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Agricultural Bioproduct Use to Raise the Physiological Quality of Seeds of Maize (*Zea mays* L.)

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Abstract

Context: One of causes of low yields in maize is the poor physiological quality of the seeds, which does not guarantee populations with the required technical parameters. Nationally, seed germination is fostered with the use of bioproducts in different species.
Objective: To determine the vigor and electric conductivity of maize seeds, using bioproducts after submitting the seeds to accelerated aging conditions.
Methods: The experiment was done in laboratory III (Bioproducts for Agricultural Use), Faculty of Agricultural Sciences, José Martí Pérez University of Sancti Spiritus, between October and December 2018. Seeds from four maize varieties provided by the Provincial Seed Company in Sancti Spiritus, were used. Francisco 28 (Fr-28), FgH, MAIG, and P-7928, and were combined with three bioproducts (*T. harzianum*, FitoMas E, and distilled water). A completely randomized experimental design was used, with factorial arrangement of 4x3x5.
Results: Bioproducts FitoMas E and *T. harzianum* proved to be effective in the recovery of seeds with physiological deterioration.
Conclusions: *T. harzianum* offered the best conditions to recover seed vigor, with 20 and 24% in relation to FitoMas E and the distilled water, respectively. The electric conductivity test proved its efficiency as a parameter to determine the vigor of seeds.

Key words: maize, germination, seed aging, Zea mays, Trichoderma.

Introducción

Maize (*Zea mays* L.) belongs to the graminaceae family, maideas tribe. It is believed to have originated in the tropics of Latin America, particularly genus Zea, Tripsacum, and Euchlaena, whose importance lies in its phytogenetic relation with genus Zea.

There is experimental evidence that *Trichoderma* spp. stimulates plant germination and growth, along with an antagonist activity against pathogen *Fusarium spp*. (Cubillos, Páez & Mejía, 2011).

Cuba produces and promotes the use of FitoMas-E®, a product containing mineral salts and highly energetic biochemical substances (amino acids, nitrogenized bases, saccharides, and biologically active polysaccharides), which increase and speed up seed germination, regardless of their botanical or agamic origin (Viñals Verde et al., 2011).

Vigor analyses have become common practice tools to determine the physiological quality of seed batches. (2004).

The electric conductivity test (EC) is suggested to provide germination and/or vigor of seeds in 24 hours or less, and it allows estimation of cell membrane integrity, whose loss and subsequent disappearance of cytoplasmic solutes with electrolytic properties indicate quick deterioration of seeds.

In the province of Sancti Spiritus, maize covers 29 355 hectares, with mean yields of 29 t ha^{-1} in the private sector, and 1.6 t ha^{-1} in the state sector. The mean national values are 2.37 t ha^{-1}, far from the world means, which account for 5.6 t ha^{-1} (ONE, 2017). One of the causes of low yields in maize is the poor physiological quality of the seeds, which does not guarantee populations with the required technical parameters.

Therefore, the aim of this paper is to evaluate the physiological quality of maize seeds from the Seed Company of Sancti Spiritus, using *Trichoderma harzianum* and FitoMas E.

Materials and Methods

The experiment was conducted in laboratory III (Bioproducts for Agricultural Use), Faculty of Agricultural Sciences, José Martí Pérez University of Sancti Spiritus, between October and December 2018. Botanical seeds from four maize varieties provided by the Provincial Seed Company in Sancti Spiritus, were used. Francisco 28 (Fr-28), FgH, MAIG, and P-7928.

A completely randomized experimental design was used, with six treatments and five replicas. Germination tests were made on sterile Petri dish with filter paper. It was run at 28 0C ± 1 ºC in a germination chamber with light photoperiod of 8 hours/light and 16 hours/dark.

The treatments are the combination of four varieties (Francisco-28, FgH, MAIG, and P-7928), and three bioproducts (distilled water, FitoMas E, and *T, harzianum*)

The seeds were previously disinfected with a commercial chlorine solution (5.25% sodium hypochlorite) at 10% for three minutes, and then it was washed three times with sterile distilled water.

Artificial deterioration was induced by accelerated aging. The seeds were chosen according to size uniformity and physical appearance; they were placed on a metallic mesh forming a layer inside a dryer containing 1L of water. The water was 2 cm away from the seed layer to prevent contact with the water, creating an aging chamber with a relative humidity of 100%.

The dryer was introduced in an incubator at 45 ± 1 °C for 72 hours. Upon completing aging, the seeds were exposed to the environment of the lab until recovering their initial contents of humidity (15%).

The doses and application time were,

T. harzianum --- 1.9 x 10^9 conidia per milliliter, the seeds were dipped for 1 hour.

FitoMas E----------solution at 2%, the seeds were dipped for 1 hour.

The following vigor tests were determined,

Germination Potency (GP). It was performed seven days after placing the seed on the dishes, according to Engels & Vissier (2007).

$$P = \frac{N}{NP} * 100$$

Where:

N: number of seeds germinated at seven days

NP: total number of seeds

Mean germination Time (MGT). It is calculated by determining the number of germinated seeds every day, considering the total number of germinated seeds (Tompsett & Pritchard, 1998)

$$MGT = \sum ni.di / N$$

Where:

ni: number of seeds germinated on d day

di: number of days since the beginning of the germination experiment

N: total number of seeds germinated at the end of the experiment

Radicle and coleoptile lengths (cm) The two tests were made on the seventh day following germination, a ruler was used for measurements. The number of secondary roots were considered in the radicle.

Electric conductivity. A completely randomized experimental design was used, with six treatments and four replicas. Overall, 100 seeds were used in each treatment, 25 per replica. After the resting time (up to 15% humidity), 25 seeds were submerged in 50 ml of distilled and deionized water for 24 hours. Then the seeds were removed and their values were read with a portable conductimeter.

A two-way ANOVA was performed for statistical analyses of germination potency, mean germination time, radicle and coleoptile lengths. To check electric conductivity, a one-way ANOVA was made after checking homogeneity and normality values, through the Levene and Kolmogórov Smirnov test, based on SPSS, 21, for Windows. The mean values were compared through Tukey's multiple range test ($p \leq 0.05$). The germination percentages mean and mean germination time were transformed by $2\,arcsen\sqrt{p}/100$ to adjust to the normal likelihood curve.

Results and discussion

The statistical analysis of germination potency showed interaction among the bioproducts with the varieties used, among the different bioproducts, and among the four varieties of maize. The highest germination potency value was achieved with FgH, using *T. harzianum*, with 82% (Table 1). It was 44% higher than the Fr-28 treatment, with distilled water, the lowest, and no different from FitoMas E, in the same variety (42%). Likewise, MAIG and P-7918 on Distilled water showed no differences with the previously mentioned treatments.

Table 1. Germination potency.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Germination potency (%)					
Bioproducts					Bioproduct mean	Typical error
Distilled water	38.0C	62.0B	40.0C	42.0C	45.5b	
T.harzianum	62.0B	82.0A	40.0C	70.0AB	65.0a	3.27
FitoMas E	42.0C	72.0AB	58.0BC	70.0AB	60.0c	
Mean of varieties	47.3c	72.0a	48.0c	60.6b	57.0	
Typical error		3.78				
VC (%)						29.0

Unequal capital letter for the means of the interactions differ ($p \leq 0.05$), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the varieties differ ($p \leq 0.05$), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the bioproducts differ ($p \leq 0.05$), according to Tukey's multiple range test.

Table 1 shows that *T. harzianum* was the bioproduct that reached the best germination potency, different from Fitomas E, and distilled water, respectively. In that sense, trichoderma raised the germination potency in FR-28 by 20 and 24%, with respect to FitoMas E and distilled water, respectively, the treatments with the lowest potency.

The previous results differ from González et al. (2014), on evaluation of accelerated aging in maize varieties in Mexico, where the percentages of germination potency were greater than 70%. It demonstrates that the seeds evaluated in the Seed Company experiment in Sancti Spiritus were physiologically unhealthy. During the experimental cycle, the incidence of contaminating fungi was remarkable, which were thought to have come from the seeds, as suggested by the disinfection actions.

The best variety was FgH, which differed from the rest. Aristizábal & Álvarez (2006) said that the seeds with germination potency higher than or equal to 80%, after accelerated aging, might be classified as of high vigor; between 60-80% as mid vigor; and 60% as low vigor. In that sense, only FgH on *T. harzianum*, came close.

Concerning mean germination time, interaction was observed between bioproducts and varieties, among varieties, but no interaction was found among the bioproducts (Table 2). In this parameter, there were no statistically different statistics among varieties FgH and P-7928, with none of the bioproducts used. In turn, the treatment with the longest mean germination time was MAIG, with distilled water, more than 1.88-fold compared to the FgH treatment (*T. harzianum*), the shortest mean germination time. These results corroborate the criteria of Olmedo and Casas (2014), who said that trichoderma stimulates plant growth and development through the production of molecules that foster plant growth.

In that direction, FgH was the best variety, though no different from P-7928. Moreover, Fr-28 and MAIG reached the highest values with no differences between them. This reveals that the Fr-28 seeds were not physiologically healthy.

Table 2. Mean germination Time.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Mean germination time					
Bioproducts					Bioproduct mean	Typical error
Distilled water	2.62ABC	2.34CDEF	3.35A	1.97EF	2.57a	
T.harzianum	2.87ABC	1.86F	2.66ABC	1.76F	2.29a	0.11
FitoMas E	2.51ABC	2.02CDEF	3.03AB	2.03CDEF	2.39a	
Mean of varieties	2.66b	2.07a	3.01b	1.92a	2.41	
Typical error		0.19				
VC (%)						25.0

Unequal capital letter for the means of the interactions differ ($p \leq 0.05$), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the varieties differ ($p \leq 0.05$), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the bioproducts differ ($p \leq 0.05$), according to Tukey's multiple range test.

Artola (2015), in a study of the use of different biopreparations of efficient microorganisms, achieved mean germination times of 2.19. Although it was higher than the treatments of varieties FgH and P-2978 with *T. harzianum and* FitoMas E, respectively, the outcome of that research was better than Fr-28 and MAIG, using the same bioproducts. All the varieties used in this study based on distilled water produced higher values, which demonstrates the effectiveness of bioproducts to favor germination in low physiological quality seeds.

The length of the radicle showed interaction among the bioproducts with the varieties used, among the different bioproducts, and among the four varieties of maize (Table 3). The worst variety and differences with the rest, was Fr-28, evidencing, as in the previous parameter, that it was the variety with the lowest physiological quality. For its part, FitoMas E produced the best results, different from the other bioproducts, where distilled water was the shortest in the radicle of all the varieties used, proving the effectiveness of FitoMas E and *T. harzianum* as growth enhancers.

Table 3. Radicle length.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Radicle length (cm)					
Bioproduct					Bioproduct mean	Typical error
Distilled water	4.10E	7.98BCD	7.68CD	8.10BCD	6.96b	
T.harzianum	6.74D	9.0ABC	7.50CD	9.96AB	7.96b	0.32
FitoMas E	8.16BCD	10.78A	9.96AB	10.68A	9.89a	
Mean of varieties	6.33b	9.25a	8.38a	9.12a	8.27	
Typical error			0.37			
VC (%)						26.3

Unequal capital letter for the means of the interactions differ (p ≤0.05), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the varieties differ (p ≤0.05), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the bioproducts differ (p ≤0.05), according to Tukey's multiple range test.

Similar stimulation results in radicle length were achieved by Santana et al. (2016) in a study with the same bioproducts, but in tomato; they explained the benefits over different morphological variables evaluated.

As in the previous aspect, the length of the coleoptile showed interaction among the bioproducts with the varieties used, among the four varieties of maize, but not among the bioproducts used (Table 4).

Table 4. Coleoptile length.

Varieties	Fr-28	FgH	MAIG	P-7928		
	Coleoptile length (cm)					
Bioproduct					Bioproduct mean	Typical error
Distilled water	4.5.40B	9.49AB	7.80B	8.0AB	7.49ab	
T.harzianum	5.70B	9.836AB	6.40B	7.20B	7,29ab	0.48
FitoMas E	6.40B	12.10A	8.90AB	7.80AB	8.80a	
Mean of varieties	5,83c	10,48a	7,46b	7,66b	7,86	
Typical error			0.56			
VC (%)						34.0

Unequal capital letter for the means of the interactions differ (p ≤0.05), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the varieties differ (p ≤0.05), according to Tukey's multiple range test.

Unequal lower case letters in the row for the means of the bioproducts differ (p ≤0.05), according to Tukey's multiple range test.

The longest treatments were the combination of Fitomas E with FgH, MAIG, and P-7928, no differences among them, and statistical differences from the rest of the treatments evaluated. The best variety, with significant differences from the rest, was FgH; it was 1.79-fold higher than Fr-28, the variety with the lowest values and statistical differences with the rest.

Santana et al. (2016) showed greater growth and development of tomato plantlets with the use of these bioproducts compared to a control. In contrast to this study, the previous authors found better results using a combination of Fitomas E and *T. harzianum*; hence, this can be considered in further research.

Figure 1. shows the electric conductivity found from seed exudates after undergoing artificial aging. FgH reached the lowest value, with statistically significant differences from the rest of the varieties evaluated, followed by P-7928, also different from the rest.

The varieties with the lowest values in this parameter were precisely the ones with the best germination potency, along with the longest radicle and coleoptile, and shortest germination time. It proves that these seeds had the best physiological conditions to withstand aging and a better response to the bioproducts used.

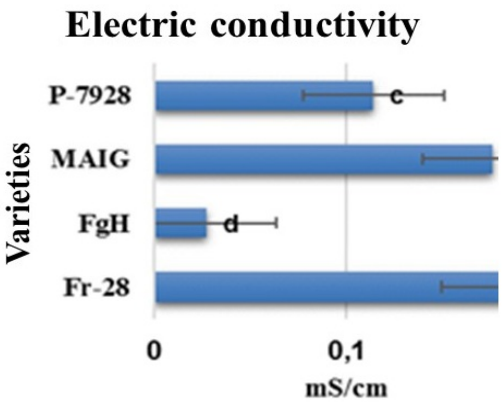

Figure 1. Electric conductivity.

The current research coincides with Hilmig & Méndez (2007), who said that the test of electric conductivity allows for the estimation of the integrity of the cell membrane. Its loss, and subsequent disappearance of cytoplasmic dissolutes with electrolytic properties indicate quick deterioration of seeds. Therefore, the evaluation of electric conductivity of the seed exudate should be a sign of seed deterioration, and consequently, seed quality.

Conclusions

1. Bioproducts FitoMas E and *T. harzianum* proved to be effective in the recovery of seeds with physiological deterioration.

2. Bioproduct *T. harzianum* offered the best conditions to recover seed vigor, with 20 and 24% in relation to FitoMas E, and distilled water, respectively.

3. The electric conductivity test proved to be an efficient parameter to determine the vigor of seeds.

Author contribution

Michell Leiva arbolaes: practical execution of the research, literature review or state of the art.

Marcos T. García González: Research planning, direction, and control of research, analysis of results, manuscript redaction, final review.

Marcia M. Rodríguez Jáuregui: Direction, advisory, and control of laboratory research.

Yander Fernández Cancio: Statistical analysis and collaboration in the practical part of research.

Conflicts of interest

There are no conflicts of interest.

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