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Original article

Combined application of chitosan and AMF in corn yield

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ABSTRACT

Two experiments were carried out in field conditions, with the objective of evaluating the QuitoMax® and EcoMic® biostimulants, alone and in combination, in the yield of two varieties of corn (*Zea mays* L.), during the years 2015 and 2016. In both Experiments, the biostimulants were applied by imbibition of grains in QuitoMax® solutions, which were then pelletized with EcoMic®, prior to sowing and, subsequently, the plants were sprinkled in the leaf area with QuitoMax®. In the first experiment, the different combinations of the bioproducts stimulated the majority of the yield components of corn, Creole variety. In the second, the treatment of the grains with QuitoMax® and EcoMic® stimulated the diameter of the ear, the number of rows of grains and the agricultural yield of the P 7928 variety, together with the foliar sprinkling of QuitoMax® in the crop. In the Creole variety, the treatment of the grains prior to sowing with QuitoMax® and EcoMic® followed by the foliar spray of QuitoMax®, increased the yield with respect to the pelletized control with EcoMic® by 62 %, while in the P 7928 variety, both the treatments of each product alone and their combinations, increased the yield with respect to the untreated control between 15 and 22 %. In general, corn yield increased significantly with the use of biostimulants, which suggests that application in the field of EcoMic® and QuitoMax®, alone and in combination, is a recommended option to increase crop yields, depending on variety.

Key words: biostimulants, biofertilizers, mycorrhizae, polymers

INTRODUCTION

Corn (*Zea mays* L.) is a cereal of great economic importance in the world for human and animal consumption [1,2], with a global production of 500 million tons. This crop covers an area greater than 120 million hectares and is grown in more than 70 countries, although its main area is the American continent. In Cuba, corn covers an area between 77,000 and 100,000 hectares, highlighting the provinces of the central and eastern regions with the largest areas of planting area [3]. In the country there are 47 commercial varieties, of which only four are traditional and the rest are cultivars from different national breeding programs. At present, the productivity of these cultivars does not exceed 1.44-2.35 t ha^{-1} on average [2]. Arbuscular mycorrhizal fungi (AMF) are mandatory biotrophic organisms associated with the roots of higher plants, which receive carbonated sources from the plant, while in these the capacity for soil exploration, the absorption of mineral nutrients and, consequently, are increased growth and development [4,5]. For these reasons they have been used as biofertilizers to increase the yields of different crops among which corn is found [6,7]. In Cuba, the EcoMic® biofertilizer (AMF) has been used in corn and other crops such as beans (*Phaseolus vulgaris* L.), sweet potato (*Ipomoea batata* (L.) Lam), banana (*Musa paradisiaca* L.), cassava (*Manihot esculenta Crantz*), tobacco (*Nicotiana tabacum* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and rice (*Oryza sativa* L.) [1,4,5,8].

Chitosan is a partially deacetylated derivative of chitin, a polysaccharide widely distributed in nature as a component of invertebrate structures. Chitosan is a linear copolymer formed by glucosamine units and to a lesser extent N-acetyl D-glucosamine linked by β 1-4 bonds [9-11]. It has been used as a biostimulant because it has a wide agricultural application based on the biological potentials that have been demonstrated, such as the promotion of plant growth and development of several crops of economic importance [9,10,12] and tolerance to abiotic stress [9] and biotic [13]. It is a compound with antimicrobial action for its bactericidal and fungicidal activity in the growth and development of fungi, bacteria and oomycetes [9, 12-15], also induces resistance in plants against pathogens [9,13,16].

In Cuba, the QuitoMax® biostimulant has been used, which is a liquid product, whose main active component is chitosan polymers. This biostimulant is applied in different crops and stimulates their development and quality. The application of QuitoMax® causes increases in yield from 20 to 55 % in corn, beans and tomato crops [2,17,18]. The combined application of QuitoMax® with EcoMic® has stimulated bean and pepper yields [8].

In Cuba, although the QuitoMax-EcoMic® combination has been in the presence of NPK fertilization in corn [8] used, the stimulatory action of both biostimulants combined or not, without the application of inorganic fertilizers is not known. In this sense, the objective of the work was to evaluate the effect of the combination of QuitoMax® and EcoMic® on the yield of the Criolla and P 7928 varieties when applied to the seeds prior to sowing, and the foliar spray of QuitoMax® at different times of crop development.

MATERIALS AND METHODS

Two experiments were carried out at the farm "El Mulato". It is located at 23° 00'34.6 "LN and 82° 08'20.5" LO, at a height of 122 meters above sea level, which belongs to the Orlando Cuellar CCS of San José de las Lajas municipality, from Mayabeque province, during the years 2015-2016 on an agrogenic leachate red ferralitic soil [19]. The first experiment was carried out with the Criolla variety (mixture of other varieties made by the producer) in the period March – June 2015, with 25.2 ± 1.61 °C average temperature, 77.15 ± 5.46 % of relative humidity and 619.8 mm of accumulated rainfall. The second experiment was carried out with the variety P 7928 [20] in the period April-July 2016, with 25.59 ± 1.81 °C average temperature, 77.82 ± 6.40 % relative humidity and 620, 7 mm of accumulated rainfall. In none of these experiments the crop was watered, so it is considered temporary corn.

In both experiments, QuitoMax® (biostimulant based on chitosan polymers) was used at a dose of 0.4 g ha^{-1} [8.17,18] and EcoMic® (AMF) at a dose of 1.35 kg ha^{-1} with a concentration of 20 g^{-1} spores equivalent to 10 % of the weight of the seeds [4.8]. The QuitoMax®, in addition to being applied by imbibition of grains (GI) prior to sowing, was applied by foliar spray (AF) at different times after sowing (das): in the first experiment at 25 and 45 das, while than in the second, at 10 and 45 days. The EcoMic® was only to the grains applied, after being embedded or not, with QuitoMax® by coating the grains (RG) [21], for four treatments in the first experiment and six treatments in the second, such as they appear shaped below.

First experiment

Control (EcoMic®) (CE)

IG with QuitoMax® + RG EcoMic® + AF with QuitoMax® (QEAFQ)

IG with QuitoMax® + RG with EcoMic® (QE)

RG with EcoMic® + AF with QuitoMax® (EAFQ)

Second experiment

Control (without EcoMic®) (CSE)

RG with EcoMic® (CE)

IG with QuitoMax® + RG with EcoMic® (QE)

IG with QuitoMax® + RG with EcoMic® + AF with QuitoMax® (QEAFQ)

RG with EcoMic® + AF with QuitoMax® (EAFQ)

AF with QuitoMax® (AFQ)

In both experiments, three blocks 2.7 m wide and 6 m long were analyzed for an area per plot of 16.2 m^2 per treatment. The planting distance of the crop was 0.90 m between rows and 0.30 m between plants. At the time of harvest the yield variables (weight (cm), length (cm) and diameter (cm)) of the ears, number of rows and number of grains per ear, the weight of 100 grains (g) and the estimated yield (t ha^{-1}), at 20 and 12 plants in the first and second experiments, respectively.

A randomized block design was performed in the first experiment, however, a completely randomized design with six rows per treatment was executed in the second. The diagonals method was used for sampling. To the data obtained, a double classification variance analysis was performed by performing the Duncan Multiple Range Test to determine significant differences between the means using the InfoStat Statistical Program [22].

RESULTS

In the first experiment, yield variables of Criolla variety of corn were stimulated with different combinations of EcoMic® and QuitoMax® evaluated, respect to mycorrhized control (CE) (Table 1).

Table 1. Behavior of yield variables of Criolla variety of corn with the combined application of QuitoMax® and EcoMic® biostimulants

Treatments	Corn cobs yield (var. Criolla)						
	Weight (g)	Length (cm)	Diameter (cm)	Number of rows	Nu. grains	P 100 grains (g)	Yield (t ha^{-1})
CE	58.81 c	12.42 b	3.90 c	13.65 b	371.53 c	15.95 c	2.15 c
QEAFQ	101.81 a	14.49 a	4.42 a	14.30 a	491.60 a	19.17 a	3.48 a
QE	80.50 b	14.06 a	4.13 b	13.63 b	437.00 b	16.95 bc	2.74 b
EAFQ	73.06 b	13.00 b	4.17 b	13.65 b	417.90 b	17.41 b	2.64 b

Equal letters in the same column do not differ statistically for $p < 0.05$, according to the Duncan test

From all the combinations of the biostimulants evaluated, the most prominent was the imbibition of grains with QuitoMax®, pelleted with EcoMic® plus the subsequent foliar

sprinkling of QuitoMax® at 25 and 45 (das) (QEAFQ). With this treatment were 62 % increases in crop yield achieved (Table 1).

Table 2 shows the results of the yield of variety P 7928 (second experiment).

Table 2. Behavior of the yield variables of the P 7928 variety of corn with the combined application of the QuitoMax® and EcoMic® biostimulants

Treatments	Corn cobs yield (var. P 7928)						
	Weight (g)	Length (cm)	Diameter (cm)	Number of rows	Nu. grains	P 100 grains (g)	Yield (t ha^{-1})
CSE	136.22 c	14.47 b	4.57 b	13.90 b	422.17 b	29.93 a	4.68 c
CE	149.41 b	15.97 a	4.60 b	14.07 ab	484.00 a	29.94 a	5.37 ab
QE	161.33 b	15.51 a	4.96 a	14.93 a	492.33 a	31.32 a	5.71 a
QEAFQ	195.75 a	15.53 a	4.71 b	14.33 ab	503.63 a	28.99 a	5.41 ab
EAFQ	164.68 b	16.37 a	4.69 b	14.03 ab	484.67 a	28.40 a	5.10 bc
AFQ	169.92 b	16.40 a	4.89 a	14.57 ab	503.77 a	30.51 a	5.69 a

Equal letters in the same column do not differ statistically for $p < 0.05$, according to the Duncan test

The weight of the cobs behaved better with the imbibition of grains with QuitoMax® plus their pelletization with EcoMic® and the subsequent foliar sprinkling of QuitoMax® (QEAFQ) (Table 2). The length of the cob was improved with biostimulants combined or not, with different values and superior to the control not mycorrhized with EcoMic®.

(CSE). The same happened with the number of grains per ear (Table 2). However, the diameter of the ears was improved only with the application of the bioproducts combined and applied to the grains, prior to planting (QE), and by foliar spraying of the crop with QuitoMax® at 10 and 45 das (AFQ), while the number of rows was only stimulated with the first treatment mentioned (QE) (Table 2). Regarding the weight of the 100 grains, there were no significant differences between the treatments.

The combinations of the biostimulants that most stimulated the corn yield were the imbibition of grains with QuitoMax® and their subsequent pelletization with EcoMic® (QE) prior to sowing with a 22 % increase and the foliar spray of QuitoMax® (AFQ) with 21 % increase, although without significant differences with the application of combined forms of the two treatments (QEAFQ) and the EcoMic® biofertilizer alone (EC) (Table 2).

DISCUSSION

In the Criolla variety, a lower yield was obtained than in the P 7928 variety of corn (Tables 1 and 2). The first variety was obtained by mixtures of other varieties made by the producer with the purpose of increasing crop productivity and which has not been achieved at present. However, with the application of biostimulants in combination, the yield of said variety was increased (Table 1). Unlike the Criolla variety, the P 7928 had the highest yields with all the combinations of the evaluated biostimulants, fundamentally, with the foliar spray of the QuitoMax® without combining, although it did not have significant differences with respect to the mycorrhized treatments with EcoMic® (Table 2). This demonstrates that it is possible to use the individual application of QuitoMax® to increase the yields of the variety.

Previously, QuitoMax® and EcoMic® were comparatively used in two varieties of white corn (Chuco and Cariaco) that raised crop yields, with a higher concentration (1 g L^{-1}) of QuitoMax® applied in grains per imbibition [(2)]. Using another chitosan foliar in corn, similar results were obtained by other authors [(23)], but using higher concentrations than those tested in this study.

In both varieties of corn, Criolla and P 7928, the most effective combination was the application to QuitoMax® grains by imbibition and then pelletizing with EcoMic® before sowing, plus foliar spraying of the first at the indistinct moments of the development of the planted crop (Tables 1 and 2). This result corroborates what was previously obtained with the combined application of biostimulants at similar doses, but in the presence of complete inorganic NPK fertilization [(8)]. The yield of corn with the application of EcoMic® plus NPK fertilization (50 %) was 2.6 t ha^{-1}, while with the addition of QuitoMax® this yield is not exceeded and higher values were achieved when applied only 50 % NPK.

On the other hand, corn cultivation is efficiently colonized by arbuscular mycorrhizal fungi (AMF) and as in other species, this symbiotic interaction significantly increases plant growth and development, phosphorus content, dry mass accumulation and the photosynthetic rate under limited phosphorus conditions (24.25). The application of AMF together with mineral fertilization in the HIMECA 3005 yellow hybrid corn variety was effective in increasing yields up to 100 % (from 2 to 4 t ha^{-1}), since nutritional requirements were met [(6)]. Something similar was found in this study even though no NPK was used. This suggests that the different combinations of the bioproducts is a recommended option to increase the yields of the corn crop, depending on the variety.

CONCLUSIONS

- The combination of the application of QuitoMax® by imbibition of the grains and its pelletization with EcoMic®, plus the subsequent foliar application of QuitoMax® at different moments of the development of corn, raises the yields of the Criolla variety and P 7928.
- The foliar spray of QuitoMax without prior application on grains of the combined biostimulants, stood out among all the combinations in the yield of the P 7928 variety of corn.

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