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RESEARCH ARTICLE

# Herbicide does not impact diazotrophic bacteria-promoting growth of red rice plants

# Aplicação de herbicidas não impacta bactérias diazotróficas promotoras de crescimento em arroz vermelho

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**Abstract: Background**: Diazotrophs bacteria that promote plant growth have been studied as a strategy to increase the productivity of rice cultivation. Herbicides are used to prevent weed infestations.
**Objective**: The evaluation of herbicides effect on diazotrophic bacteria strain associated with the growth of Red Small rice.
**Methods**: Three experiments were conducted: I - *in vitro* growth of bacterias (*Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H, Z67, Z94, ZA25 and ZAL95); II - germination of red rice seeds; III - growth and production of red rice plants inoculated with diazotrophic bacteria and treated with quinclorac, penoxsulam and cyhalofop-butyl herbicides.
**Results**: I - All bacterial strain growth was inhibited under the highest herbicide doses, except strain 245. II – The number of roots reduced upon increasing herbicide concentration for strain 245. III - The dry mass increased in plants inoculated with strains 245 and ZA25. Besides, strains 245, 26H and ZAL95 provided better performance for dry root mass. At 60 days, the same behavior of the first evaluation was observed. At final cycle, increases in fresh and dry root mass and dry mass of grains were evidenced, especially on rice inoculated with strains 245 and ZA25.
**Conclusions**: The herbicides do not impact *Azospirillum* and *Herbaspirillum* bacterial strains. Besides, strains 245 and ZA25 show better performance in promoting plant growth of rice.

**Keywords**: plant growth-promoting bacteria, non-target organism intoxication, *Oryza sativa*

**Resumo: Introdução**: Bactérias diazotróficas que promovem o crescimento de plantas têm sido estudadas como estratégia para aumentar a produtividade do cultivo de arroz. Herbicidas são usados para prevenir infestações de ervas daninhas.
**Objetivo**: Avaliar o efeito de herbicidas sobre cepas de bactérias diazotróficas associadas ao crescimento de arroz vermelho.
Métodos: Foram realizados três experimentos: I - crescimento *in vitro* de bactérias (*Azospirillum brasilense* cepa 245 e *Herbaspirillum seropedicae* cepas 26H, Z67, Z94, ZA25 e ZAL95); II - germinação de sementes de arroz vermelho; III - crescimento e produção de plantas de arroz vermelho inoculadas com bactérias diazotróficas e tratadas com os herbicidas quinclorac, penoxsulam e cyhalofop-butyl.
**Resultados**: I - O crescimento de todas as linhagens bacterianas foi inibido nas maiores doses de herbicida, exceto a linhagem 245. II - O número de raízes diminuiu com o aumento da concentração do herbicida para a linhagem 245. III - A massa seca aumentou nas plantas inoculadas com as linhagens 245 e ZA25. Além disso, as linhagens 245, 26H e ZAL95 proporcionaram melhor desempenho para massa seca de raízes. Aos 60 dias, observou-se o mesmo comportamento da primeira avaliação. No ciclo final, foram evidenciados aumentos na massa fresca e seca da raiz e na massa seca dos grãos, principalmente no arroz inoculado com as linhagens 245 e ZA25.
**Conclusões**: Os herbicidas não afetam as cepas bacterianas *Azospirillum* e *Herbaspirillum*. Além disso, as linhagens 245 e ZA25 apresentam melhor desempenho na promoção do crescimento de plantas de arroz.

**Palavras-chave**: bactérias promotoras de crescimento de plantas, intoxicação por organismos não-alvo, *Oryza sativa*.

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## 1. Introduction

Rice (*Oryza sativa* L.) is a Poaceae family member most cultivated in several countries worldwide. It is an important food crop used to feed more than half of the global population and paddy to feed animals as fodder (Banik et al., 2019; Kumar et al., 2022). In the 2024 Brazil rice crop, more than 11 million tons of this grain are expected to be produced (Conab, 2023). However, the lack of biological nitrogen fixation (BNF) capacity (non-leguminous plant), promotes deprivation in N for growth and development. On the other hand, the low N use efficiency for plants increases use of agrochemicals, such as N fertilizers. Nitrogen is the major element required for plant growth, among the macronutrients (Shabanamol et al., 2018).

There is the need to increase agricultural production (60%), especially rice production to meet the demand for food consumption expected by global population increase (Shabanamol et al., 2018). To meet these requirements, studies of plant-associated with microbiota has been increasing, especially bacteria form endophytic diazotrophs class. In plants, the association with bacteria has been reported an ecofriendly and cost-effective way to provide N as much as necessary for host plants to promote plant growth. Besides, bacteria also contribute with the production of plant growth hormones, solubilization of soil nutrients and protection against pathogens (Hayat et al., 2010; Hossain et al., 2023).

These comprise a wide range of prokaryotic microorganisms commonly called plant growth-promoting rhizobacteria (PGPR). Among the bacterias, the genus *Azospirillum* is the most common bacteria used in agriculture to promote plant growth (Hossain et al., 2023). Besides, there as other bacteria such as *Acetobacter*, *Azoarcus*, *Enterobacter*, and *Herbaspirillum* with the ability to colonize grasses (monocot plants) and cereal crops (Mehnaz et al. 2001). Efforts on the role of PGPR in enhancing plant growth upon stress conditions have been paid.

Of the abiotic stress factors with the potential to reduce the productivity of rice crops, weeds are highlighted. Weeds compete with the crop for growth resources such as light, water, and nutrients that are usually found in limited quantities in the environment which can cause losses in grain productivity over 52% (Concenço et al., 2012). A rapid way to maintain food production and concomitantly eliminate or avoid weeds, pests and diseases is the large use of pesticides. Among them, herbicides are the mostly pesticides applied (70%) in the world (Kniss, 2017).

Besides the benefits of pesticides in cropping systems, the use for long-term can contribute to modify soil characteristics, such as diazotrophic bacterial communities. A negative impact on diazotrophs in soil due to herbicide largely used in crops has also been reported (Ma et al. 2023). On the other hand, pesticides do not impact negatively the diazotrophs communities weather applied in correct concentrations and can be benefit in low concentrations by stimulating plant growth (Shen and Luo, 2011). The answer to the question of whether herbicide use impacts diazotrophs bacteria in soil remains controverse. Therefore, the aim was to evaluate the impact of herbicides (recommended for the management of weeds in rice cultivation) in diazotrophs *Azospirillum brasilense* and *Herbaspirillum seropedicae* strains in promoting plant growth of red rice crop.

## 2. Material and Methods

Three experiments were carried out. Bioassay I - To evaluate the growth of bacterial strains in a medium containing herbicides used in rice culture. Bioassay II - Germination of red rice seeds inoculated with diazotrophic bacteria and treated with herbicides. Bioassay III - Growth and production of inoculated red rice subjected to herbicides. In all experiments, the diazotrophs used were: *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H, Z67, Z94, ZA25 and ZAL95 and the herbicides quinclorac, penoxsulam and cyhalofop-butyl.

### Bioassay I – Bacterial growth upon herbicide

The growth of bacterial strains (above described) was performed in medium containing herbicides [Quinclorac 375, 750, 1125 and 1500 g a.i. $ha^{-1}$ (15, 30, 45 and 60 mg $L^{-1}$), Penoxsulam 60, 120, 180 and 240 g a.i. $ha^{-1}$ (2.4, 4.8, 7.2 and 9.6 mg $L^{-1}$) and cyhalofop-butyl 225, 450, 675 and 900 g a.i. $ha^{-1}$ (9, 18, 27 and 36 mg $L^{-1}$)] , added in a volume equivalent to 200 L $ha^{-1}$ of syrup, and the controls without herbicide, with four replications. The bacterial cells were activated in 250 mL Erlenmayer flasks containing 50 mL of DYGS medium and incubated at 30° C under constant agitation of 150 rpm for 24-48 h. Then, each strain was grown in Petri dishes with solid culture medium NFB, JNFb and medium Potato (Döbereiner et al., 1995) in order to verify the purity. After checking the purity, a colony of each isolate was also inoculated in 250 mL Erlenmayer under the same conditions mentioned above. After growth, 10 μL aliquot of these strains were inoculated into penicillin tubes, containing 5 mL of semi-solid NFB and JNFb culture medium, for the cultivation of *Azospirillum* and *Herbaspirillum* strains, respectively. After that, the herbicide solutions were prepared by mixing the commercial product with distilled water and applied in the strains. The tubes were immediately placed in an incubation chamber at 30 ºC, in the dark. The bacterial growth was then evaluated after seven days, by the presence or absence of the typical aerotaxic film close to the surface of the medium and by the change in the color of the culture medium, due to the change in pH provided by the growth of the bacteria (Kuss et al., 2007).

### Bioassay II – Germination of red rice seeds inoculated with diazotrophic bacteria and treated with herbicides

The germination test was performed with Small Red Rice variety. The herbicides were tested, but only at the commercial recommended doses: Quinclorac 375 g a.i. $ha^{-1}$, (15 mg $L^{-1}$) Penoxsulam 60 g a.i. $ha^{-1}$ (9.6 mg $L^{-1}$) and Cyhalofop-butyl 225 g a.i. $ha^{-1}$ (9 mg $L^{-1}$), considering each herbicide as a treatment. The treatments were arranged in a factorial scheme (2 x 6 +1): the first being composed of the presence or absence of herbicide and the second by the same strains mentioned above and one control without herbicide and inoculation, in a fully randomized design with four replications. The working solutions were prepared corresponding to a spray volume of 200 L $ha^{-1.}$ The seeds were dipped for 10 seconds in the solutions containing herbicides and then inoculated. For this process, the peat was weighed in a proportion of 10 g $Kg^{-1}$ seed and a solution of water and sugar (10%) in the proportion of 6 mL was added per $Kg^{-1}$ seed for better adherence between the seed and the peat.

After seed inoculation, the microorganisms present in the inoculant were counted using the most likely number (NMP) as described by McCrady table (Döbereiner et al., 1995), as established in an official protocol of the Ministry of Agriculture, Livestock and Supply (MAPA), which recommends a population of at least 106 cells $g^{-1}$ seed. Subsequently, the seeds were placed to germinate in Petri dishes containing Germitest paper. Each plate received 25 seeds, according to the seed analysis rule (MAPA, 2009). At 14 days after germination, the number of germinated seeds, fresh and dry mass of the aerial part, fresh and dry mass of the root, number of secondary roots, length of the root and the aerial part were evaluated.

### Bioassay III – Growth and production of inoculated red rice subjected to herbicides

The growth and production of inoculated red rice subjected to herbicides were performed. The experiment was conducted in unprotected environment conditions, in a dry planting system cultivated in pots with a capacity of 5 $dm^3$ containing Planossolo soil, from the superficial layer (0-20 cm). The soil used was a mix of 10 samples collected randomly, dried in the shade, and sieved in a 2 mm mesh. The physical-chemical analysis of the soil is shown in Table 1. From the results, fertilization was defined according to the recommendation for rice cultivation (Freire, 2013), except for the supply of nitrogen, in order to verify the possible increase of this nutrient comes from the tested bacteria strains.

**Table 1**. Chemical analysis of soil used in the experiment.

| Sample | pH (water) | $Al^{+3}$ | H+ Al | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | P |
|---|---|---|---|---|---|---|---|---|
| | | ------------------------cmol$_c$/dm$^3$------------------------ | | | | | -----mg $L^{-1}$----- | |
| *Average | 5.27 | 0 | 0.7 | 1 | 2.05 | 0.018 | 43 | 8.5 |

*Recommendation based on the average value of the 2 composed samples.

The local climate is classified as Aw (Köppen classification), with hot and rainy summer, with rains concentrated between November to March and dry winter. The same herbicides and diazotrophs strains were used as in the bioassay I. When sowing, rice seeds were inoculated with peat containing the respective strains. Counting was also performed on the inoculated seeds using the NMP technique. Four seeds were sown per pot, after germination and establishment of the seedlings, they were thinned, leaving one plant per pot. Irrigation was carried out whenever necessary, uniformly, with a volume of 100 mL of water applied to each pot, to maintain at approximately 80% of the field capacity.

At 25 days after sowing, when the critical period of weed competition in rice culture usually begins, herbicides were applied. For the application of these, the plants were sprayed using a $CO_2$-pressurized backpack sprayer equipped with four TeeJet XR110.015 flat-fan nozzles (TeeJet Technologies), which delivered 200 L ha$^{-1}$ of spray solution at 250 kPa. There was also the use of adjuvant (vegetable oil) in the applications of the cyhalofop-butyl and quinclorac herbicides. At the time of application, in the late afternoon, the recorded temperature was 22.2 ° C, the relative humidity was 77% and the minimum and maximum wind speed were 2.4 and 4.1 km h- 1, respectively.

At 5 days after herbicide application (30 days after sowing), at the flowering stage and the end of the crop cycle were evaluated: the fresh and dry mass of the shoot, fresh and dry mass of root, the number of tillers, leaf area, N-total of the shoot and the components of production: mass of 100 grains and total mass of grains. As nitrogen did not show significance in the two initial collections, it was decided not to determine the leaf nitrogen content in the third collection. On those same evaluation dates (5 days after herbicide application (30 days after sowing), at the flowering stage and the end of the crop cycle were evaluated), the quantification of diazotrophic bacteria (BD) was performed per gram of fresh tissue from the shoot and root. For that, the plants were harvested and separated into shoots and roots with cutting in the base of the stem. The different parts of the plants were packed in plastic bags and transported to the Laboratory. The roots, without rhizospheric soil, were washed in three successive passes in distilled water. Afterward, 5 g of each vegetable sample was crushed and placed under agitation for 60 min in saline solution, in a volume of 45 mL, which consisted of a $10^{-1}$ dilution. These saline suspensions were diluted in series, transferring 1 mL of the previous suspension to test tubes containing 9 mL of saline until the $10^{-7}$ dilution. Then, a 0.1 mL aliquot from the $10^{-3}$ dilutions to the $10^{-7}$ dilution was used to inoculate, in triplicate, vials containing 5 mL of the semi-solid and semi-selective NFB and JNFb media for diazotrophic bacteria of the genus *Azospirillum* and *Herbaspirillum* (Döbereiner et al, 1995). The inoculated flasks were incubated at 30 °C for 7 days and the formation of a film on the surface of the medium, a characteristic common to associative diazotrophic bacteria, was observed for their quantification by the NMP method.

The normality of the data was tested using the Shapiro-Wilk test and the homogeneity of the variances by the Bartlett test. Next, all experimental data, except experiment 1 (qualitative data), were subjected to analysis of variance (ANOVA), using the *F* at $p > 0.05$ and, when significant, a comparison was made between means by the Scott-Knott clustering criterion, $p > 0.05$. The statistical program R was used, using the software Rbio.

## 3. Results and Discussion

### Bioassay I – Bacterial growth upon herbicide

The herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl did not impact the bacteria, showing normal growth of the tested strains at doses 375, 750 and 1125 g a.i. ha$^{-1}$ for quinclorac; doses of 60, 120 and 180 g a.i. ha$^{-1}$ for penoxsulam; and 225, 450 and 650 g a.i. ha$^{-1}$ for Cyhalofop-butyl. *Azospirillum brasilense* strain 245 grew in all tested doses and herbicides. Growth inhibition of bacteria of the genus *Herbaspirillum* was observed when the dose of 1500 g a.i. ha$^{-1}$ was applied for Quinclorac, 240 g a.i. ha$^{-1}$ for Penoxsulan and 900 g a.i. ha$^{-1}$ for Cyhalofop-butyl (Table 2).

The evaluation of 18 herbicides recommended for sugar cane cultivation, the growth of *Gluconacetobacter diazotrophicus* was inhibited only with paraquat, diuron, metribuzin, MSMA and 2,4-D upon their respective commercial doses (Procópio et al., 2013). The insecticides imidacloprid, fipronil and thiamethoxam used in their recommended commercial doses of 250, 400 and 480 g a.i. ha$^{-1}$, respectively, did not harm growth or on the biological fixation of nitrogen *in vitro* of *Herbaspirillum seropedicae* (Fernandes et al., 2012). The development of resistance to the most varied types of pesticides is a complex process that in most cases may be linked to changes in the physiology or even in the genetic makeup of microorganisms (Rice and Bonomo, 2005). Microorganisms are reported to transform pesticides into forms not toxic by diverse enzymatic biochemical reactions of oxidation, reduction, or hydrolytic type, providing the survival of these microorganisms. The effects of pesticides on the growth of microorganisms, as variable as possible, are discussed. The soil bacteria such as species of the genera *Azotobacter*, *Artrobacteria*, *Rhodococcus*, *Burkholderia* and *Pseudomonas* are reported to tolerate and/or be involved in the detoxification of pesticides which amplify their use in studies of biodegradation and bioremediation programs (Castillo et al., 2011).

**Table 2**. Growth of bacterial strains in medium containing the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl, used in the culture of rice.

| | Quinclorac (g a.i. ha$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Bacteria | 0 | 375 | 750 | 1125 | 1500 |
| 245 | +* | + | + | + | + |
| 26H | + | + | + | + | -* |
| Z67 | + | + | + | + | - |
| Z94 | + | + | + | + | - |
| ZA25 | + | + | + | + | - |
| ZAL95 | + | + | + | + | - |
| | Penoxsulam (g a.i. ha$^{-1}$) | | | | |
| Bacteria | 0 | 60 | 120 | 180 | 240 |
| 245 | + | + | + | + | + |
| 26H | + | + | + | + | - |
| Z67 | + | + | + | + | - |
| Z94 | + | + | + | + | - |
| ZA25 | + | + | + | + | - |
| ZAL95 | + | + | + | + | - |
| | Cyhalofop-butyl (g a.i ha$^{-1}$) | | | | |
| | 0 | 225 | 450 | 675 | 900 |
| 245 | + | + | + | + | + |
| 26H | + | + | + | + | - |
| Z67 | + | + | + | + | - |
| Z94 | + | + | + | + | - |
| ZA25 | + | + | + | + | - |
| ZAL95 | + | + | + | + | - |

*(+) Bacterial growth verified by the presence of the typical aerotaxic film and color change of the culture medium; * (-) Absence of the typical film and medium without color change. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H, Z67, Z94, ZA25 and ZAL95.

Kong et al. (2014) reported the capacity of *Rhodopseudomonas palustres* bacteria to degrade the quinclorac molecule by removing the chlorine molecule and mineralizing the 4-Chlorophenol. This process can be done in two ways: (i) quinclorac acts by receiving an electron that is donated by the co-substrate used to reduce the inhibition of microbial growth. (ii) quinclorac receives electrons donated by the microorganism that acts as a catalyst. Studies show that several other aromatic compounds are converted due to the action of microorganisms in intermediate products, by breaking the aromatic ring and converting it into non-cyclic metabolites that are used in the metabolism of the microorganism.

Regarding quinclorac degradation by *Pseudomonas putida*, Yang et al. (2020) reported four possible pathways for the degradation of quinolones. Based on the analysis of the *P. putida* genome, the pathway of anthranilate may be responsible for the degradation of the compound quinolone. The first step in the degradation of quinolone (including its derivatives) is the conversion of the quinolone substrate into a 1H-2-oxo- or 1H-4-oxo- product, which can be accumulated in the culture medium temporarily. In subsequent steps, the authors report a gene responsible for encoding the production of an enzyme that can catalyze the detoxification of quinolone heterocycles into N-acetylanthranylate and carbon monoxide. Besides the causes of the non-harmful effect of pesticides on microbial growth in this work cannot be precisely specified, such reported pathways can be acting to inactivate the herbicides used.

There has proven the ability of microorganisms to degrade cyhalofop-butyl right after the inoculation process. Wu et al. (2019) observed that the degradation of cyhalofop-butyl by *Rhodopseudomonas palustris* bacteria started after twenty-four hours of inoculation. This period is the need of the time by bacteria to acclimate to the environment contaminated with herbicide, which also represent the time required for the synthesis of the enzyme that acts in the degradation process to occur. After this period, the synthesis of carboxylic acid, active in the hydrolysis process of cyhalofop-butyl is also observed.

## Bioassay II – Germination of red rice seeds inoculated with diazotrophic bacteria and treated with herbicides

Upon 14 days, there was no influence of the herbicides and no positive response to inoculation, nor the interaction between these two factors for the variables root and shoot fresh weight, root and shoot dry mass, root and shoot length were observed, as well as the number of seeds germinated. No significant effects of herbicides and interaction of strain x herbicide were identified for the number of roots (Figure 1); however, a significant difference was found for the strain effect, with a higher average being observed for the *Azospirillum brasilense* strain 245. Rice from upland regions showed an increase in both the number of secondary roots and the number of root branches, with similar positive responses for *Azospirillum* species used as inoculants (Herrera et al., 2016). These results are possibly associated with the production of plant regulators belonging to the auxin group, as auxin is considered the main substance that promotes adventitious rooting. In addition, auxin is the main phytohormone produced by the rhizobacteria of the genus *Azospirillum* and has a high synthesis *in vitro*. Cassán et al., (2014) reported values of auxin production ranging from 5 to 50 μg mL$^{-1}$, varying according to the culture medium and the tested strain. Studies show a positive effect of *Azospirillum* sp. on the initial growth of grass seedlings (Wu et al., 2019). These bacteria have an important effect on agriculture as they provide a better initial performance of seedlings, a decisive factor in the formation of the crop stand and with an impact on yield. Seedlings with more vigorous can compete more efficiently for light, nutrients, and water, with better population establishment and greater grain production (Farooq et al., 2006). When related to competitive capacity, rice seedlings with initial development higher to weeds can partially suppress the development of invasive species, contributing to less investment in technologies to control weeds.

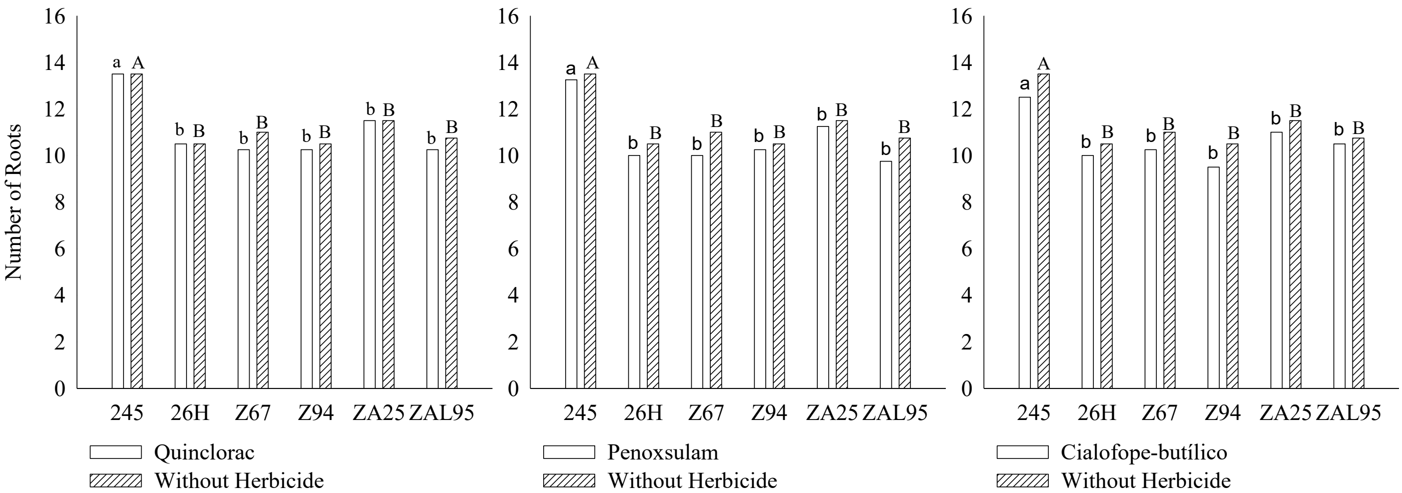


**Figure 1**. Number of root of Red Small rice seedlings inoculated with strains of diazotrophic bacteria, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H, Z67, Z94, ZA25 and ZAL95. Lower case and upper case letters, respectively, in the presence or absence of each herbicide, do not differ by the Scott -Knott test ($p > 0.05$).

## Bioassay III – Growth and production of inoculated red rice subjected to herbicides

Upon 30 days, the total nitrogen of shoot, leaf area, number of tillers, bacterial cells of root and shoot did not show the difference for the effect of herbicides as for bacteria; there was also no interaction between them. The results achieved in this work demonstrate that there was a statistical difference for the variables root and shoot fresh mass, root and shoot dry mass (Tables 3 and 4). There was no significant response from the herbicides and no interaction between the bacteria x herbicides. The strains 245 and ZA25 showed increases in root and shoot fresh weight compared to other bacteria. Besides, the rice cultivar IAC4440 was inoculated alone with the Cd strain of *Azospirillum brasilense* and the mixture of strains ZAE94 and M2 of *Herbaspirillum seropedicae*, presented shoot fresh mass higher than control not inoculated (Sabino , 2012). As well as inoculation with a mixture of three *Azospirillum* species (*A. brasilense*, *A. lipoferum* and *A. oryzae*) increased the productivity of the maize hybrid P32R48 (De Quadros et al., 2014).

**Table 3**. Average values shoot and root fresh mass (SFM and RFM), of Small Red rice plants with 30 days, inoculated with diazotrophic bacteria strains, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl.

| | SFM (g) | | RFM (g) | |
|---|---|---|---|---|
| | Quinclorac (375 g a.i ha$^{-1}$) | | | |
| Bacteria | With | Without | With | Without |
| 245 | 12.25 a | 11.97 a | 34.85 A | 31.87 a |
| 26H | 10.72 c | 10.53 b | 25.95 B | 25.10 b |
| Z67 | 10.34 c | 10.41 b | 27.87 B | 25.75 b |
| Z94 | 11.14 b | 10.86 b | 25.85 B | 27.12 b |
| ZA25 | 11.34 b | 11.63 a | 27.60 B | 33.20 a |
| ZAL95 | 9.36 d | 8.93 c | 27.32 B | 25.97 b |
| C.V. (%) | 5.12 | | 10.73 | |
| | Penoxsulam (60 g a.i ha$^{-1}$) | | | |
| Bacteria | With | Without | With | Without |
| 245 | 11.86 a | 11.98 a | 36.32 A | 31.87 a |
| 26H | 11.11 a | 10.54 b | 25.47 B | 25.10 a |
| Z67 | 10.59 b | 10.41 b | 24.82 B | 25.75 a |
| Z94 | 11.05 a | 10.86 b | 24.50 B | 27.12 a |
| ZA25 | 11.46 a | 11.63 a | 36.12 A | 33.20 a |
| ZAL95 | 9.63 c | 8.94 c | 27.70 B | 25.97 a |
| C.V. (%) | 5.78 | | 16.72 | |
| | Cyhalofop-butyl (225 g a.i. ha$^{-1}$) | | | |
| Bacteria | With | Without | With | Without |
| 245 | 11.61 a | 11.97 a | 34.07 A | 31.87 a |
| 26H | 10.81 a | 10.53 b | 27.80 B | 25.10 b |
| Z67 | 11.31 a | 10.41 b | 27.50 A | 25.75 b |
| Z94 | 11.13 a | 10.86 b | 24.02 B | 27.12 b |
| ZA25 | 11.52 a | 11.63 a | 33.100 A | 33.20 a |
| ZAL95 | 8.98 b | 8.98 c | 24.27 B | 25.97 b |
| C.V. (%) | 5.01 | | 12.63 | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test (p ≤ 0.05. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

**Table 4**. Average values shoot and root dry mass (SDM and RDM), of Small Red rice plants with 30 days, inoculated with diazotrophic bacteria strains, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl.

| | SDM (g) | | RDM (g) | |
|---|---|---|---|---|
| | Quinclorac (375 g a.i. ha$^{-1}$) | | | |
| Bacteria | With | Without | With | Without |
| 245 | 12.25 a | 11.97 a | 5.65 A | 5.57 a |
| 26H | 10.72 c | 10.53 b | 4.96 A | 5.09 a |
| Z67 | 10.34 c | 10.41 b | 5.16 A | 3.85 b |
| Z94 | 11.14 b | 10.86 b | 3.74 B | 3.86 b |
| ZA25 | 11.34 b | 11.63 a | 3.91 B | 3.56 b |
| ZAL95 | 9.36 d | 8.93 c | 4.78 A | 4.80 a |
| C.V. (%) | 4.86 | | 16.31 | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test (p ≤ 0.05. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

**Table 4**. Continuation.

| | ---------------------SDM (g)--------------------- | | | | ---------------------RDM (g)--------------------- | | | |
|---|---|---|---|---|---|---|---|---|
| | Penoxsulam (60 g a.i. ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 11.86 | a | 11.98 | a | 5.58 | A | 5.57 | a |
| 26H | 11.11 | a | 10.54 | b | 5.00 | A | 5.10 | a |
| Z67 | 10.59 | b | 10.41 | b | 3.19 | B | 3.85 | b |
| Z94 | 11.05 | a | 10.86 | b | 3.93 | B | 3.87 | b |
| ZA25 | 11.46 | a | 11.64 | a | 3.52 | B | 3.56 | b |
| ZAL95 | 9.63 | c | 8.94 | c | 5.08 | A | 4.80 | a |
| C.V. (%) | 2.52 | | | | 15.36 | | | |
| | Cyhalofop-butyl (225 g a.i ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 11.61 | a | 11.97 | a | 5.26 | A | 5.57 | a |
| 26H | 10.81 | a | 10.53 | b | 4.71 | A | 5.10 | a |
| Z67 | 11.31 | a | 10.41 | b | 3.44 | A | 3.85 | b |
| Z94 | 11.13 | a | 10.86 | b | 4.09 | B | 3.87 | b |
| ZA25 | 11.52 | a | 11.63 | a | 4.31 | B | 3.56 | b |
| ZAL95 | 8.98 | b | 8.98 | c | 4.59 | A | 4.80 | a |
| C.V. (%) | 2.00 | | | | 15.23 | | | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test ($p \leq 0.05$. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

During the flowering stage, at 60 days after sowing, similar results from 30 days were observed. With statistically non-significant values for the variables total nitrogen, the number of tillers, count of bacteria present and leaf area. The variable root and shoot fresh weight were statistically different for the comparison of strains, with better performance when inoculated with *Azospirillum brasilense* 245 and *Herbaspirillum seropedicae* ZA25 (Table 5). As well as for root and shoot dry mass, which presented a better performance for the treatments inoculated with the referred strains (Table 6). Probably the significant results for these variables are related to the same causes mentioned above, no negative influence on the growth of bacteria or even growth stimulus by the tested herbicides, and promotion of plant growth due to inoculation with the rhizobacteria strains. This response can be attributed to the bacteria for providing a beneficial environment, increasing the root proliferation, consequently increasing the absorption of water and nutrients, allowing the plant a better performance. In the flowering stage, significant differences between treatments were observed for the accumulation of dry matter, due to the positive effects of inoculation of the plants with *H. seropedicae*, with dry mass higher than the control treatment, which did not receive inoculation and fertilization, being statistically equal to the treatments with doses of 50 and 100 kg N. ha$^{-1}$ (Guimarães et al., 2010).

**Table 5**. Average values shoot and root fresh mass (SFM and RFM), of Small Red rice plants with 60 days, inoculated with diazotrophic bacteria strains, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl.

| | ---------------------SFM (g) --------------------- | | | | ---------------------RFM (g) --------------------- | | | |
|---|---|---|---|---|---|---|---|---|
| | Quinclorac (375 g i.a ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 31.05 | a | 30.55 | a | 41.875 | A | 41.85 | a |
| 26H | 22.78 | b | 24.93 | a | 32.175 | B | 33.03 | b |
| Z67 | 21.83 | b | 24.63 | a | 27.375 | B | 27.83 | b |
| Z94 | 28.18 | a | 24.63 | a | 34.875 | B | 33.08 | b |
| ZA25 | 30.38 | a | 28.80 | a | 40.475 | A | 41.90 | a |
| ZAL95 | 24.78 | b | 24.98 | a | 31.475 | B | 30.13 | b |
| C.V. (%) | 16.70 | | | | 4.48 | | | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test ($p \leq 0.05$. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

**Table 5**. Continuation.

| | SFM (g) | | | | RFM (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Penoxsulam (60 g i.a ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 28.90 | a | 30.55 | a | 42.025 | A | 41.85 | a |
| 26H | 24.78 | b | 24.93 | a | 31.925 | B | 33.03 | b |
| Z67 | 22.20 | b | 24.63 | a | 27.625 | B | 27.83 | b |
| Z94 | 28.83 | a | 24.63 | a | 35.525 | A | 33.08 | b |
| ZA25 | 30.93 | a | 28.80 | a | 42.175 | A | 41.90 | a |
| ZAL95 | 22.50 | b | 24.98 | a | 32.35 | B | 30.13 | b |
| C.V. (%) | 15.42 | | | | 15.43 | | | |
| | Cyhalofop-butyl (225 g i.a ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 29.65 | a | 30.55 | a | 43.38 | A | 41.85 | a |
| 26H | 24.95 | a | 24.93 | a | 32.85 | B | 33.03 | b |
| Z67 | 24.08 | a | 24.63 | a | 27.65 | B | 27.83 | b |
| Z94 | 25.40 | a | 24.63 | a | 34.35 | B | 33.08 | b |
| ZA25 | 29.75 | a | 28.80 | a | 42.40 | A | 41.90 | a |
| ZAL95 | 24.58 | a | 24.98 | a | 32.58 | B | 30.13 | b |
| C.V. (%) | 16.14 | | | | 4.34 | | | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test ($p \leq 0.05$. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

**Table 6**. Average values shoot and root dry mass (SDM and RDM), of Small Red rice plants with 60 days, inoculated with diazotrophic bacteria strains, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl.

| | SDM (g) | | | | RDM (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Quinclorac (375 g i.a ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 31.05 | a | 30.55 | a | 5.83 | A | 5.89 | a |
| 26H | 22.78 | b | 24.93 | b | 4.55 | B | 4.61 | b |
| Z67 | 21.83 | b | 24.63 | b | 3.81 | B | 3.91 | b |
| Z94 | 28.18 | b | 24.63 | b | 4.87 | B | 4.65 | b |
| ZA25 | 30.38 | a | 28.80 | a | 5.73 | A | 5.79 | a |
| ZAL95 | 24.78 | b | 24.98 | b | 4.40 | B | 4.25 | b |
| C.V. (%) | 16.70 | | | | 16.33 | | | |
| | Penoxsulam (60 g i.a ha$^{-1}$) | | | | | | | |
| Bacteria | With | | Without | | With | | Without | |
| 245 | 28.90 | a | 30.55 | a | 5.84 | A | 5.89 | a |
| 26H | 24.78 | b | 24.93 | b | 4.47 | B | 4.61 | b |
| Z67 | 22.20 | b | 24.63 | b | 3.83 | B | 3.91 | b |
| Z94 | 28.83 | b | 24.63 | b | 4.99 | B | 4.65 | b |
| ZA25 | 30.93 | a | 28.80 | a | 5.82 | A | 5.79 | a |
| ZAL95 | 22.50 | b | 24.98 | b | 4.57 | B | 4.25 | b |
| C.V (%) | 15.42 | | | | 9.65 | | | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test ($p \leq 0.05$. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

**Table 6**. Continuation.

| | SDM (g) | | RDM (g) | |
|---|---|---|---|---|
| | Cyhalofop-butyl (225 g i.a ha-1) | | | |
| Bacteria | With | Without | With | Without |
| 245 | 29.65 a | 30.55 a | 6.08 A | 5.89 a |
| 26H | 24.95 b | 24.93 b | 4.64 B | 4.61 b |
| Z67 | 24.08 b | 24.63 b | 3.84 B | 3.91 b |
| Z94 | 25.40 b | 24.63 b | 4.80 B | 4.65 b |
| ZA25 | 29.75 a | 28.80 a | 5.93 A | 5.79 a |
| ZAL95 | 24.58 b | 24.98 b | 4.59 B | 4.25 b |
| C.V. (%) | 16.14 | | 9.59 | |

Means followed by equal letters in the column. do not differ by the Scott-Knott test ($p \leq 0.05$. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H. Z67. Z94. ZA25 and ZAL95.

At the end of the bioassay, no changes in the results in relation to the first and second harvesting was observed, with statistically non-significant values for number of tillers, leaf area and also for the count of bacteria present in the shoot and root. In addition, no significance for root and shoot of fresh and dry mass were observed and can be associated with the grain filling phase, in which there is a decrease in the accumulation of biomass, at the time of leaf senescence (Machado et al., 2006). There was no difference for the variable weight of 100 grains in rice plants when inoculated with bacteria and herbicide application. However, there was a difference in the variable total grain mass (Figure 2), with higher performance of Small red rice plants inoculated with 245 and ZA25 strains. This result may be related to the dependence of grain yield on the various yield components (numbers of spikelets and grains per panicle, panicle length, etc.), which are controlled by genetic factors, by the environment and by plant nutrition (Cargnin et al., 2010). For plants inoculated with the other strains, no differences were found for the grain mass.

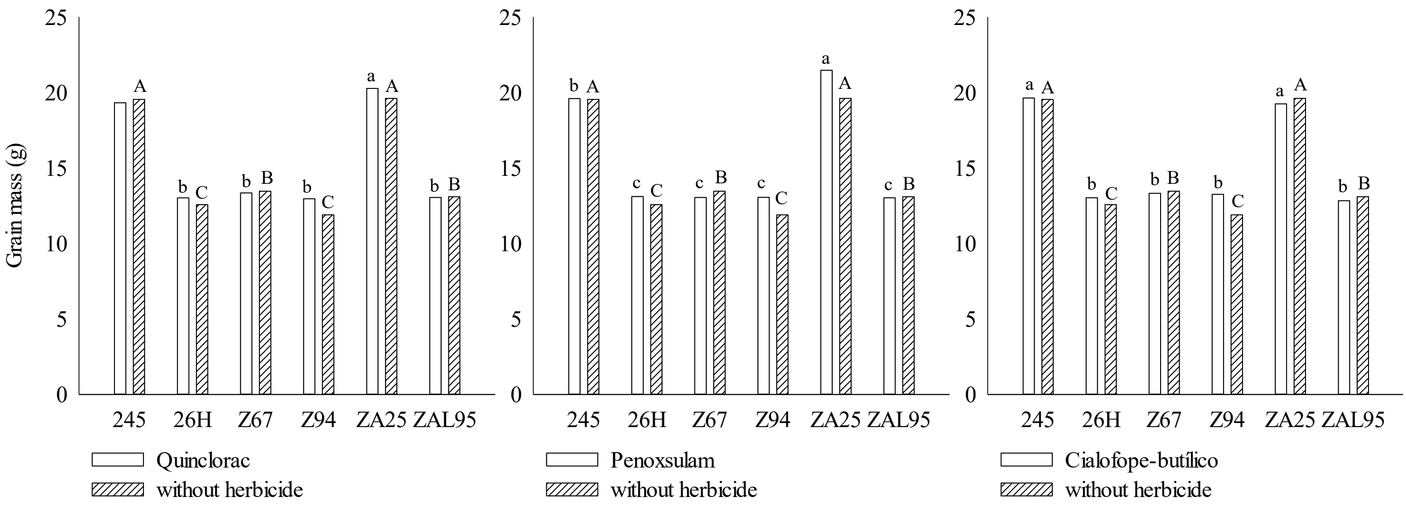


**Figure 2**. Grain mass of Red Small rice seedlings inoculated with strains of diazotrophic bacteria, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl. *Azospirillum brasilense* strain 245 and *Herbaspirillum seropedicae* strains 26H, Z67, Z94, ZA25 and ZAL95. Lower case and upper case letters, respectively, in the presence or absence of each herbicide, do not differ by the Scott-Knott test ($p > 0.05$).

The auxin produced by bacteria can stimulate the activity of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase involved in the biosynthesis of ethylene in root plants. In balanced concentrations auxin hormone promotes the growth of root hair in plants (Cendales et al., 2017), resulting in an increase in the uptake of both, water and nutrients by the root system of plant, and consequently the growth, which can reflect in greater crop productivity. Other authors have also described the importance of phytohormones, such as auxin biosynthesized from tryptophan transamination and decarboxylation. Auxin control cell division, apical dominance, root initiation, phototropism, and geotropism in plants (Gupta, 2015). The inoculation with *Azospirillum* provided increases of 7 to 14%

in the productivity of corn grains, even without the addition of N (Lana et al., 2012). Corn grain yield was also increased when the plants were inoculated, together with doses of 20-24 kg ha$^{-1}$ of N. It is evident the importance of using such bacteria as plant growth promoters to contribute to better crop performance, mainly the growth of the root system. The increase in soil explored can increase the efficiency of nutrient absorption, which may even reduce the use of mineral fertilizers.

When comparing the factorial averages with the control treatment, no statistical significance was observed for the number of germinated seeds, the number of roots, fresh and dry weight of shoot and root, length of shoot and root of rice seedlings. As well as, leaf area, number of tillers, total nitrogen over the harvesting (Table 7). At 30 days, the shoot fresh mass was significant, with a yield of14% above the control, as well as the root fresh mass with 16%, dry mass of shoot with 12% and dry mass of root with 27% of superiority. At 60 days, the same trend of significance was observed, and in the last one, significative values were observed for root mass (fresh and dry), with an increase of 13% and 7%, respectively, showing the potential of these bacteria in promotion of plant growth. Besides, studies on field conditions should be conducted to better understand the genotype interaction of the plant and microorganisms. It is noteworthy that the excessive use of herbicides in Brazilian agriculture is due to the rapid response, reduced need for labor and the great efficiency of this method in the control of weeds. Thus, the results obtained in the present study demonstrated that there is no impact of the tested herbicides on the evaluated microbiological variables. However, more detailed studies, with other active ingredients and with a longer duration, should be developed in order to guarantee the microbiological role upon herbicide application on arable lands.

**Table 7**. Average values of the factorials compared to the control treatment of Small Red rice plants, inoculated with diazotrophic bacteria strains, in the presence or absence of the herbicides Quinclorac, Penoxsulam and Cyhalofop-butyl.

| | SFM | | RFM | | SDM | | RDM | |
|---|---|---|---|---|---|---|---|---|
| | | | | 30 days | | | | |
| F | 10.8 | A | 28.5 | a | 0.8 | a | 4.4 | A |
| C | 9.2 | B | 23.9 | b | 0.7 | b | 3.2 | B |
| | | | | 60 days | | | | |
| F | 35.0 | A | 35.0 | a | 2.0 | a | 4.9 | A |
| C | 27.5 | B | 28.4 | b | 1.7 | b | 3.8 | B |
| | | | | End cycle | | | | |
| F | 63.6 | A | 46.1 | a | 7.0 | a | 7.9 | A |
| C | 58.9 | A | 39.8 | b | 7.3 | a | 7.3 | B |

Means followed by equal letters in the column, do not differ by the F test of the analysis of variance ($p \leq 0.05$). SFM: shoot fresh mass; RFM: root fresh mass; SDM: shoot dry mass; RDM: root dry mass; F: average of the factorial; C: mean of the control.

## 4. Conclusions

The herbicides quinclorac, penoxsulam and cyhalofop-butyl do not impact the bacterial strains of *Azospirillum* and *Herbaspirillum*. The inoculation with bacterial strains contributes to increasing the number of roots, shoot and root biomass, and grain mass of rice. The variety Small Red shows better performance when inoculated with strains 245 and ZA25.

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