for all treatments (common practice in the area) and the climatic characteristics of the study area. This irrigation was carried out by sprinklers. Independently of the irrigation that all plots received, the plants that were foliar-fertilized received the same quantity of water but different byproduct rates. The climatic characteristics of the study area are typical for a Mediterranean climate.

All plots were additionally fertilized with 300 kg $N \cdot ha^{-1}$ (as urea), 80 kg $P \cdot ha^{-1}$ [as $(NH_4)H_2PO_4$)] and 120 kg $K \cdot ha^{-1}$ (as K_2SO_4), which is the common practice in the area. The mineral fertilizers were incorporated on 10 March 2001 to a 25-cm depth.

Maize (*Zea mays*, L. cv. Tundra) was sown at a rate of 100 000 $plants \cdot ha^{-1}$ in 75-cm inter-row spacing, which is common practice in the area. The sowing date was 19 March 2001. Prophylactic applications of herbicide (MCPA) and herbicide insecticide (α-cipermethrine) were applied.

Foliar treatments were applied four times, from 15 April to 30 August 2001 (15 April, 14 May, 16 June and 14 July). Visual ratings of leaf injury due to the fertilizer application were

Table II. Irrigation plan carried out and climatic characteristics during the experiment.

Irrigation plan			Climatic characteristics	
Week	No. irrigation	m^3	rainfall (m^3)	air temperature (°C)
1 (sowing)	3	42	0	16.3
2	3	42	0	16.5
3	3	52	0	16.6
4	3	88	8.7	16.2
5	3	120	5.3	15.8
6	3	150	0	16.0
7	3	165	0	16.4
8 (maize was 40 cm high)	3	185	4.5	16.2
9	3	190	0	19.5
10	3	230	8.1	18.3
11	3	250	2.3	19.0
12	3	230	0	19.4
13 (maize was 70 cm high)	3	220	3.4	21.2
14	3	200	2.5	23.4
15	3	192	0	24.5
16	3	192	4.8	26.1
17	3	192	0	27.2
18	3	192	0	27.8
19	3	190	0	28.3
20 (tasseling)	3	190	0	28.9
21	3	160	0	29.8
22	3	140	0	30.4
23	3	120	0	32.3
24	3	100	0	30.5
25	2	95	0	27.0
26	2	90	0	26.9
27	2	80	10.8	26.0
28	2	70	2.3	26.1
29 (harvest)	1	65	0	23.8

collected from all trials by two independent observers. Leaf injury was expressed as the percentage of leaf area damaged. Potential treatment effects on the duration of green leaf area were estimated by visual ratings of the proportion of green and yellow leaves before leaf drop began.

Triplicate leaf samples were taken from each of the plots at four stages during the maize growth cycle: (i) when the maize was 40 cm high, 2 May 2001; (ii) when the maize was 70 cm high, 10 June 2001; (iii) at tasseling, 27 July 2001, and (iv) at harvest, 28 September 2001, by selecting the spike leaves for the dates. The aerial parts of eight plants were collected from all replications of the same treatments at the same growth stage. Leaf samples were washed, frozen in a liquid N_2 and stored in a freezer at 20 °C until analyzed. Leaf samples were mineralized [3] and subjected to the following analyses: K, Ca, Mg, Fe, Cu, Mn and Zn by atomic absorption spectrophotometry; P by the Williams and Stewart method, described by [10], and N by the Kjeldahl method [20] on fresh matter. Chlorophylls and total carotenoids in the lyophilized leaf samples were measured by extraction with methanol and quantified by the Lichtenthaler method [12]. Leaf soluble carbohydrate contents were measured using the anthrone method [45]. About 50 g samples were collected from each plot. Dried leaf samples were extracted in 5 cm^3 80% (v/v) ethanol (30 min, 30 °C). The extract was centrifuged (10 min, 2650 × g) and the pellet was extracted again with ethanol. After centrifugation, chlorophyll was removed from the combined supernatants by chloroform extraction. The samples were analyzed colorimetrically for soluble carbohydrates using the anthrone method.

Crop yield ($kg \cdot ha^{-1}$), number of grains per corncob and protein content [20] were determined on samples collected in each plot on 28 September 2001. Grain mineral composition was characterized by analyzing N, P, K, Ca, Mg, Fe, Cu, Mn and Zn by techniques described previously. Also, grain soluble carbohydrate contents were characterized by a technique described previously.

2.4. Statistical analysis

Analysis of variance was performed on leaf mineral nutrient and soluble carbohydrate contents, chemical composition of pigments, grain mineral nutrient and soluble carbohydrate contents, and protein content and crop yield parameters' response to foliar byproduct fertilizer using the PROC MIXED procedure in the Statgraphics v. 5.0 software package [35] and considering the treatment as the independent variable. The means were separated by the Tukey's test, considering a significance level of $P < 0.05$ throughout the study.

3. RESULTS AND DISCUSSION

3.1. Leaf injury and plant maturity

The foliar fertilization treatments produced little leaf injury and, therefore, the results are not shown. The A1 and A2 treatments produced no visual injury in any trial. The A3 treatment produced moderate leaf injury because of the acid pH of the byproduct. This byproduct has a very high capacity buffer [9], and therefore, when mixing 50 cm^3 of the byproduct with 100 L of water (treatment A3), the dissolution continues, presenting an acid pH. Since the quantity of byproduct used in this treatment is bigger, and due to this pH characteristic, when applying by foliar fertilization on the plant, the structures of some cells are damaged. The percentage of leaf area affected was only 4% or less. Foliar fertilization did not affect the maturity of the maize plants and, therefore, the data are not shown.

3.2. Leaf mineral nutrient content of the maize growth cycle

Table III shows the dynamics of leaf mineral contents during the maize cycle, expressed on a dry matter basis. The A3 treatment had the highest leaf average N levels for the first

Table III. Leaf mineral nutrient content of the maize growth cycle (on a dry matter basis).

	N†	P	K	Ca	Mg	Fe	Mn	Cu	Zn
	($g \cdot kg^{-1}$)					($mg \cdot kg^{-1}$)			
maize was 40 cm high (2 May)									
A0 treatment	34.7	2.4	41.0	7.6	6.4	448	63	10	33
A1 treatment	35.9	2.5	42.6	7.7	6.2	450	62	11	35
A2 treatment	36.4	2.5	43.7	7.8	6.2	453	60	11	36
A3 treatment	37.2	2.6	44.9	7.8	6.1	459	59	12	38
maize was 70 cm high (10 June)									
A0 treatment	33.8	2.2	39.7	7.7	5.9	438	64	11	34
A1 treatment	34.4	2.3	40.1	7.8	6.0	441	61	12	36
A2 treatment	35.3	2.3	40.9	7.9	6.0	446	60	12	37
A3 treatment	35.9	2.4	41.2	7.9	6.1	450	58	13	39
tasseling (27 July)									
A0 treatment	29.4	2.0	24.1	7.9	3.7	356	79	11	36
A1 treatment	30.1	2.1	24.6	8.2	3.4	364	72	12	39
A2 treatment	30.8	2.2	25.3	8.3	3.3	371	70	13	40
A3 treatment	31.8	2.2	26.1	8.4	3.2	379	69	14	42
harvest (28 September)									
A0 treatment	14.3	1.8	10.9	8.1	2.9	317	86	10	37
A1 treatment	16.1	1.9	11.8	8.5	3.1	324	83	11	41
A2 treatment	16.8	2.0	12.6	8.5	3.2	335	80	11	42
A3 treatment	17.3	2.0	13.4	8.6	3.3	341	78	12	43
Average dates									
A0 treatment	28.1a‡	2.1a	29.1a	7.8a	4.7a	390a	73a	10a	35a
A1 treatment	29.1b	2.2a	29.8a	8.1a	4.7a	395b	69b	11a	38b
A2 treatment	29.8c	2.3a	30.6b	8.1a	4.7a	401c	67b	12a	39b
A3 treatment	30.6d	2.3a	31.4c	8.2a	4.8a	408d	66b	13a	41c

† Fresh matter. ‡ Letters were assigned to show treatment means separation at the 0.05 probability level. Within columns, identical lowercase letters indicate no significant difference between treatments.

data (2 May). The average values of N in leaves for the fertilizer treatments were higher than those of [32] after the application of 15 $t \cdot ha^{-1} \cdot yr^{-1}$ of this byproduct to soil. The statistical analysis indicated that significant differences existed with regard to fertilizer treatments. This increase in plant N in the plots fertilized with humic substances coincided with the results of [4, 8, 19, 30, 37] on green asparagus crop, [22] on oat plants, and [31] on pumpkin plants, where the humic substances resulted in increased plant N. Leaf N levels decreased gradually during the maize cycle, because of N transfers from leaves to spikes and grains for protein synthesis. Absolute values observed at the different stages suggest that crop N nutrition was adequate [32, 39].

The highest values of P, Ca and Mg were observed in plots treated with the highest dose of byproduct, although differences were not statistically significant. Like N, the average values of P, Ca and Mg in leaves for the fertilizer treatments were higher than those of [32]. Lastly, an interaction was observed between P and N, coinciding with [38, 40, 42].

The A3 treatment had the highest leaf average K levels. Like N, the average values of K in leaves for the fertilizer treatments were higher than those of [32] after the application of 15 $t \cdot ha^{-1} \cdot yr^{-1}$ of this byproduct to soil. The statistical analysis indicated that significant differences existed with regard to fertilizer treatments. This higher K content may exert a beneficial effect on maize produced on these plots as this element has had a positive influence on the transfer of carbohydrates to the corncob [32, 39], and improves the yield by more efficient grain filling [21].

With respect to micronutrients, the highest values were observed in plots foliar-fertilized with a higher dose of byproduct, except for the Mn, mainly due to the antagonistic effect of this micronutrient with Fe [40, 41]. Like macronutrients, the average values of micronutrients in leaves for the fertilizer treatments were higher than those of [32] after the application of this byproduct to soil. The statistical analysis indicated significant differences in Fe and Zn with respect to fertilizer treatments. This increased plant Fe and Zn in the plots fertilized with humic substances coincided with the results of [3, 8, 13, 15, 26, 30]. These results indicate that foliar fertilization with humic substances may be helpful in soils that are deficient in Fe or Zn.

3.3. Leaf pigments and soluble carbohydrates analysis

Table IV shows the leaf pigments and soluble carbohydrate contents during the maize cycle. The statistical analysis indicated significant differences in leaf pigments and soluble carbohydrate contents with respect to fertilizer treatments. The highest values of chlorophyll A and B, carotenoids and soluble carbohydrate contents were obtained in the foliar-fertilized plots, mainly where there was a higher supply of byproduct, according to [7, 8, 33]. Leaf pigments and soluble carbohydrate contents increased gradually during the maize cycle until harvest (28 September). Starting from this date, these values in the leaf decreased. These results are of great importance, because photosynthesis could be increased over a longer period of time as the levels of pigments in the leaf increase, resulting in a higher production of soluble carbohydrates and thereby increased yield and grain quality (Tab. VI). These results are in line with [8].

3.4. Chemical analysis of the grains, grain soluble carbohydrates, protein content and crop yield parameters

Table V shows the chemical analysis of the grains from the different treatments. The average values of chemical analysis of the grains for the fertilizer treatments were higher than those of [32] after the application of 15 $t \cdot ha^{-1} \cdot yr^{-1}$ of this byproduct to soil. The most significant differences were found in N and P. For these macronutrients, the highest values were observed with the A3 treatment. The P levels were lower and the N levels higher than the values previously reported [39]. The K, Ca and Mg levels did not show any significant differences with the fertilizer treatments and their values were lower than the values reported by [32] for the same maize variety. These values were higher in the A3 treatment. With respect to the analyzed micronutrients, the most significant differences were observed in Fe and Zn. For these micronutrients, the highest values were observed with the A3 treatment. With respect to the grain soluble carbohydrate contents, the highest values were observed in the plots foliar-fertilized with a higher dose of byproduct. This may be due to transfers of soluble carbohydrates from leaves to grains, coinciding with [28, 29].

Table IV. Leaf pigments and soluble carbohydrate contents.

	Chlorophyll A	Chlorophyll B	Carotenoids	Soluble carbohydrate contents
	($mg \cdot kg^{-1}$)			
	maize was 40 cm high (2 May)			
A0 treatment	2954	1269	1108	115
A1 treatment	3068	1373	1142	124
A2 treatment	3196	2286	1196	139
A3 treatment	3398	2725	1223	150
	maize was 70 cm high (10 June)			
A0 treatment	3978	1308	1119	126
A1 treatment	3197	1410	1163	136
A2 treatment	3285	2493	1201	149
A3 treatment	3501	2882	1246	161
	tasseling (27 July)			
A0 treatment	2986	1317	1136	132
A1 treatment	3205	1475	1198	141
A2 treatment	3378	2539	1277	157
A3 treatment	3582	2905	1316	170
	harvest (28 September)			
A0 treatment	2990	1326	1172	95
A1 treatment	2936	1478	1162	106
A2 treatment	2978	2126	5228	119
A3 treatment	2994	2034	1198	127
	Average dates			
A0 treatment	2977a‡	1305a	1134a	117a
A1 treatment	3099a	1434a	1166a	127a
A2 treatment	3209b	2361b	1213b	141b
A3 treatment	3369c	2606c	1246b	152bc

‡ Letters were assigned to show treatment means separation at the 0.05 probability level. Within columns, identical lowercase letters indicate no significant difference between treatments.

Table VI shows the protein content and crop yield parameters for the different treatments. The average values of protein content and crop yield parameters for the fertilizer treatments were higher than those of [32] after the application of this byproduct to soil. The highest protein content was in the

Table V. Chemical analysis of the grains.

	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	SCC
	($g \cdot kg^{-1}$)					($mg \cdot kg^{-1}$)				($mg \cdot kg^{-1}$)
A0 treatment	13.6a‡	2.3a	4.3a	0.3a	0.9a	17.6a	11.5a	3.2a	19.6a	179a
A1 treatment	14.7ab	2.5a	4.4a	0.4a	1.0a	18.3ab	11.4a	3.3a	20.8a	198ab
A2 treatment	15.6b	2.9ab	4.6a	0.4a	1.0a	19.6b	11.3a	3.3a	21.7ab	211b
A3 treatment	16.b8c	3.2b	4.6a	0.4a	1.1a	21.2bc	11.3a	3.4a	22.6b	226bc

SCC: soluble carbohydrate contents.
‡ Letters were assigned to show treatment means separation at the 0.05 probability level. Within columns, identical lowercase letters indicate no significant difference between treatments.

Table VI. Protein content and crop yield parameters.

	Protein content (%)	No grains corncob^{-1}	Yield (kg·ha^{-1})
A0 treatment	8.5a‡	457a	11357a
A1 treatment	9.2ab	498b	13002b
A2 treatment	9.8b	536c	13879b
A3 treatment	10.5bc	589d	14875c

‡ Letters were assigned to show treatment means separation at the 0.05 probability level. Within columns, identical lowercase letters indicate no significant difference between treatments.

A3 treatment, while the lowest corresponded to the A1 treatment. The values were higher than those reported by [32] for the same maize variety fertilized with this byproduct applied to soil. The fertilizer treatments increased the number of grains per corncob, essentially when the highest rate was applied. Finally, maize yield increased significantly at each level of the byproduct.

4. CONCLUSIONS

There is great agricultural interest in olive oil mill byproduct as a soil additive, mainly due to its organic matter content and the improvement of soil physical, chemical and biological properties and increase in crop yield and quality [32, 40, 41]. However, this byproduct added to soil, while greatly improving its physical properties, needs a certain time to mineralize and supply the nutrient needed by the crops; moreover, a large quantity of product is needed to fulfil the nutritional requirement of the crops. Also, the dynamics of plant nutrient uptake are quite complex and are dependent on crop growth stages. There is a time lag between fertilizer application and the uptake of applied nutrient by plant roots. This is the reason why some authors suggest foliar fertilization for supplying the nutrients that the plant requires in the early stages of development.

In this study the mineral elements that were increased by foliar fertilization with the byproduct (rich in humic substances) were Fe, Zn, N and K. The analysis of leaf pigments indicated the highest values of chlorophyll A and B, and carotenoids in the plots foliar-fertilized with this byproduct. Yield, grain number, grain protein content and grain soluble carbohydrate contents were all increased by foliar fertilization.

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