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# Effects of foliar fertilization of a biostimulant obtained from chicken feathers on maize yield



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ARTICLEINFO	A B S T R A C T
<i>Keywords:</i> Biostimulant Chicken feathers Foliar fertilization Maize crop	Due to the important contribution that it makes to human nutrition, maize is one of the most widely-consumed cereals in the world. There is, therefore, high demand for fertilizers that will maintain maize production at both high yield and quality levels. The objective of this work was to study the effect of foliar fertilization using a biostimulant, obtained by enzymatic hydrolysis from chicken feathers, on the productivity and quality of maize crops ( <i>Zea mays</i> , L. cv PR32W86 Pioneer), located in Trujillanos (Extremadura, Spain), over two consecutive seasons. Foliar biostimulant/biofertilizer was applied three times each season and at two rates (3.6 and $7.2  lha^{-1}$ ). At the higher rate and for both seasons, foliar fertilization significantly increased the leaf concentrations of macro- and micronutrients, while grain protein content and yield increased by 26% and 14%. These results suggest that the foliar use of this

#### 1. Introduction

Foliar fertilization is currently a highly efficient agronomic crop fertilization technique since it favours the assimilation of the nutrients in the plant and consequently, the utilisation of the nutrients applied with the fertilizer, thus increasing crop yields and quality (Tejada and Gonzalez, 2004; Abbas and Ali, 2011; Osman et al., 2013). Since it significantly reduces the effects of groundwater contamination caused by applying inorganic fertilizers to the soil it is, moreover, a technique that contributes to sustainable, environmentally friendly agriculture (Tejada and González, 2003; Fernández and Eichert, 2009).

In recent years, foliar fertilization has been used to apply macronutrients, micronutrients and humic substances. This results in a great number of positive effects in the plant, principally at physiological level (respiration and photosynthesis), at morphological level, (root length and leaf area index), and the yield of various crops such as rice, tomato, pepper and maize (Tejada and González, 2003a; Tejada and Gonzalez, 2004; Karakurt et al., 2009; Tejada et al., 2016).

The use of biostimulants (BS) obtained from various organic residues (carob germ, sewage sludge) by enzymatic hydrolysis processes via foliar fertilization is increasing. This is because these organic compounds are easily assimilated by crops and therefore improve crop nutrition, increasing both the productivity and the quality of the grain or fruit harvested (Parrado et al., 2008; Tejada et al., 2016). Several authors have tested the effectiveness of a BS obtained from chicken feathers by enzymatic hydrolysis processes in the bioremediation of polluted soils with organic xenobiotics (Gómez et al., 2014; Rodríguez-Morgado et al., 2015a, 2015b). However, there are no studies concerning the use of this type of organic compound via foliar fertilisation in order to increase both crop yield and quality.

Maize (*Zea mays* L.) is one of the world's major cereal crops, ranking third in importance after wheat and rice (Lashkari et al., 2011). Most of the maize produced worldwide is used for animal feed, although it is also part of the basic diet in human nutrition, as it is a good source of starch, proteins, lipids, polyphenols, carotenoids, vitamins and dietary fibre (Nuss and Tanumihardjo, 2010; Blandino et al., 2017). Consequently, studying the response of this crop to foliar fertilization of a new BS could be of great interest to the farmer.

The main objective of this paper is to study the effect of a BS obtained from chicken feathers by enzymatic hydrolysis processes when it is applied via foliar in a corn crop, observing both maize yield and grain quality.

#### 2. Material and methods

biostimulant could be of great interest to the farmer for improving both maize crop yield and quality.

#### 2.1. Site and properties of the biostimulant

The study was carried out during two consecutive experimental seasons (from April to October in 2014 and 2015) at Trujillanos,

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#### Table 1

Initial soil physico-chemical characteristics (mean  $\pm$  standard error). Data are the means of three samples.

pH (soil:H <sub>2</sub> O ratio 1:2.5)	$7.1 \pm 0.3$
Electric conductivity (soil: $H_2O$ ratio 1:5) (dS m <sup>-1</sup> )	$0.071 \pm 0.06$
Coarse sand $(g kg^{-1})$	$418 \pm 21$
Fine sand $(g kg^{-1})$	$154 \pm 18$
Silt $(g kg^{-1})$	$246 \pm 20$
Clay $(g kg^{-1})$	$182 \pm 17$
Total C ( $g kg^{-1}$ )	$8.7 \pm 1.5$
Kjeldahl-N (g kg <sup>-1</sup> )	$0.78 \pm 0.13$
Olsen P (mg kg <sup><math>-1</math></sup> )	$11.0 \pm 1.3$
Available K (mg kg <sup>-1</sup> )	$86.4 \pm 10.7$
Available Ca (mg kg <sup>-1</sup> )	$2103~\pm~21$
Available Mg (mg kg <sup>-1</sup> )	$428 \pm 13$
Available Fe (mg kg <sup>-1</sup> )	$80.1 \pm 7.9$
Available Cu (mg kg <sup><math>-1</math></sup> )	$4.6 \pm 1.1$
Available Mn (mg kg $^{-1}$ )	$119 \pm 22$
Available Zn (mg kg <sup><math>-1</math></sup> )	$1.8 \pm 0.3$

(Extremadura, Spain). The climatic characteristics of the study area are detailed in the supplemental material (Table S1) (AEMET, 2017). Total annual rainfall was 342.3 mm in 2015 and 458.4 mm in 2016. Average air temperature averaged 17.8  $^{\circ}$ C in 2015 and 17.5  $^{\circ}$ C in 2016.

The soil used was the same as that described in Tejada et al. (2016). The main soil characteristics (0–25 cm) are described in Table 1. The methodology used for determining each parameter is described in Tejada et al. (2016).

The BS used was obtained from chicken feathers by the enzymatic hydrolysis. The obtaining process is described in Rodríguez-Morgado et al. (2014). This process was carried out in a bioreactor under the following conditions: (a) substrate concentration: 10%; (b) solvent: water; (c) catalytic agent: subtilisin, 0.15% (v/v) (d) Enzymatic concentration:  $1 \text{ mll}^{-1}$  substrate; (e) temperature: 55° C; (f) pH: 9, controlled by the addition of 10 M NaOH; (g) time: 180 min. Finally, the hydrolysed product was centrifuged obtaining the biostimulant. The organic compound's chemical composition is described in Table 2. The methodology used for determining each parameter is described in Rodríguez-Morgado et al. (2015b).

Amino acid composition was determined by reversed-phase HPLC analysis of 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) derivatives, with  $\gamma$ -aminobutyric acid as internal standard (Table 3). The methodology used for determining each parameter of these amino acids is described in Parrado et al. (2008).

Table 2

Chemical composition (mean  $\pm$  standard error) of the biostimulant used for each experimental season. Data are the means of three samples (oven wet basis).

Organic matter $(g kg^{-1})$	459 ± 39
Kjeldahl-N (g kg <sup>-1</sup> )	$15.7 \pm 1.9$
Total carbohydrates (g kg $^{-1}$ )	$69 \pm 10$
Total P (g kg <sup>-1</sup> )	$29.2 \pm 2.2$
Total K (g kg $^{-1}$ )	$1.5 \pm 0.5$
Total S (g kg $^{-1}$ )	$18 \pm 1.9$
Total Ca (g kg <sup><math>-1</math></sup> )	$110 \pm 4$
Total Mg (g kg <sup>-1</sup> )	$23.9 \pm 5.2$
Total Fe (mg kg <sup>-1</sup> )	$13.5 \pm 2.1$
Total Cu (mg kg <sup>-1</sup> )	$2.1 \pm 0.6$
Total Mn (mg kg <sup>-1</sup> )	$33.8 \pm 6.2$
Total Zn (mg kg <sup>-1</sup> )	$0.59 \pm 0.17$
Total Ni (mg kg <sup>-1</sup> )	$0.53~\pm~0.11$
Molecular weight (Da) (%)	
> 10000	$23.4 \pm 2.1$
10000-5000	$8.8 \pm 1.0$
5000-1000	$23.2 \pm 3.1$
1000–300	$6.9 \pm 1.1$
< 300	$37.7 \pm 3.9$

#### Table 3

Amino acid composition (mean  $\pm$  standard error) of the experimental biostimulant. Data are the means of three samples. Results are expressed as grams per 100 g of proteins.

Ala	$5.0 \pm 0.6$	Arg	$7.1 \pm 0.4$
Asp	$10.9 \pm 1.0$	His	$1.6 \pm 0.3$
Cys	ND	Ile	$6.1 \pm 0.7$
Glu	$11.8 \pm 1.2$	Leu	$9.1 \pm 0.7$
Gln	$15.6 \pm 1.9$	Val	$8.9 \pm 0.8$
Gly	$8.4 \pm 0.8$	Lys	$2.9 \pm 0.5$
Pro	$9.5 \pm 0.6$	Met	$1.1 \pm 0.2$
Ser	$10.9 \pm 1.1$	Phe	$5.5 \pm 0.6$
Tyr	$1.1 \pm 0.2$	Thr	$4.2 \pm 0.9$

ND: not determined.

#### 2.2. Experimental layout and treatments

For each experimental season, the experimental layout was a randomized complete block with three treatments and three replicates per treatment. Each plot size was  $9 \text{ m} \times 7 \text{ m}$ . The treatments were the following:

- (1) A0 treatment, plots fertilized with 300 kg N ha<sup>-1</sup> (as urea), 80 kg P ha<sup>-1</sup> + 41.7 kg N ha<sup>-1</sup> [as  $(NH_4)H_2PO_4$ ] and 120 kg K ha<sup>-1</sup> (as K<sub>2</sub>SO<sub>4</sub>), which is common practice in the area
- (2) A1 treatment, plots fertilized with the A0 treatment mineral fertilizers and foliar fertilized with BS at a dose of 3.61 ha<sup>-1</sup>
- (3) A2 treatment plots fertilized with the A0 treatment mineral fertilizers and foliar fertilized with BS at a dose of 7.21 ha<sup>-1</sup>

The doses used in the BS are those described by Tejada et al. (2016) when they applied a BS obtained from sludge and hydrolytic processes. The inorganic fertilizers were incorporated on April 13th 2015 and 18th April 2016, respectively, to a depth of 20–25 cm.

Similar to Tejada et al. (2016), BS was applied three times during the maize vegetative cycle and for each experimental season. In this regard, the BS was applied on July 13th, July 27th and August 17th during the 2015 season, and July 11th, July 25th and August 22nd during the 2016 season. Therefore, the total doses used in the experiment were  $10.8 \, lha^{-1}$  or A1 and  $21.6 \, lha^{-1}$  for A2 in each experimental season.

Maize (*Zea mays* cv PR32W86 Pioneer) was sown at a rate of 100,000 seeds ha<sup>-1</sup> with 75-cm inter-row spacing. The planting dates were April 14th 2015 and April 19th 2016, respectively. Once the harvest was collected during the first experimental season, all of the residues generated were also collected. This was done to prevent these organic residues interfering with plant nutrition.

The irrigation system, irrigation time and amount of water applied to the crop were similar to that described by Tejada et al. (2016). Table 4 shows the chemical composition of the irrigation water used. Values were obtained from the arithmetic mean of 6 samples per year during each vegetative cycle of the plant.

Table 4Chemical composition of the water used in the irrigation crop(mean  $\pm$  standard error) for each experimental season. Dataare the means of six samples.

pН	$6.4 \pm 0.2$
$Ca^{2+}$ (mgl <sup>-1</sup> )	$96.7 \pm 3.4$
$K^{+}$ (mg1 <sup>-1</sup> )	$50.2 \pm 3.5$
$Cl^{-}$ (mg $l^{-1}$ )	$3.5 \pm 1.6$
$SO_4^{2-}$ (mgl <sup>-1</sup> )	$33.2 \pm 4.1$
$HCO_3^{2-}$ (mgl <sup>-1</sup> )	$314 \pm 10$
$NO_3^{-}$ (mg l <sup>-1</sup> )	$22.4 \pm 2.1$

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