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# Effect of the applications of a biological formulation (*Azotobacter salinestris, Bacillus amyloliquefaciens, Rhizophagus intraradices*) on the yield of *Allium cepa* 'Century'

## Efecto de las aplicaciones de un formulado biológico (*Azotobacter salinestris, Bacillus amyloliquefaciens, Rhizophagus intraradices*) en el rendimiento de *Allium cepa* 'Century'

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**Abstract**

This research was conducted from September to December 2016 on an agricultural farm in the district of Santa Rita de Siguas, Arequipa Province, Peru. The concentrations used were 2, 4, and 6 kg ha$^{-1}$ with 4 applications by means of impregnation to the transplant and via drench every 15 days. The statistical design was through a DBCA with factorial arrangement randomly distributed in four blocks. The tests used were the orthogonal contrast test and Duncan's multiple comparison test ($\alpha = 0.05$). The findings showed that the treatment concentrations of 4 kg ha$^{-1}$ and 6 kg ha$^{-1}$ resulted in a lower incidence of *Fusarium oxysporum*, at 6.71% and 9.36%, an ABCPE of 637.78 and 950.14 units, and an exportable yield of 66.34 t ha$^{-1}$ and 65.42 t ha$^{-1}$, respectively, displaying significant differences from the control. The first application was statistically significant to the treatments with the highest number of applications, showing a greater exportable yield of 67.54 t ha$^{-1}$. The best interactions between concentrations and applications were 6 kg ha$^{-1}$ with 1 application, 4 kg ha$^{-1}$ with 2 applications, and 4 kg ha$^{-1}$ with 1 application, with exportable yields of 70.66, 69.61, and 69.11 t ha$^{-1}$, respectively.

***Keywords:*** *Biological formulation, Azotobacter salinestris*, *Bacillus amyloliquefaciens*, *and Rhizophagus intraradices.*

**Resumen**

La investigación se realizó entre los meses de Setiembre y Diciembre del 2016, en la zona de Santa Rita de Siguas – Arequipa. Las dosis usadas fueron dos, cuatro y seis kg ha$^{-1}$ con cuatro aplicaciones mediante impregnación al transplante y vía drench cada 15 días. El diseño estadístico fue un DBCA con arreglo factorial repartidos aleatoriamente en 4 bloques. Se utilizó la prueba de contrastes ortogonales y la prueba de comparación múltiple de medias Duncan ($\alpha = 0,05$). En los resultados las dosis de los tratamientos de 4 kg ha$^{-1}$ y 6 kg ha$^{-1}$ presentaron una menor incidencia a *Fusarium oxysporum* con 6,71 % y 9,36%, un ABCPE de 637,78 y 950,14 unidades y un rendimiento exportable de 66,34 t ha$^{-1}$ y 65,42 t ha$^{-1}$ respectivamente mostrando diferencias significativas respecto al testigo. La primera aplicación fue estadísticamente significativa a los tratamientos con más número de aplicaciones mostrando un mayor rendimiento exportable de 67,54 t ha$^{-1}$. Las mejores interacciones entre dosis y aplicaciones fueron 6 kg ha$^{-1}$ con 1 aplicación, 4 kg ha$^{-1}$ con 2 aplicaciones y 4 kg ha$^{-1}$ con 1 aplicación, con rendimientos exportables de 70,66, 69,61 y 69,11 t ha$^{-1}$ respectivamente.

***Palabras clave:*** *Formulado biológico, Azotobacter salinestris, Bacillus amyloliquefaciens y Rhizophagus intraradices.*

## Introduction

In Peru, 19 thousand hectares of land is cultivated with onions, representing 0.4% of the global harvested area, and 748 thousand tons of onions are produced, which represents 0.9% of the global production (Apcho, Caballero, & Miranda, 2017).The highest production of all Peruvian departments is reported from Arequipa, where there are 438,323 tons of produce and 9,164 hectares of planted area, representing 58% of the national production. This is followed by Ica, where 154,111 tons are produced and 2,423 hectares are planted, representing 20% of national production. In addition to these coastal locations, onions are produced in Lima, La Libertad, Tacna, and Lambayeque, and also in Ayacucho and Junín, in the mountains (Apcho *et al.*, 2017). Currently, excessive amounts of agrochemicals are used in producing yellow onions, and this is causing serious environmental and economic problems. This is exemplified in the progressive decay of the quality of the soils used for agricultural

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production, largely due to excessive application of pesticides and chemical fertilizers. It is also worth noting that the indiscriminate use of pesticides is having a negative impact on yellow onion exports to the international market, in which the tendency is to consume organic products because of their superior nutraceutical properties. One of the chief alternatives to application of agrochemicals is the application of biological formulations, prepared from beneficial microorganisms, which, according to research findings, have positive effects both on the agronomic performance of the cultivated plants and on the economic returns. Nonetheless, biological formulations are used rarely in producing yellow onions, if at all, because there are no biological formulations in our market whose effectiveness and efficiency in producing yellow onions have been demonstrated through research. Given this problem, this study was conducted to evaluate the effect of different concentrations and applications of a biological formulation (*Azotobacter salinestris*, *Bacillus amyloliquefaciens*, *Rhizophagus intraradices*) on the yield of *Allium cepa* 'Century' under the edaphoclimatic conditions of Santa Rita de Siguas.

## Material and methods

***Duration and location of the experiment:*** Onion sets were planted on September 9th 2016, and the onions were harvested on December 16th 2016. The experiment took place in the field called Fundo Samuel Zegarra, located in Santa Rita de Siguas district, in the province and region of Arequipa at latitude 16° 29' South, longitude 72° 11' West, and at 1,277 meters above sea level.

***Biological material:*** We used the product BIOKIT, formulated as 1 kg of dry powder. It is a biological formula containing strains of the bacterial *A. salinestris* and *B. amyloliquefaciens* and the arbuscular mycorrhiza *R. intraradices* in the following composition:

*A. salinestris* UFC …….0.035%
*B. amyloliquefaciens* UFC ……. 0.775%
*R. intraradices* …..……..... more than 90 IMP
Inerts .....…….…….…… 99.19%
Total ….....… ……..100%

***Edaphic characteristics of the experimental area:*** Analysis of a representative soil sample revealed a strongly alkaline soil, with a high organic matter content, high to very high phosphorus and potassium contents, and a sandy loam texture class.

***Land preparation:*** The soil was milled using a polydis, and stones that make farming difficult were removed from the soil surface. The floor was leveled using a disc and rail plow, and the beds were marked with a roller, with two lines of drip irrigators placed per bed.

***Transplantation:*** Transplantation was conducted when the seedlings were 15 cm high and 6 mm in diameter. Four rows of seedlings were placed in each bed for each drip tape. Molasses that served as a food source for the inoculated microorganisms were applied to the drip irrigation system by injection.

*Plant material*: seedlings of yellow onion (*A. cepa* 'Century').

***Treatments*:** The treatments using the biological product are shown in Table 1.

Table 1. Fertilization levels (kg ha−1) used in the farm and the experimental area

| Levels | N | $P_2O_5$ | $K_2O$ | CaO | Mg | Zn | $B_2O_4$ |
|---|---|---|---|---|---|---|---|
| Fundo América. (2015) | 219.1 | 76.7 | 270.8 | 34.3 | 23.2 | 2.2 | 0.8 |
| Experimental area. (2016) | 40 | 50 | 80 | 32 | 2 | 2.6 | 2 |

The first application of the biological product was by impregnation and before transplantation. This application was made in all treatments except for the conventional control, in which the seedlings were disinfected with a chemical fungicide based on carbendazim.

The second application, by drenching, was conducted 15 days after the first application. A washer was used for this application, which was made in all treatments except for the T1 (control), T2 (2 kg $ha^{-1}$), T6 (4 kg $ha^{-1}$), and T10 (6 kg $ha^{-1}$) treatments, as these treatments only required 1 application per impregnation.

The third application, by drenching using a backpack, was conducted 15 days after the second application for the treatments T4 (2 kg $ha^{-1}$), T5 (2 kg $ha^{-1}$), T8 (4 kg $ha^{-1}$), T9 (4 kg $ha^{-1}$), T12 (6 kg $ha^{-1}$), and T13 (6 kg $ha^{-1}$). There was no third application to T1 and the other treatments as they involved either one or a maximum of 2 applications.

The fourth application, by drenching, was conducted 15 days after the third application for the treatments T5 (2 kg $ha^{-1}$), T9 (4 kg $ha^{-1}$), and T13 (6 kg $ha^{-1}$) and was not conducted for T1 and the other treatments that received only up to 3 applications.

***Fertilization:*** Fertilizers were applied by fertigation. The fertilizer levels were lower than those applied ordinarily at the farm in different crop seasons; it is important because N and $K_2O$ levels were reduced to one-third and $P_2O_5$ levels by 25 units less than the levels applied normally. (Table 1)

***Irrigation:*** An irrigation program was established, taking into account the crop coefficient (Kc), according to the stages of development of the onion crop, and the daily evapotranspiration recorded by the Fundo América weather station. The experimental field was drip irrigated every other day.

***Harvest:*** The field was undercut 99 days after transplant, when the onion plants reached 60% of bland necks.

***Experimental design:*** We used the completely randomized blocks design, in a 3 × 4 factorial arrangement, plus 1 conventional control; factor 1: N = concentration of the biological formulation (2, 4, and 6 kg ha$^{-1}$), and factor 2: A = number of applications (1 application, 2 applications, 3 applications, and four applications), totaling 13 treatments with four repetitions (Table 2). The results were compared using the Analysis of Variance (ANOVA). The between-treatment differences were determined using the orthogonal contrasts test, at the level of α = 0.05, and the Duncan's multiple comparison test (α = 0.05).

(days) the area under the progress curve of the disease (AUDPC), determining the accumulation of disease for each treatment. Campbell &Madden, 1990.

*Plant height:* We took 10 plants at random from the central lines and measured their height from the neck of the plant to the apex of the longest leaf. This was evaluated after transplantation, with a frequency of 21 days.

*Number of leaves:* We counted the number of leaves at 6, 21, 42, 63, and 84 days after transplantation, selecting 10 plants at random from each experimental unit.

Table 2. Description of the treatments, Irrigation of Santa Rita de Siguas–Arequipa, 2016

| Number | Symbol | Concentration | Application Number (Days after transplant) | Application Form |
|---|---|---|---|---|
| T1 | T0 | 0 | Any | - |
| T2 | N1A0 | 2 kg ha$^{-1}$ | 1 application (at zero ddt) | Impregnation |
| T3 | N1A1 | 2 kg ha$^{-1}$ | 2 applications (at 0 and 15 ddt) | Impregnation and drench |
| T4 | N1A2 | 2 kg ha$^{-1}$ | 3 applications (at 0, 15, and 30 ddt) | Impregnation and drench |
| T5 | N1A3 | 2 kg ha$^{-1}$ | 4 applications (at 0, 15, 30, and 45 ddt) | Impregnation and drench |
| T6 | N2A0 | 4 kg ha$^{-1}$ | 1 application to (0 ddt) | Impregnation |
| T7 | N2A1 | 4 kg ha$^{-1}$ | 2 applications (at 0 and 15 ddt) | Impregnation and drench |
| T8 | N2A2 | 4 kg ha$^{-1}$ | 3 applications (at 0, 15, and 30 ddt) | Impregnation and drench |
| T9 | N2A3 | 4 kg ha$^{-1}$ | 4 applications (at 0, 15, 30, and 45 ddt) | Impregnation and drench |
| T10 | N3A0 | 6 kg ha$^{-1}$ | 1 application to (0 ddt) | Impregnation |
| T11 | N3A1 | 6 kg ha$^{-1}$ | 2 applications (at 0 and 15 ddt) | Impregnation and drench |
| T12 | N3A2 | 6 kg ha$^{-1}$ | 3 applications (at 0, 15, and 30 ddt) | Impregnation and drench |
| T13 | N3A3 | 6 kg ha$^{-1}$ | 4 applications (at 0, 15, 30, and 45 ddt) | Impregnation and drench |

Where:
N: Is the application level or concentration (N1, N2, and N3) or (2, 4, and 6 kg ha$^{-1}$)
A: Number of applications (A1, A2, A3, and A4) or (1 application, 2 applications, 3 applications, and 4 applications).

***Evaluated variables***

*Surviving plants after transplanting:* two evaluations were performed, at 17 and 35 days after transplantation (ddt), respectively. The percentage of plants surviving in 3 linear meters of bed was calculated (out of a total of approximately 83 plants).

*Incidence of Fusarium oxysporum f.sp cepae*: The number of plants damaged by *F. oxysporum* in 3 meters of bed were counted at 28, 42, 56, and 70 ddt, when the first symptoms of the disease appeared. Two sampling points measuring 1 linear meter each were taken for each experimental unit. The disease symptoms were basal plate and root rot.

*Area under the disease progress curve (AUDPC) of F. oxysporum f.sp cepae:* Based on the incidence values (proportion of disease) of *F. oxysporum* recorded in time

*Diameter of the bulb:* The diameters of 20 bulbs chosen at random after harvest were measured using a digital verrnier.

*Colonization by R. intraradices* (%): Samples were taken at random from the roots of three onion plants for each experimental unit, at 93 ddt. We used the methodology of Philips and Hayman (1970), which consists of the staining of roots with Trypan blue for microscopic observation.

*Spore Frequency*: Soil samples weighing approximately 2 kg were taken from each experimental unit after harvest. We used the modified wet sieving and decantation protocol proposed by Gerdemann and Nicolson (1963), followed by centrifugation in saccharose proposed by Walker and Mizecw, 1982.

*Total yield:* Immediately after harvest, all the onion bulbs were weighed separately according to each experimental unit. The yield is expressed in . The bulbs were then classified according to the categories of the American export market.

*Economic analysis:* To determine the profitability of each of the treatments under study, economic analysis was carried out, taking into account the variable costs (such as the costs of applying the treatments) and the fixed costs.

## Results

***Survival (%):*** The treatments with the lowest percentages of Survival at 35 ddt were T5 (2 kg ha$^{-1}$, with four applications) and T13 (6 kg ha$^{-1}$, with four applications) at 82.33% and 87.45%, respectively, where as the treatments with the highest percentages of arrest were T6 (4 kg ha$^{-1}$, with one application) and T8 (4 kg ha$^{-1}$with three applications), at 95.55% and 94.92%, respectively.

For the statistical analysis, the original data were transformed using the formula (arcsen $\sqrt{x}$ 10) * 180 π, where x represented each of the obtained data. According to ANOVA, we determined that there were no significant differences between treatments, with a coefficient of variability of 10.22 % (Table 3).

***Incidence:*** The treatments T4 (2 kg ha$^{-1}$, with three applications) and T5 (2 kg ha$^{-1}$, with four applications) had the highest rates of incidence of *F. oxysporum*, with incidences of 19.57% and 14.39%, respectively. Meanwhile, the treatments with the lowest percentages of incidence of *F. oxysporum* were T6 (4 kg ha$^{-1}$, with 1 application) and T8 (4 kg ha$^{-1}$, with three applications) with incidences of 4.17% and 4.61%, respectively. The ANOVA of the incidence of *F. oxysporum* determined that there are significant differences for treatments (Table 4). Duncan's multiple comparison test (α = 0.05) revealed significant differences in the main dose factor, with doses of 4 and 6 kg ha$^{-1}$ for the factor number of applications presented significant differences being the first application that had a

Table 3. Results of the variables evaluated and comparison test of means Duncan (α = 0.05)

| Treatments | Survival (%) | Number of leaves | Plant height (cm) | Bulb diameter (mm) | Colonization *Rhizophagus intraradices (%)* | Spore frequency (%) |
|---|---|---|---|---|---|---|
| T1 (Control) | 87.96 a | 8.64 a | 72,48 a | 80.01 a | 27.50 a | 9.75 a |
| T2 (2 kg ha$^{-1}$ with one application) | 92.92 a | 8.77 a | 72,78 a | 79.79 a | 52.50 a | 17.25 a |
| T3 (2 kg ha$^{-1}$ with two applications) | 87.57 a | 8.88 a | 72,29 a | 80.59 a | 50.00 a | 14.75 a |
| T4 (2 kg ha$^{-1}$ with three applications) | 89.12 a | 8.68 a | 73,25 a | 81.41 a | 35.00 a | 9.50 a |
| T5 (2 kg ha$^{-1}$ with four applications) | 82.33 a | 8.58 a | 74,20 a | 79.75 a | 45.00 a | 12.75 a |
| T6 (4 kg ha$^{-1}$ with one applications) | 95.55 a | 8.62 a | 74,43 a | 81.16 a | 55.00 a | 15.75 a |
| T7 (4 kg ha$^{-1}$ with two applications) | 89.70 a | 8.50 a | 73,58 a | 80.83 a | 40.00 a | 14.25 a |
| T8 (4 kg ha$^{-1}$ with three applications) | 94.92 a | 8.69 a | 73,62 a | 80.07 a | 45.00 a | 12.50 a |
| T9 (4 kg ha$^{-1}$ with four applications) | 90.55 a | 8.84 a | 73,84 a | 82.71 a | 52.50 a | 17.25 a |
| T10 (6 kg ha$^{-1}$ with one applications) | 90.19 a | 8.68 a | 75,22 a | 83.18 a | 50.00 a | 18.75 a |
| T11 (6 kg ha$^{-1}$ with two applications) | 89.07 a | 8.70 a | 73,36 a | 81.53 a | 32.50 a | 13.75 a |
| T12 (6 kg ha$^{-1}$ with three applications) | 87.96 a | 8.87 a | 72,35 a | 81.40 a | 22.50 a | 11.25 a |
| T13 (6 kg ha$^{-1}$ with four applications) | 87.45 a | 8.77 a | 72.35 a | 81.54 a | 40.00 a | 12.25 a |
| C.V | 10.22% | 3.97%, | 4.38% | 4.24% | 29.09% | 18.54% |

lower percentage of incidence of *F. oxysporum*. As for the simple effect of the number of applications in doses, lower incidences of *F. oxysporum* (figure 1) were observed with the N1 concentration (2 kg ha$^{-1}$), with two applications and one application of the biological formulation; the N2 concentration (4 kg ha$^{-1}$), with three applications, 1 application, and four applications of the biological formulation; and the N3 concentration (6 kg ha$^{-1}$), with one application, 2 applications, and three applications of the biological formulation (Table 5).

***Area under the curve of disease progress (AUDPC):*** A higher AUDPC was observed with the T4 (2 kg ha$^{-1}$, with three applications) treatment and the control, with 1524.09 units and 1101.67 units of *F. oxysporum*, respectively, whereas a lower AUDPC was observed with treatments T8 (4 kg ha$^{-1}$, with three applications) and T6 (4 kg ha$^{-1}$, with one application) with 512.01 units and 564.22 units of *F. oxysporum*, respectively. The original data were transformed using the formula $\sqrt{x}$ for the ANOVA, which showed that there is no significance between treatments, with a coefficient of variability of 27.67%. When conducting the Duncan's multiple comparison test ($\alpha = 0.05$), the main factor "dose" showed the lowest AUDPC of *F. oxysporum* with the dose of 4 kg ha$^{-1}$ (637.78 units) (Table 5).

***Number of leaves:*** The plants inoculated with the microorganisms had practically the same number of leaves as did the control. The ANOVA did not reveal significant differences between treatments, with the coefficient of variability being 3.97% (Table 3).

Table 4. Results of the variables evaluated and comparison test of means; Duncan ($\alpha = 0.05$)

| Treatments | Incidence of *Fusarium oxysporum (%)* | AUDPC | Yield (t ha$^{-1}$) | | |
|---|---|---|---|---|---|
| | | | Exportable | Discard | Total |
| T10 (6 kg ha$^{-1}$ with one applications) | 5.53 bcd | 679.79 ab | 70.66 a | 10.23 a | 80.89 a |
| T7 (4 kg ha$^{-1}$ with two applications) | 11.44 abc | 722.02 ab | 69.61 a | 12.78 a | 82.39 a |
| T6 (4 kg ha$^{-1}$ with one applications) | 4.61 cd | 564.22 ab | 69.11 a | 9.54 a | 78.65 ab |
| T13 (6 kg ha$^{-1}$ with four applications) | 11.90 abc | 1042.05 ab | 67.18 ab | 8.19 a | 75.37 ab |
| T8 (4 kg ha$^{-1}$ with three applications) | 4.17 d | 512.01 b | 67.14 ab | 14.37 a | 81.51 a |
| T11 (6 kg ha$^{-1}$ with two applications) | 9.45 abcd | 1093.98 ab | 66.50 ab | 11.86 a | 78.36 ab |
| T5 (2 kg ha$^{-1}$ with four applications) | 14.39 ab | 1063.11 ab | 65.41 ab | 10.32 a | 75.73 ab |
| T2 (2 kg ha$^{-1}$ with one applications) | 10.48 abcd | 1010.30 ab | 62.84 abc | 13.23 a | 76.05 ab |
| T1 (Control) | 12.59 abc | 1101.67 ab | 62.46 abc | 9.60 a | 72.06 bc |
| T9 (4 kg ha$^{-1}$ with four applications) | 6.63 bcd | 752.89 ab | 59.52 bc | 12.13 a | 71.65 bc |
| T12 (6 kg ha$^{-1}$ with three applications) | 10.58 abcd | 984.73 ab | 57.33 c | 13.53 a | 70.86 bc |
| T4 (2 kg ha$^{-1}$ with three applications) | 19.56 a | 1524.09 a | 55.40 c | 10.08 a | 65.48 c |
| T3 (2 kg ha$^{-1}$ with two applications) | 5.67 bcd | 776.18 ab | 55.10 c | 11.87 a | 66.97 c |
| C.V | 27.00% | 27.67% | 7.82% | 7.45% | 6.59% |

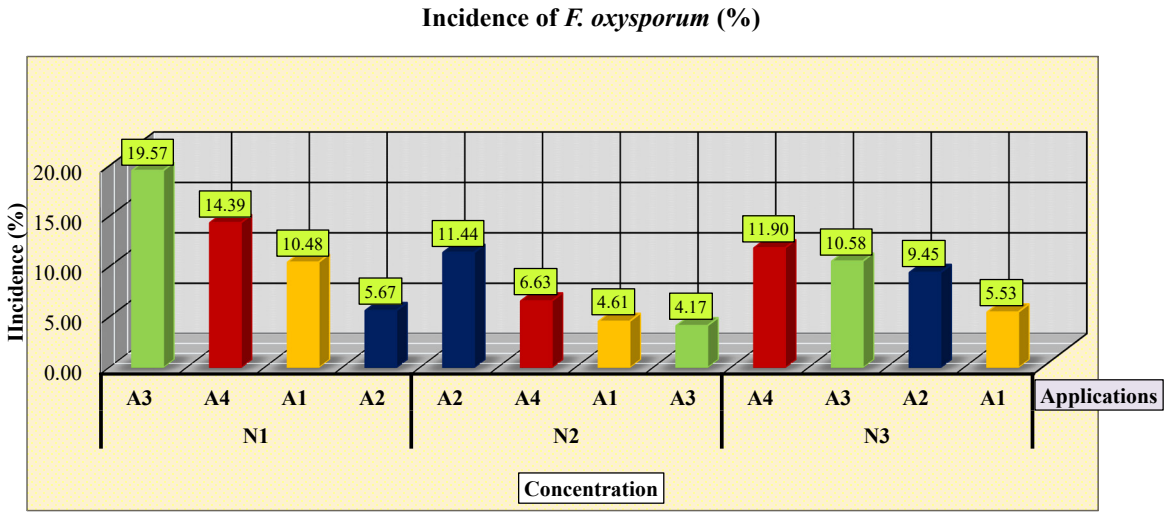


Figure 1. Simple effects on incidence of *Fusarium oxysporum* (%) at 70 days after transplant, Irrigation of Santa Rita–Arequipa, 2016

Table 5. Simple and main effect of concentration and number of applications on the incidence and AUDPC of *Fusarium oxysporum* (%) at 70 (ddt), Irrigation of Santa Rita–Arequipa, 2016

| Concentration (kg ha$^{-1}$) | Incidence of *F. oxysporum* (%) | AUDPC | Number of applications | Incidence of *F. oxysporum* (%) |
|---|---|---|---|---|
| 0 (Control) | 12.59 a | 1101.67 a | None (Control) | 12.59 a |
| 2 | 12.53 a | 1093.42 ab | 3 applications | 11.44 ab |
| 6 | 9.36 ab | 950.14 ab | 4 applications | 10.97 ab |
| 4 | 6.71 b | 637.78 b | 2 applications | 8.51 ab |
| | | | 1 application | 6.87 b |

***Plant height:*** The treatments with the highest plants at 84 ddt were T10 (6 kg ha$^{-1}$, with one application), T6 (4 kg ha$^{-1}$, with one application), T5 (2 kg ha$^{-1}$, with four applications) with plant heights measuring 75.22, 74.43, and 74.20 cm, respectively. Treatments with low plant height included T3 (2 kg ha$^{-1}$, with two applications) T13 (6 kg ha$^{-1}$, with four applications), with plant heights of 72.29 cm and 72.35 cm, respectively. The ANOVA revealed significant differences, and the coefficient of variability was 4.38% (Table 3).

***Bulb Diameter:*** Bulb diameter was greatest in the treatments with the highest concentrations of the biological formulation; T10 (6 kg ha$^{-1}$, with one application) showed the largest average diameter (83.18 mm) with respect to other treatments. The bulb diameters for T5 (2 kg ha$^{-1}$, with four applications), T2 (2 kg ha$^{-1}$, with one application), and the control were 79.75, 79.79, and 80.01 mm, respectively; these treatments resulted in smaller equatorial bulb diameters. The statistical analysis revealed no significant differences for blocks and treatments, and the coefficient of variability was 4.24% (Table 3).

***Rate of colonization by R. intraradices* (%):** The highest rate of settlement by *R. intraradices* (55%) was with treatment T6, in which 4 kg ha$^{-1}$ was in a single application. This was followed by treatments T2 (4 kg ha$^{-1}$, with four applications), and T9 (2 kg ha$^{-1}$, with one application), with a rate of 52.5% for each, and the colonization rate of the control was 27.50%. For the statistical analysis, the original data were transformed using the formula (arcsen √x 10) * 180 π. The ANOVA revealed significant differences between blocks, but no significant differences were found in terms of treatments. The coefficient of variability was 29.09%, (Table 3).

***Number of R. intraradices spores***: The spore count revealed that *R. intraradices* spores were present in all treatments, including control. The treatment with the highest number of spores was T10 (6 kg ha$^{-1}$, with one application), which had an average of 18.75 spores per gram of soil, and the treatments with the least number of spores were the control and T4 (2 kg ha$^{-1}$, with three applications), with an average count of 9.75 and 9.50 spores per gram of soil, respectively. The ANOVA revealed significant differences between blocks but no significant differences among treatments (Table 3).

***Yield:*** We weighed all the bulbs harvested from each experimental unit, and the weights obtained were projected to tons per hectare (t ha$^{-1}$). The best total yields were T7 (4 kg ha$^{-1}$, with two applications), with 82.39 ha$^{-1}$; T8 (4 kg ha$^{-1}$, with three applications), with 81.50 t ha$^{-1}$; and T10 (6 kg ha$^{-1}$, with one application), with 80.90 t ha$^{-1}$. The lowest total yields were found in T3 (2 kg ha$^{-1}$, with two applications), with 66.97 t ha$^{-1}$ and T4 (2 kg ha$^{-1}$, with three applications), with 65.48 t ha$^{-1}$. The best treatments for exportable yield were T10 (6 kg ha$^{-1}$, with 1 application), T7 (4 kg ha$^{-1}$, with two applications), and T6 (4

kg ha$^{-1}$, with one application), with 70.66, 69.61, and 69.11 t ha$^{-1}$, respectively. The lowest exportable yields were the treatments T4 (2 kg ha$^{-1}$, with three applications), with 55.40 t ha$^{-1}$; T12 (6 kg ha$^{-1}$, with three applications), with 57.33 t ha$^{-1}$; and T3 (2 kg ha$^{-1}$, with two applications), with 55.10 t ha$^{-1}$. Figure 2. The ANOVA for exportable yield and for total yield revealed significant differences between treatments, concentration and number of applications, factors of the biological formula, and the interactions of both factors. The coefficient of variability for exportable yield was 7.82% and that for total yield was 6.59% (Table 4).

The ANOVA of orthogonal contrasts for total yield revealed that treatments T10 (6 kg ha$^{-1}$, with one application), T11 (6 kg ha$^{-1}$, with two applications), and T13 (6 kg ha$^{-1}$, with 4 applications) were statistically different from the control. The first application resulted in the highest

Table 6.Concentration and number of applications of the variable total yield (t ha $^{-1}$) of the treatments under study, Irrigation of Santa Rita–Arequipa, 2016

| Concentration (kg ha $^{-1}$ ) | Yield (t ha -1 ) | | N. of A.* | Yield (t ha -1 ) | |
|---|---|---|---|---|---|
| | Exportable | Total | | Exportable | Total |
| 4 | 66.34 a | 78.55 a | 1 | 67.54 a | 78.53 a |
| 6 | 65.42 a | 76.37 ab | 4 | 64.03 ab | 74.25 ab |
| 0 | 62.46 ab | 72.06 b | 2 | 63.73 ab | 75.91 ab |
| 2 | 59.68 b | 71.06 b | 0 | 62.46 ab | 72.06 b |
| | | | 3 | 59.96 b | 72.62 b |

*N. of A.: Number of Applications

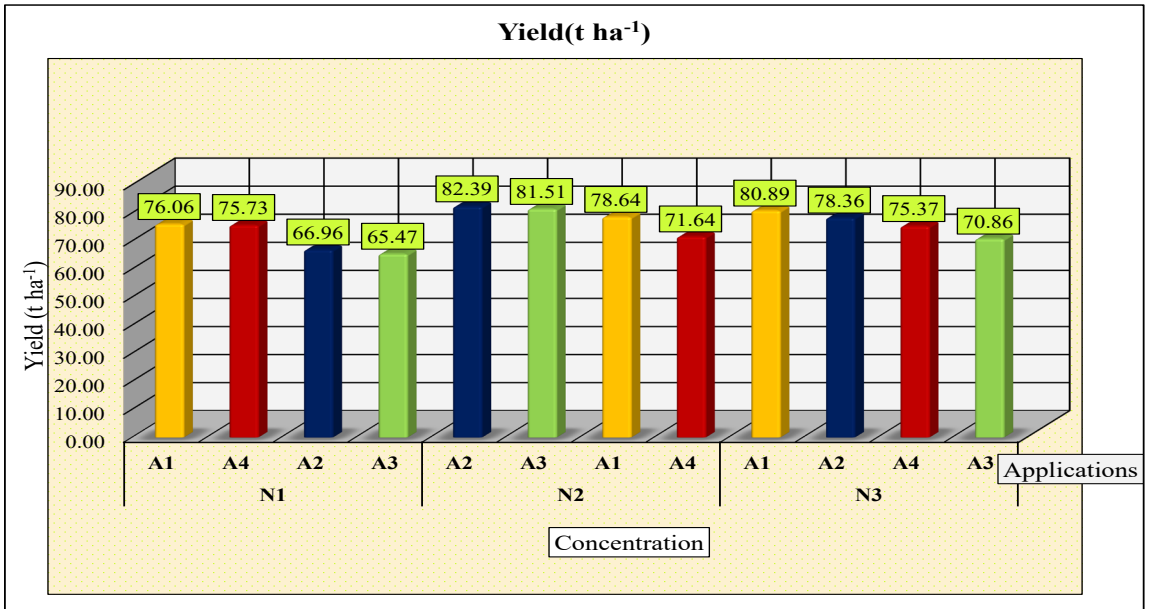


Figure 2. Main effects of treatments under study on yield (t ha$^{-1}$), Irrigation of Santa Rita–Arequipa, 2016

performance, and the highest yields were obtained with dose concentrations of 4 kg ha$^{-1}$ and 6 kg ha$^{-1}$, and these were statistically different from the concentration of 2 kg ha$^{-1}$ and the control (Table 6).

Evaluation of the main factor concentration showed that the concentrations of the biological formula with the greatest effects on yield were of 4 kg ha$^{-1}$ and 6 kg ha$^{-1}$. Regarding the number of applications of the main factor, the first application had the greatest effect on yield.

## Discussion

**Survival:** As prehension of the onion seedlings was affected by the presence of *Fusarium* in all the experimental units, we inferred that the biological formula does not have a significant effect on the onion seedlings when there are high rates of incidence of *F. oxysporum*.

The development of the disease was promoted by the warm temperatures, with maximum temperatures of 28.57 °C, which, according to Koike, Gladders, and Paulus (2007), is the optimal temperature for development of the disease.

In addition to being present in the field of commercial onion growing, there is a record of the presence of the disease in the previous seasons, which reaffirms the ability of the disease to survive for decades in the form of chlamydospores, with a resistance structure that allows it to continue even in the absence of the host crop or in unfavorable environmental conditions (Ploetz, 2015).

***Biometric variables***: With respect to the biometric variables we considered in the investigation, such as the number of leaves, the height of the plant, and the diameter of the bulb, there were no significant differences found in the results of

any treatment in relation to the control treatment, For this reason, it is considered that this complex of rhizobacterial microorganisms (PGPR) that promote plant growth has not shown any influence on the development of onion plants, although it is recognized that the use of the biological formulation has the potential to improve both production and yields in the final results. This can be supported by the authors Cakmakçi, Dönmez, Aydın and Şahin. (2006), who mention the conditions by which PGPR strains have limits on their ability to colonize the rhizosphere, these conditions include soil type, temperature, moisture content, organic matter and pH. Banerjee, Yesmin and Vessey (2006); and Bly, Woodard and Gelderman (2009) have reported that the reason why the use of PGPR is not yet a widespread practice is largely due to the inconsistency in the promotion of plant growth by most of the PGPR strains in different field conditions. . These finding agree with those of Sánchez, Ley, Ricardo, and Collazo (2015), who evaluated the effect of the arbuscular mycorrhizal fungi (AMF) *Glomus spp*., *Glomus clarum*, and *Glomus intraradices* in the tomato crop (*Solanum lycopersicum L.* Var., Amalia) and found no significant differences in the number of leaves in the tomato plants inoculated with the AMF and the control plants.

***Incidence:*** In the treatments in which the biological formulation was applied, there was a lower incidence of *F. oxysporum* with respect to the control; this observation is relevant, as the control treatment seedlings were disinfected with a chemical fungicide based on carbendazim before transplantation. This would seem to demonstrate that the biological formula plays an important role in inhibiting the development of phytopathogens of the root system, as a chemical fungicide would do, which coincides with the findings by Hsiang, Jamie, and McDonald (2008), who inoculated onion plants with biological formulations based on *G. intraradices* and reduced the incidence of *F. oxysporum*. Furthermore, application of the biological formula will have a greater effect if it is done at the time of either sowing or transplantation (Bashan, De-Bashan, Prabhu, & Hernandez, 2014). This was corroborated by Qingxiao (2014), who performed an assay to ascertain the effect in the biological control of *Streptomyces spp.* in radish plants and reported that the disease was suppressed completely by application before planting. The AUDPC was lower with a dose of 4 kg $ha^{-1}$, which corroborates the results obtained previously in the incidence evaluation, the dose of 4 $kg.ha^{-1}$ could have a favorable effect in reducing the development of the disease caused by *F. oxysporum*, possibly by the induction of systemic resistance or competition for nutrients of the rhizosphere microorganisms (Nadeem, Zahir, Naveed, & Ashraf, 2010). According to Alarcón, Ferrera, González and Villegas (2000), in addition to the nutritional benefit of the symbiosis, the Arbuscular mycorrhizal fungi also act as agents of biological control of the phytopathogens present in the ground.

The colonization percentages reached during the trial did not differ statistically between the inoculated treatments, and the conventional control may likely have influenced the high phosphorus content in the experimental area. Authors such as Rodríguez *et al.* (2010) mention that mycorrhization is generally inhibited in soils with a high phosphorus content, as high phosphorus concentrations determine the permeability of cell membranes and the radical exudation of carbohydrates and amino acids available as metabolites for the fungus [Kurle and Pfleguer (1994) cited by Regalado, (2002)]. The spore count revealed the presence of *R. intraradices* spores in all treatments, including control. This would seem to corroborate the existence of native *Rhizophagus spp.* spores in the soils of the cultivated fields in the irrigation of Santa Rita that would also explain the colonization observed in the control treatment. Fernández (2006) suggests that AMF form symbiotic relationships with the underground organs of most higher plants, enabling the survival of the spores of *Rhizophagus spp.* in the soils of the cultivated fields.

***Yield:*** The effect of the biological formulation was important in increasing the yield of yellow onions. The possible direct mechanisms by which PGPR microorganisms increased the yields of this crop include its control effect on the incidence of plants infected with F. oxysporum, due to their antagonistic action against pathogenic microorganisms present in the soil, in addition to their components Antibiotics and siderophores production.

According to Soesanto *et al.* (2010), each microbial antagonist, including PGPR, has its own mechanism of inhibition in control of plant pathogens and may have more than one mechanism. It is likely that the yields obtained from the treatments are due not only to a lower incidence of *F. oxysporum*. They may also result from the action or effect of the mycorrhiza and the rhizobacteria either interacting with each other or independently producing changes in the chemical composition in the rhizosphere and its interaction with the plant roots, given the ability of PGPR to Increase the availability of nutrients for the plant by eliminating the chemical phosphate bonds to become soluble phosphate and be absorbed by the roots, reaching optimal yields similar to high levels of nitrogen, phosphorus and potassium fertilization.. Finally, the PGPR of the biological formulation may have increased the yield of the yellow onions because *Bacillus spp*. solubilize the inorganic phosphorus present in the soil, thereby enhancing the entry of this macronutrient to the plant, which results in a larger amount of biomass (Rajankar, Tambekar & Wate, 2007).

## Conclusions

A biological formula consisting of the microorganisms *Azotobacter salinestris*, *Bacillus amyloliquefaciens*, and

*Rhizophagus intraradices* had a positive effect on the yield of the yellow onion in the spring–summer season under the edaphoclimatic conditions of the Santa Rita of Siguas–Arequipa irrigation.

The concentrations of the biological formula with the most significant effect on the onion yield were 4 kg $ha^{-1}$ and 6 kg $ha^{-1}$, producing exportable and total yields of 66.34 t $ha^{-1}$ and 65.42 t $ha^{-1}$, respectively, which were superior to those obtained using other concentrations. Moreover, the two concentrations also resulted in a lower incidence of *Fusarium oxysporum* disease and a smaller AUDPC of the disease.

The first application of the biological formula had the greatest effect on the yield of the yellow onion crop. The yield obtained with this application was 67.54 t $ha^{-1}$, which was superior to those obtained with other applications. This corroborated the theory that applying the biological formula before transplantation has a greater effect than the later applications via drenching.

Regarding the best combination of concentration and number of applications, T6 treatments (4 kg $ha^{-1}$, with one application), T7 (4 kg $ha^{-1}$, with two applications) and T10 (6 kg $ha^{-1}$, with one application) treatments stand out, with exportable yields of 69.11, 69.61, and 70.66 t $ha^{-1}$, respectively. These combinations produced higher exportable yields and a lower incidence and AUDPC of *F. oxysporum*.

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