

1 **Effects of foliar fertilization of a biostimulant obtained from chicken feathers on maize**
2 **yield**

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4 Manuel Tejada^{1*}, Bruno Rodríguez-Morgado², Patricia Paneque¹, Juan Parrado²

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6 ¹Grupo de Investigación Edafología Ambiental, Departamento de Cristalografía, Mineralogía y
7 Química Agrícola, E.T.S.I.A. Universidad de Sevilla, Crta de Utrera km. 1, 41013 Sevilla, Spain

8 ²Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de
9 Sevilla, C/ Prof. García González 2, 41012 Sevilla, Spain

10

11 * Corresponding author (E-mail address: mtmoral@us.es)

12 Tel.: +34 954486468

13 Fax: +34 954486436

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15 **Abstract**

16 Due to the important contribution that it makes to human nutrition, maize is one of the
17 most widely-consumed cereals in the world. There is, therefore, high demand for fertilizers that
18 will maintain maize production at both high yield and quality levels. The objective of this work
19 was to study the effect of foliar fertilization using a biostimulant, obtained by enzymatic
20 hydrolysis from chicken feathers, on the productivity and quality of maize crops (*Zea mays*, L. cv
21 PR32W86 Pioneer), located in Trujillanos (Extremadura, Spain), over two consecutive seasons.
22 Foliar biostimulant/biofertilizer was applied three times each season and at two rates (3.6 and 7.2 l
23 ha⁻¹). At the higher rate and for both seasons, foliar fertilization significantly increased the leaf
24 concentrations of macro- and micronutrients, while grain protein content and yield increased by
25 26% and 14%. These results suggest that the foliar use of this biostimulant could be of great
26 interest to the farmer for improving both maize crop yield and quality.

27

28 **Keywords:** biostimulant; chicken feathers; foliar fertilization; maize crop

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30 **1. Introduction**

31 Foliar fertilization is currently a highly efficient agronomic crop fertilization technique
32 since it favours the assimilation of the nutrients in the plant and consequently, the utilisation of the
33 nutrients applied with the fertilizer, thus increasing crop yields and quality (Tejada and González,
34 2004; Abbas and Ali, 2011; Osman et al., 2013). Since it significantly reduces the effects of
35 groundwater contamination caused by applying inorganic fertilizers to the soil it is, moreover, a
36 technique that contributes to sustainable, environmentally friendly agriculture (Tejada and
37 González, 2003a; Fernández and Eichert, 2009).

38 In recent years, foliar fertilization has been used to apply macronutrients, micronutrients
39 and humic substances. This results in a great number of positive effects in the plant, principally at

40 physiological level (respiration and photosynthesis), at morphological level, (root length and leaf
41 area index), and the yield of various crops such as rice, tomato, pepper and maize (Tejada and
42 Gonzalez, 2003a, 2004; Karakurt et al., 2009; Tejada et al., 2016).

43 The use of biostimulants (BS) obtained from various organic residues (carob germ, sewage
44 sludge) by enzymatic hydrolysis processes via foliar fertilization is increasing. This is because
45 these organic compounds are easily assimilated by crops and therefore improve crop nutrition,
46 increasing both the productivity and the quality of the grain or fruit harvested (Parrado et al.,
47 2008; Tejada et al., 2016).

48 Several authors have tested the effectiveness of a BS obtained from chicken feathers by
49 enzymatic hydrolysis processes in the bioremediation of polluted soils with organic xenobiotics
50 (Gómez et al., 2014; Rodríguez-Morgado et al., 2015a, b). However, there are no studies
51 concerning the use of this type of organic compound via foliar fertilisation in order to increase
52 both crop yield and quality.

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

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

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65 **2. Material and methods**

66 *2.1. Site and properties of the biostimulant*

67 The study was carried out during two consecutive experimental seasons (from April to
68 October in 2014 and 2015) at Trujillanos, (Extremadura, Spain). The climatic characteristics of the
69 study area are detailed in the supplemental material (Table S1) (AEMET 2017). Total annual
70 rainfall was 342.3 mm in 2015 and 458.4 mm in 2016. Average air temperature averaged 17.8 °C
71 in 2015 and 17.5 °C in 2016.

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

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

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

87 *2.2. Experimental layout and treatments*

88 For each experimental season, the experimental layout was a randomized complete block
89 with three treatments and three replicates per treatment. Each plot size was 9 m × 7 m. The
90 treatments were the following:

- 91
- 92 (1) A0 treatment, plots fertilized with 300 kg N ha⁻¹ (as urea), 80 kg P ha⁻¹ + 41.7 kg N ha⁻¹ [as
93 (NH₄)H₂PO₄] and 120 kg K ha⁻¹ (as K₂SO₄), which is common practice in the area
- 94 (2) A1 treatment, plots fertilized with the A0 treatment mineral fertilizers and foliar fertilized
95 with BS at a dose of 3.6 l ha⁻¹
- 96 (3) A2 treatment plots fertilized with the A0 treatment mineral fertilizers and foliar fertilized
97 with BS at a dose of 7.2 l ha⁻¹

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

102 Similar to Tejada et al. (2016), BS was applied three times during the maize vegetative
103 cycle and for each experimental season. In this regard, the BS was applied on July 13th, July 27th
104 and August 17th during the 2015 season, and July 11th, July 25th and August 22nd during the 2016
105 season. Therefore, the total doses used in the experiment were 10.8 l ha⁻¹ for A1 and 21.6 l ha⁻¹ for
106 A2 in each experimental season.

107 Maize (*Zea mays* cv PR32W86 Pioneer) was sown at a rate of 100000 seeds ha⁻¹ with 75-
108 cm inter-row spacing. The planting dates were April 14th 2015 and April 19th 2016, respectively.
109 Once the harvest was collected during the first experimental season, all of the residues generated
110 were also collected. This was done to prevent these organic residues interfering with plant
111 nutrition.

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

116

117 *2.3. Plant sampling and analytical determinations*

118 In each fertilizer treatment and for each experimental season, the leaves of 10 plants
119 located in the central area of each plot were selected. Leaf samples were collected in two stages of
120 growth: (1) at tasselling [R1 physiological state according to Hanway scale (Ritchie et al., 1986)],
121 occurring on August 8th 2015 and August 5th 2016; and (2) at harvest [R6 physiological state
122 according to Hanway scale (Ritchie et al., 1986)], which took place on October 16th 2015 and
123 October 20th 2016, by selecting the spike leaves (Tejada and González, 2003a; Tejada et al., 2016).

124 Before their analysis, the leaves were subjected to a washing and drying process, described
125 in Tejada et al. (2016). Furthermore, the macro- and micronutrients in the leaves were determined
126 according to the methods described in Tejada et al. (2016).

127 For each season and fertilizer treatment, all the corncobs located in each experimental plot
128 were collected. Number of grains per corncob and crop yield (kg ha^{-1}) was determined in samples
129 collected from each plot on October 14th 2015 and October 20th 2016, respectively.

130 On the other hand, protein concentration, macro- and micronutrients in the grain were
131 determined according to the methodology described in MAPA (1986) and Tejada et al. (2016).

132

133 *2.4 Statistical analysis*

134 With the data obtained, an analysis of variance (ANOVA) was performed considering the
135 treatment as independent variable followed by Tukey's significant difference as a post hoc test,

136 considering a significance level of $p < 0.05$ throughout the study and using Statgraphics Plus 2.1
137 software package.

138

139 **3. Results**

140 Table 5 shows the macro- and micronutrient leaf contents during the maize cycle for each
141 experimental season expressed on a dry matter basis.

142 Regarding N, at harvest and for both experimental season, the A2 treatment showed the
143 highest levels of N in leaf. Compared with the A0 treatment, foliar N concentration was 14.4%
144 and 39.1% higher in the A1 and A2 treatments for the 2015 season, whereas it was 15% and
145 33.3% higher in A1 and A2 treatments for the 2016 season.

146 In the same way, and at harvest, the A2 treatment showed the highest values of P,
147 highlighting the effect of BS on the contents of this macronutrient in leaf. Compared with A0
148 treatment, leaf P concentration was 32.8% and 52.2% higher in A1 and A2 treatments for the 2015
149 season, whereas it was 43.5% and 51.1% higher in the A1 and A2 treatments for the 2016 season
150 (K, Ca and Mg in leaf) are also higher in the foliar fertilized plots with A2, followed by A1,
151 demonstrating the effect of the BS dosage on the concentration of the macronutrients analysed in
152 maize leaf.

153 The micronutrients analysed in leaf show a similar evolution to the macronutrients. At
154 harvest and for both experimental seasons, the highest values were obtained in the A2 treatment,
155 followed by the A1 treatment. The statistical analysis showed significant differences with A2
156 treatment only, again highlighting the importance of the BS rate used in the experiment.

157 The chemical composition of the grains presented a very similar behaviour to the nutrient
158 content in the leaves (Table 6). For both experimental seasons, there was a significant increase in
159 macro- and micronutrients analyzed, principally when the higher BS rate was used.

160 Compared with the A0 treatment, grain protein concentration significantly increased by
161 26.5% in the A2 treatment in the first season and by 25.3% in the second (Table 7). Moreover, the
162 number of grains per corncob significantly increased by 15% in the A2 treatment in the 2015
163 season and by 15.8% in the 2016 season. Finally, the higher application rate significantly
164 increased yield by 13.4% and by 14.6% in the first and the second seasons.

165

166 **4. Discussion**

167 Our results suggest that there is a positive effect on the mineral nutrition of corn when a
168 BS obtained from chicken feathers was applied via foliar application. These results are in
169 agreement with those obtained by Tejada and González (2003a) and Tejada et al. (2016), who
170 observed an increase in the plants' mineral nutrition after the application of a BS obtained from
171 sewage sludge or from a by-product of the two-step olive oil milling process via foliar to a corn
172 crop.

173 In the same way, other authors have obtained similar results after the foliar application of
174 different organic substances and amino acids on rice, corn, tomato, pepper, cucumber, wheat,
175 asparagus and green beans (Tejada and González, 2003b, 2004; Yildirim, 2007; Karakurt et al.,
176 2009; Katkat et al., 2009; Abdel-Mawgoud et al., 2011; El-Nemr et al., 2012).

177 This improvement in the plant mineral nutrition after the foliar fertilization of humic
178 substances and amino acids is mainly due to an improvement in the plants' uptake of nutrients
179 (Tejada et al., 2003b, 2016). Several studies have shown that the foliar application of humic
180 substances increases leafcuticle permeability, favouring the entry of ions attached to these
181 molecules within the plant cell (Fageria et al., 2009; Çelik et al., 2010).

182 Numerous studies have shown the importance of amino acids in the plant's physiological
183 activities, mainly at the cellular level. Since they are highly water-soluble, the positive effects of
184 applying amino acids might be due to their internal function within the cell as an osmo-regulator.

185 This increases the concentration of cellular osmotic components (Abdel-Mawgoud et al., 2011),
186 stimulating cell growth and consequently increasing the plants' chemical composition, as well as
187 the growth, yield and quality of the harvest (Awad et al., 2007; Abdel Aziz, 2009; Thomas et al.,
188 2009; Abd El-Aal et al., 2010). Also and due to the chelating effect of amino acids on
189 micronutrients, when applied together with micronutrients they facilitate the absorption and
190 transport of these micronutrients inside the plant, since they also positively affect cell membrane
191 permeability (Ibrahimn et al., 2010).

192 Some authors propose different formulations of humic acids, amino acids, hydrolysed
193 proteins, etc. as growth promoters, thus improving plant nutrition (Thomas et al., 2009). Our
194 results confirm these judgments, since the BS, with the mixture of substances used in this
195 experiment, favours the mineral nutrition of corn.

196 The increase in grain macro- and micronutrient concentrations is possibly due to the
197 improvement in the plant's mineral nutrition. These results are in agreement with those obtained
198 by Tejada et al. (2016), who found a significant increase in the concentration of macro- and
199 micronutrients in corn grain when they applied the same doses of a BS obtained from sewage
200 sludge, also composed of a mixture of humic substances, low molecular weight peptides, amino
201 acids and macro- and micronutrients. In the same way, these data are in agreement with those
202 obtained by other authors, who found a significant increase in macronutrients in rice and maize
203 grains after the foliar application of different humic substances (Tejada et al., 2006; Osman et al.,
204 2013).

205 The increase in micronutrients in grain is a consequence of the micronutrient-rich BS foliar
206 fertilization. These results are in agreement with those obtained by other authors when applying
207 different micronutrients via foliar to crops such as wheat, cowpea and rice (Simoglou and Dordas,
208 2006; Dordas, 2009; Zeidan et al., 2010; Mabesa et al., 2013; Manzeke et al., 2017). For many

209 authors, foliar fertilization can be used to satisfy the essential micronutrient requirements in crop
210 grains, increasing yields and the quality of production (Fang et al., 2008).

211 The significant increase in the concentration of micronutrients in the grain after the foliar
212 application of the experimental biostimulant is very important. This is because, in general terms,
213 cereal crops usually present a low concentration of such micronutrients in their grain (Cakmak,
214 2010).

215 Finally, the increase in plants' nutrient uptake may be responsible for the increase in the
216 maize yield, highlighting again the influence of the dose of the biostimulant applied to the plant.

217 Many authors consider N as the essential element that directly influences the number of
218 grains per corncob, weight of grains and, consequently, in crop yield (Osborne et al., 2002; Ma et
219 al., 2006; Jin et al., 2012). In our experiment, the high concentration of N that was applied to the
220 plant from the BS in the form of N or as amino acids could be responsible for this increase in the
221 crop yields, number corncob and grains per corncob.

222

223 **5. Conclusions**

224 Foliar fertilization with biostimulants obtained from chicken feathers (rich in organic
225 matter, low molecular weight peptides and amino acids) significantly increased the maize nutrition
226 and, consequently, maize yield and grain quality. This increase was higher when the said product
227 was applied three times during the maize vegetative cycle at a dose of 7.2 l ha^{-1} . It is, however,
228 necessary to continue studying the behaviour of this biostimulant on crops in order to optimise
229 both the application rates and the number of applications needed with the aim of obtaining the
230 maximum responses from the crops when using this compound. In the same way, it is also
231 necessary to study the behaviour of this new organic compound on different soils, since the
232 different physical-chemical properties of the soils can also influence the response of the crop when
233 applying these biostimulants.

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