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RESEARCH ARTICLE

DIFFERENTS DOSES OF A BIOSTIMULANT BASED ON PLANT EXTRACTS IN TOMATO AND PEPPER SEEDS

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ABSTRACT

Aiming to increase the yield and quality of tomato and pepper fruits, alternatives are sought, such as the use of biostimulants applied to the seed. The objective was to evaluate the effect of the application of different doses of a biostimulant on the physiological quality of tomato and pepper seeds. The research was carried out in the period between March and July 2016 in the Laboratory of Seed Quality Analysis of the Faculty of Agrarian Sciences of the National University of Asunción. The experimental design was completely randomized in a factorial scheme 2x4, with four replications for each treatment, where factor A corresponded to the species and factor B corresponded to the doses used, totaling 32 experimental units. The variables studied were germination (%), germination rate, germination rate index, germination rate coefficient, root length, stem length and emergence. The collected data were subjected to analysis of variance and in the case of significant statistical differences, Tukey' stest for comparison of meanswas performed at 1% error probability. The results showed that for tomato, no significant differenceswere observed in the variables germination and emergence, nevertheless, there were differences between the other variables. Likewise, for pepper, no statistical differences were observed regarding the emergence, in contrast with whatwas verified for the remaining variables. It is concluded with this work that the biostimulant application does not influence the physiological quality of the tomato and pepper crops.

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INTRODUCTION

In order to supply domestic and foreign markets, the industrialization of vegetables is expanding in Brazil. Aiming to provide the raw materials necessary for the agro-industry, it was developed a new type of research on the extensive cultivation of vegetables, with the purpose of obtaining a huge volume of production (Filgueira, 2008). The success of horticulture generally depends on a suitable emergence for each crop. Otherwise, there may be reductions in the quantity and variations in the quality of the final product (Silva & Vieira, 2006). Seed quality includes genetic, phytosanitary, physical and physiological aspects. The latter include viability, germination capacity and vigor, which are affected both by the growth conditions of the mother plant during seed development, the degree of maturity of the seed at the time of harvest, the harvesting technique and theprocessing conditions (Pittcock, 2008). Seed is the most important input for successful agriculture (Meneghello *et al.,* 2016). In the case of the vegetables, the majority of seeds have reduced their size and their reserve contents, reason why the conditions for the germination thereof must be optimized in order to guarantee a good level of emergence and the production of vigorous seedlings (Soares *et al.,* 2013). In order to increase the expression of the set of characteristics that establish the yield potential of a seed after sowing or during storage, seed treatment has been used, which is a practice of great value for its ease of execution, low cost and efficiency on several aspects (Nascimento, 2009). Biostimulants comprise a variety of products that can be applied to both the seeds and the shoots of plants, whose common denominator is that they contain active principles, which act on the physiology of plants,

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increasing their development and yield, reflecting in the quality of the vegetables and contributing to improve the resistance of the vegetable species to various diseases (González *et al.,* 2015). The majority of vegetables are produced by small producers, who do not have a proper infrastructure and advanced technology, leading to a trend of decreased yield, affecting the quality thereof. With the aid of seed treatment with organic products such as biostimulants, it is possible to reduce the transplantationtime and to obtain plants with greater vigor, allowing later to obtain a better yield, since the action of the product on the seeds can provide greater germination capacity and germination rate. The objective of the research was to evaluate the effect of the application of different doses of a biostimulant based on plant extracts and activated water on the physiological quality of tomato (*Lycopersicumesculentum*) and pepper (*Capsicum annuum*) seeds.

MATERIALS AND METHODS

The seeds used were obtained from local markets, belonging to two different species of the family Solanaceae: tomato (*Lycopersicumesculentum*) and pepper (*Capsicum annuum* L). The biostimulant was also obtained from local markets, composed of activated water and plant extracts from*Quercusfalcata* (red oak), *Opuntialindheimeri* (cactus), *Rhusaromatica* and *Rhizophora mangle* (red mangrove). The experimental design was a randomized complete block,in a factorial scheme 2x4, where factor A corresponded to the two species that were evaluated and factor B corresponded to the four different doses of the biostimulant, in addition to the control (Table 1). Each treatment had 4 replications per crop, totaling 32 experimental units; each experimental unit had 100 seeds.

Table 1. Doses of the biostimulant in ml applied to the seeds of two species belonging to the Solanaceae family

Treatments	Factor A (species)	Factor B (dosis)
Control		0
T2	Tomato (*L.*	2,5
T3	*esculentum*)	5
T4		10
Control		0
T2	Pepper (*C. annuum*L)	2,5
T3		5
T4		10

A germination test was carried out prior to the beginning of the experiment, in order to know the germinative capacity of the seeds to be used. In the test, a 95% germinationwas observed for pepper seeds, this value being of 92% for tomato seeds. For the treatment with thebiostimulant, firstly, the preparation of the solution was performed, which consisted in the dissolution of the product in distilled water (Table 2). Then, in each treatment, the seeds were soaked during a period of 10 minutes. Subsequently, the product was removed, leaving the seeds to dry, and sowing was performed. The procedure was the same for both crops. All the elements to be usedin the experiment, such as gerboxboxes, tweezers and absorbent papers, were previously sterilized with 70% alcohol to avoid any possible contamination of the analyzed samples. The sieved gross sand, used tomeasure seedling emergence, was subjected to an oven temperature of 200 °C during 2 hours for sterilization. Records were taken daily on sheets prepared for each variable assessed. To count the different variables, the trays were manipulated with latex gloves in order to avoid contamination of the experimental units. The temperature of the germinator was controlled daily so that it remained constant for the entire time until the experiment was finished.

Table 2. Description of thepreparation of thebiostimulantsolution in distilledwaterbytreatment. San Lorenzo, FCA / UNA, 2016

Treatments	Biostimulant (%)
Testigo	0
T2	2,5
T3	5
T4	10

For the germination percentage, two absorbent papers were initially moistened with distilled water (2.5 times the dry paper weight) and placed in gerbox boxes, the seeds were treated in advance with the corresponding doses of each treatment (Table 1); then, 100 seeds were sown in eachgerbox. These were placed in the germinator at an alternating temperature of 20-30 °C. The final reading of both species was performed at 14 days after sowing, after which the percentage of normal seedlings was determined. The result of normal seedlings was obtained by the following formula, which was expressed in percentage:

$$\text{Germination (\%)} = \frac{N_{o.}\text{ Germ Sds}}{N_{o.}\text{Sown Sds}} \times 100$$

$N_{o.}$Germ Sds: number of germinated seeds
$N_{o.}$ Sown Sds: number of sown seeds

For the germination rate, daily count of the number of germinated seeds was made in the same experimental units used for the germination analysis, until the day of the final reading. The data obtained were submitted to the formula proposed by (Edmond &Drapalha, 1958) and the result showed the number of mid-days required for the germination of the seedlings. It was considered as germinated seeds those which presented 2 mm root length.

GR= [(G1*N1) + (G2*N2) + (Gn*Nn)] / (G1+G2+Gn)

GR: germination rate in mid-days

N1, N2, Nn: Number of days from the sowing to the first and second counts, and so on until the last count

G1, G2, Gn: Number of germinated seeds in the first and second counts, and so on until the last count

For the germination rate index, the aforementioned procedure was again performed, the GRI was obtained using the formula proposed by (Maguire, 1962), whose result indicated the number of germinated seeds per day.

GRI= (N1/G1 + N2/G2 + Nn/Gn)

GRI: germination rate index
N: number of germinated seeds per day
G: period in days

The same procedure used for the variables GR and GRI was usedfor the germination rate coefficient. The daily data obtainedwere used for the calculation of the GRC by the formula described by (Furbeck, 1993).

$$GRC = \frac{(G1+G2+\ldots+Gn)}{(N1*G1)+(N2*G2)+\ldots+(Nn*Gn)} \times 100$$

GRC: germination rate coefficient
N: number of germinated seeds per day
G: period in days

To measure stem and root length, 20 seeds of each species were used for each replication per treatment, these being previously treated with the doses corresponding to each treatment, then distributed on germitest paper. These papers were weighed and then moistened with distilled water to a volume corresponding to 3 times their dry weight. Two germitest papers were used as a basis, on which the 20 seeds distributed alternately were placed to 2 cm of the upper edge; soon the seeds were covered with another sheet, leaving the micropyle directed toward the bottom part of the sheet, so that the roots and stems had room to develop. Once the seeds were covered, they were rolled, being subsequently placed in transparent bags and fastened with rubber bands to facilitate handling. After the procedure, they were placed in a germinator at a constant temperatureof 25 °C for a period of 10 days. At the end of this time, the stems and roots of normal plants were measured using a centimetric rule. These measurements were averaged by treatment and the results were expressed in cm.

$$X = \frac{\text{leng } 1 + \text{leng } 2 + \ldots\ldots \text{ leng } n}{\text{Number of normal seedlings}}$$

X: stem or root length
Leng: stem or root length

In order to measure seedling emergence, the sterilized gross sand was placed in polyethylene pots under uncontrolled conditions, in which 3 seeds were sown; each treatment had 4 replications, respectively. Daily temperature data were taken, which were averaged at the end of a 10-day period. The pots were watered as needed. The data collected from each variable were submitted to ANOVA (analysis of variance) and in case of statistical difference.

RESULTS AND DISCUSSION

The results of the analysis of variance are shown in Table 3, which demonstrate that there was no significant statistical difference for the variable germination percentage in tomato seeds, contrary to what was observed in pepper seeds, which presented significant statistical differences; each species was evaluated independently. The different biostimulant doses used in the treatment of tomato seeds presented the same behavior, together with the control. While in the treatments applied to pepper seeds, it was observed that the 0% dose obtained a higher percentage of germinated seeds.A similar behavior was observed for the seeds treated with a dose of 2.5%, exceeding 90% germination.On the other hand, in T4, only 10% of the seeds germinated, indicating that from a concentration of 5%, there is impairment, inhibiting seed germination. Meneghello *et al.* (2016) state that when evaluating the physiological quality of eggplant, bell pepper and tomato seeds treated with a bioactivatorunder six different doses (0; 0.2; 0.4; 0.6; 0.8; 1.0 ml) of the product per thousand seeds, they obtained an overall positive effect on germination, where the three species presented high germinationcapacity using the doses of 0.4 and 0.6 ml; from the following dose, germination was slightly decreasing, but remained with average values above the control for all lots and species of the study.

Table 3. Effect of different doses of biostimulant on the germinative power of tomato and pepperseeds. San Lorenzo, Paraguay, 2016

Treatment	Germination (%)	
	Tomato	Pepper
1	89 A	97 A*
2	86 A	94 A
3	92 A	49 B
4	85 A	10 C
Mean	88	62,5
CV (%)	5,7	5,7

* Means followed by different letters on the vertical differ from each other by theTukey test at 1%.

Table 4. Mean germinationrate (VG), germinationrateindex (IVG), germinationratecoefficient (CVG) of pepperseeds. San Lorenzo, Paraguay. 2016

Treat.	VG	IVG	CVG
1	5,35 A*	19,15 A	18,68 A
2	5,26 A	19,37 A	19,27 A
3	8,04 B	7,52 B	12,44 B
4	9,58 C	1,61 C	10,47 B
Means	7,05	11,91	15,21
CV (%)	5,92	10,24	6,12

*Means followed by different letters on the vertical differ from each other by theTukey test at 1%

Table 5. Means of germinationspeed (VG), germinationrateindex (IVG), germinationratecoefficient (CVG) of tomatoseeds. San Lorenzo, Paraguay. 2016

Trat.	VG	IVG	CVG
1	6,31 B*	16,60 B	15,87 C
2	5,46 A	21,39 A	18,36 A B
3	5,29 A	22,30 A	18,92 A
4	6,03 A B	18,02 B	16,75 B C
Means	5,77	19,57	17,47
CV (%)	5,92	10,24	6,12

*Means followed by different letters on the vertical differ from each other by theTukey test at 1%

Which agrees with the results for tomato in this study, since no differences were shownbetween treatments. Regarding the species *L. esculentum*, the present experiment corroborates Almeida *et al.* (2009), who evaluated the physiological performance of carrot seeds treated with different doses of a bioactivator, using three different concentrations (0.0; 0.05 and 0.4 ml), and found no significant differences betweentreatments, since the obtained results oscillate between 70 and 75% germination. Moreover, Alfonso et al. (2014), who evaluated the agrobiological effectiveness of a bioactive product in radish seeds, using doses of 5, 10 and 15 mg, obtained 90% germination with the maximum dose and 83% for the control, being inferior to the result obtained in this research, where in pepper seeds, the control presented 97% germination and with the dose of 2.5%, 94% of the seeds were germinated. Tables 4 and 5 show the data obtained in the analysis of variance, in which it was found that there were statistical differences regarding the variables germination rate, germination rate index and germination rate coefficient for the studied species. For the variables under study (GR, GRI and GRC), in pepper seeds, the results show that the control, together with treatment 2, behave optimally, standing out from the other treatments; on the other hand, using the dose of 10%, the lowest results are observed. Evaluating the vigor of tomato seeds submitted to the same biostimulant doses, it is observed that treatments 2 and 3 present the best results, contrary to the control, which had a negative behavior. Thus, the treatment with 2.5% dose is highlighted for both species under study. For the variable germination rate, it was observed that both for pepper and tomato seeds, 5 mid-days were necessary for the germination to start, which was obtained with the 2.5% dose and the control for pepper, and only with treatment 2 for tomato. In pepper seeds, the germination rate index was 19 plants per day in treatments 1 and 2, decreasing to only 2 plants per day when using the 10% dose. On the other hand, in tomato seeds, values of up to 22 plants per daywere obtainedin treatments 2 and 3.

Regarding the germination rate coefficient, the best values were 19.27 and 18.92, for pepper and tomato sedes, respectively, corresponding to treatment 2, in pepper, and treatment 3, in tomato. In an experiment carried out with carrot seeds, using different doses of a bioactivator to evaluate their physiological behavior, Almeida *et al.* (2009) observed that the seeds treated with 0.05 and 0.4 ml doses showed a germination rate of 3 days, in contrast with the control, with germination at 6 days. Although the results were similar, the treated seeds germinated, on average, one day faster than thosewhich did not receive any treatment, which is in agreement with the present study. The aforementioned authors emphasize the importance of the variable germination rate index, since it is related to seed vigor, by taking into account the number of germinated seeds per day and the time required to achieve it (Alfonso *et al.,* 2014). On the other hand, it is suggested that even in water, not all seeds germinate simultaneously, with differences between seeds of the same population, and furthermore the distribution over time is an indicator of the uniformity of the lot of those seeds. As for the product studied, it is suggested that it can stimulate the biochemical processes that originate the germination of seeds, favoring its acceleration. The data related to the analysis of variance expressed in Table 6 show the existence of statistical differences in the variables stem and root length in both species for the different doses applied, analyzed independently.

Table 6. Effect of different doses of a biostimulantonthemeans of stem and rootlength. San Lorenzo, Paraguay. 2016

Treatment	Length of stems (cm)		Rootlength (cm)	
	Tomato	Pepper	Tomato	Pepper
1	4,36 B C	0,46 B	8,99 C	3,20 C
2	4,81 A	2,97 A	10,48 A	5,72 A
3	4,76 A B	0,88 B	9,85 B	4,98 B
4	4,19 C	0,53 B	9,88 B	3,12 C
Mean	4,53	1,21	9,83	4,25
CV (%)	7,32	7,32	4,11	4,11

*Means followed by different letters on the vertical differ from each other by theTukey test at 1%

For the variables stem and root length, both species under study presented higher results in treatment 2; in tomato, the average stem length was 4.81 and the average root length was 10.48, differing from other treatments. In terms of stem and root length, Alfonso et al. (2014) observed in radish seeds treated with a bioactive product, using doses of 5, 10 and 15 mg, that the product used in their research exerted a positive effect on the length of stems and roots, yielding significant differences among the different treatments; the dose of 5 mg showed superior results in relation to the other doses, differing from the present work for both species. Likewise, the work presented by Huez-Lopez et al. (2008), who evaluated the effect of a biostimulant based on plant extractson the seed germination of chilli (*Capsicum annuum*), using doses of 0.0; 0.5; 1 and 1.5 cm^3/L of the product, showed that their results, in terms of stem and root length, had highly significant differences, the highest result being observed with the dose of 0.5 cm^3/L. According to data from the analysis of variance, shown in Table 7, no statistically significant differences were shown between treatments for any of the species under study.

Table 7. Effect of different doses of biostimulant based on plant extracts and activated water in themeans of emergency percentage in tomato and peppers seeds. San Lorenzo, Paraguay. 2016

Treatment	Emergence(%)	Emergence (%)
	Tomato	Pepper
1	100 A	42 A
2	67 A	67 A
3	50 A	84 A
4	42 A	25 A
Mean	64,75	54,5
CV (%)	59,9	59,9

*Means followed by different letters on the vertical differ from each other by theTukey test at 1%

As for the emergence of both crops under uncontrolled conditions, similar behavior was observed among the different treatments.Regarding emergence, Almeida et al. (2009), who carried out a study on the physiological behavior of carrot seeds with the application of different doses, comprised between 0.0; 0.05 and 0.4 ml, found that there is a significant difference between the treated seeds and the control, obtaining greater results in the seeds treated with 0.4 ml of the product. Therefore, the data acquired in the present study differ from the results of those authors, since both species were not statistically different among their treatments.

Conclusion

The biostimulant did not affect the germination of the seeds under study. Using the doses of 2.5 and 5 ml in the pepper crop, fewer days were required for germination, and more plants were germinated per day. The emergence was not affected by the use of biostimulants in any of the species under study.

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