Article

Effectiveness of biofunguisides for the control of rust in coffee seedlings

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Abstract

Rust (*Hemileia vastatrix*) is the most destructive and economically important disease in coffee worldwide, especially in *Coffea arabica* L. It occurs throughout the plant cycle and chemical, biological and cultural methods are used to control it. integrated management and genetic resistance; although some are ineffective, expensive and polluting. The objective was to evaluate the incidence and severity of rust in coffee seedlings var. Geisha under the effect of different biofungicides. The research was carried out at the El Nueve farm, Santa María Huatulco, Oaxaca; during 2018 in nursery. Different treatments were evaluated: control, products based on microorganisms: Baci-Sur *subtilis*, Bacit-Sur, Michoderma, Blite Free F-07/Guanobras; the minerals: Copper Oxychloride, Bordeaux Broth, Visous Mineral Broth, Sulfocal and a homeopathic (Nat-Rx), plus some combinations, for a total of 24 treatments. The design was completely randomized with 10 repetitions. The variables to assess incidence were total leaves, healthy leaves and rust damaged. Photographs of damaged leaves were used to assess visual severity using the logarithmic-diagrammatic scale. To assess digitized severity using Adobe Photoshop® CC 2017 software, total leaf area, rust-damaged area, and healthy area were measured. They were analyzed by the Kruskal-Wallis test in SAS software. Significant statistical differences were found between the biofungicides at 150 dda on the incidence of rust (IR), without significant differences in severity. The treatments with the lowest IR were the combination of microorganisms Bacit-Sur+Nat-Rx, Baci-Sur *subtilis*+Blite FreeF-07/Guanobras and Michoderma+Blite Free F-07/Guanobras.

Keywords: *Coffea arabica*, *Hemileia vastatrix*, Biofungicides, incidence, seedlings, severity.

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Introduction

Coffee production (*Coffea arabica* L.) has a high economic, social and environmental value in the countries where it is grown (Flores, 2015). However, the crop is affected by the attack of pests and diseases, aggravating due to climatic conditions (Waller, 1982; SARH, 1993). The affectation goes from the germination of the plant to the productive stage, which generates large losses in production and a decrease in the quality of the grain (CEPAL and CAC/SICA, 2014; Chemura *et al*., 2017).

Rust (*Hemileia vastatrix* Berk. & Br.) is the most important coffee disease (Haddad *et al*., 2014; Oliveira, *et al*., 2014; Barka *et al*., 2017), when using this crop as its main host (Brown and Hovmøller 2002). It generally causes the loss of leaves of up to 50% (Avelino *et al*., 2004; Bonilla, 2018) and 30% of the yield in some varieties of *C*. *arabica* L., which means a great economic impact worldwide (Oliveira, *et al*., 2014; Barka *et al*., 2017). In the 2013-2014 cycle, production in Central America and Mexico was affected by 3.3 million bags (Flores, 2015), so in 2016 Mexico implemented the Phytosanitary Epidemiological Surveillance Program in the states of Hidalgo, Jalisco, State of Mexico, Nayarit, Querétaro, Chiapas, Veracruz, Puebla, Guerrero, San Luis Potosí and Oaxaca to determine the incidence, severity and implement preventive management actions against rust (SENASICA, 2016).

H. *vastatrix* is controlled through the use of agrochemicals, fungicides, biofungicides, resistant varieties, biological control, cultural control and integrated management (Obando *et al*., 2013; Hernandez-Martinez and Velazquez-Premio, 2016). Agrochemicals generate pollution to the environment and increase production costs (Romero, 2010; Gonza *et al*., 2013), therefore, viable alternatives have been sought that guarantee sustainability in agricultural production (Gonza *et al*., 2013).

An example of this is the use of cupric fungicides (copper oxychloride and mineral broths) considered the most efficient for the control of rust, since they do not alter the biota of the agroecosystem (Capucho *et al*., 2013; Melchor *et al*., 2018), these penetrate the leaf tissue and have curative effects (McCook, 2009) attack the fungus during mycelial growth and pustule formation (Duicela and Ponce, 2015) as well as the use of microorganisms such as fungi and bacteria (Gómez-De La Cruz *et al*., 2017), with the ability to survive at the expense of the fungus (Boosalis, 1964), affecting the reproductive structures of the pathogen (Barros *et al*., 1999).

The incidence and severity of rust is obtained through visual evaluations by trained personnel (Zambolim, 2016). However, the use of logarithmic scales with illustrations of leaves in different degrees of damage is common, being a method to quantify visual severity (Nascimento *et al*., 2005; Hernández and Sandoval, 2015). Digitized severity is obtained with a leaf area integrator (Chavarra *et al*., 2015) and the use of software such as ImageJ, Image Tool and Adobe Photoshop, allowing image areas to be determined with greater accuracy and precision (Sussel *et al*., 2009; Rincón *et al*., 2012).

Based on the above, the objective of the present study was to evaluate the incidence and severity of rust (*Hemileia vastatrix* Berk. & Br.) in coffee seedlings (*Coffea arabica* L.) var. Geisha under the effect of different biofungicides.

Materials and methods

The study was carried out from February to August 2018 at the El Nueve farm, Santa María Huatulco, Oaxaca, located at coordinates 15° 55’ 56’’ north latitude and 96° 17’ 08’’ west longitude, with an altitude range of 1 200 to 1 300 m. The type of vegetation is median sub-evergreen forest (INEGI, 2019). To know the fertility level of the substrate in the nursery (mixture of crop residues and forest soil ratio 1:1), a physical-chemical analysis was carried out according to NOM-021-RECNAT-2000 (SEMARNAT, 2002).

Three-month-old var. Geisha coffee seedlings were used, transplanted into 13 x 20 cm polyethylene bags, standardized in terms of height (10 cm) and size. 24 treatments with 10 repetitions were established, each repetition was considered a seedling. The products evaluated were: a single effect control, four based on microorganisms, four based on minerals, one homeopathic and the combinations with the same dose (Bss+Nr, Bs+Nr, M+Nr, Bfg+Nr, Oc+Nr, Cb+Nr, Cmv+Nr, S+Nr, Bss+Bfg, Bs+Bsg, M+Bfg, Bss+Nr+Bfg, Bs+Nr+Bfg and M+Nr+Bfg) (Table 1).

Table 1. Products evaluated for their simple and combined effect on the incidence and severity of *H. vastatrix* Berk. & Br. in seedlings of *C. arabica* L. var. Geisha.

Treatment		Composition	*Dose
T	Control	-	Without application
Bss	Baci-Sur *subtilis*	*Bacillus subtilis* $5x10^{20}$ colonies ml	0.15 ml + 15 ml water
Bs	Bacit-Sur	*Bacillus subtilis* $5x10^{20}$ colonies ml, *Trichoderma* spp. $1.3x10^{12}$ spores ha^{-1}	0.15 ml + 15 ml water
M	Michoderma	*Trichoderma harzianum* $1.2x^{8}$ g/l/spore dose	0.01 g + 15 ml water
Bfg	Blite Free F-07/Guanobras	*Streptomyces* spp. 1x10 CFU/ml 60%, glucosamine 1%, OM 3%, ac. humic 0.65%, ac fulvic 0.15%	0.2 ml + 0.15 ml + 15 ml water
Oc	Copper oxychloride	Metallic copper59%	0.01 g + 4 ml water
Cb	Bordeaux soup	Copper sulfate, calcium hydroxide,	0.04 g $CuSO_4$ + 0.04 g $Ca(OH)_2$ + 4 ml water
Cmv	Broth mineral visosa	Copper sulfate, calcium hydroxide, zinc sulfate, magnesium sulfate, ac. boric	0.02 g $CuSO_4$ + 0.02 g $Ca(OH)_2$ + 0.02 g $ZnSO_4$ + 0.02 g $MgSO_4$ + 0.02 g H_3BO_3 + 4 ml water
S	Sulfocal	Sulfur and quicklime	0.1 ml + 7.5 ml water
Nr	Nat-Rx	Hepar sulphur 15%, *Bovista plumbea* 5%, omeopathic plant extract 10%, water	0.7 ml + 30 ml water

*= dose per seedling per application.

The treatments were applied at two times one month apart (February 15 and March 15). The applications were made in the morning and the dosages were adjusted to 10% of what is applied in the field based on the recommendations issued by the manufacturers and specified on the label of the products.

The incidence of rust (INR) was evaluated using the methodology of Samayoa-Juarez and Sanchez-Garita (2000), adapted to the research work that consisted of counting the total of diseased leaves for each repetition and dividing by the total number of leaves, multiplying the result by 100. The following variables were considered for each repetition: total leaves (HT), healthy leaves (HS) and leaves damaged by rust (HDR). Five evaluations were made every 30 days after applying the biofungicides (dda). At 150 dda, the visual severity (SVR) of the HDR was determined, using the logarithmic-diagrammatic scale proposed by SENASICA (2016) (Figure 1).

Clase	0	1	2	3	4	5	6
Severidad	(0%)	Puntos cloróticos	(2%)	(7%)	(20%)	(45%)	(≥70%)

Figure 1. Logarithmic-diagrammatic scale to assess the severity of leaf rust.

At the same time, HDR photographs were taken to determine the digitized severity (SDR), considering the total leaf area (AFT), rust damaged area (ADR) and the healthy area (AS), analyzing the photographs with Adobe Photoshop® software CC 2017. For this, 1 cm of the image was measured by standardizing the measurement scale (370 pixels= 1 cm), for all images. A completely randomized design was used. The assumptions of normality and homogeneity were applied to the data. As the test was not completed, even after the data had been transformed, they were analyzed using nonparametric statistics using the Kruskal-Wallis test using statistical software SAS/STAT 9.3 (SAS Institute Inc., 2011).

Results and discussion

The results of the analysis indicate that the texture of the substrate is loamy sand (Ac), pH of 6.9, electrical conductivity of 0.23 dS m^{-1} (negligible effects of salinity) organic matter of 5.82% (high levels), phosphorus of 20.86 mg kg^{-1} (average levels), calcium of 0.0011 cmol kg soil (low), magnesium of 0.0016 cmol kg soil and nitrogen of 0.38%.

The percentage of leaves with rust (PHR) showed significant differences (0.041) between biofungicides up to 150 dda, with a value of $p\leq 0.05$. However, from 30 to 120 dda there was no response from biofungicides on the incidence of *H. vastatrix* Berk. & Br. in INR microorganism-based biofungicides were more effective. Combinations stand out Bacit-Sur + Nat-Rx; Baci-Sur *subtilis* + Blite Free F-07/Guanobras and Michoderma + Blite Free F-07/Guanobras, which generated the greatest control over the INR of *H. vastatrix* with average ranges of 93.5 (Figure 2).

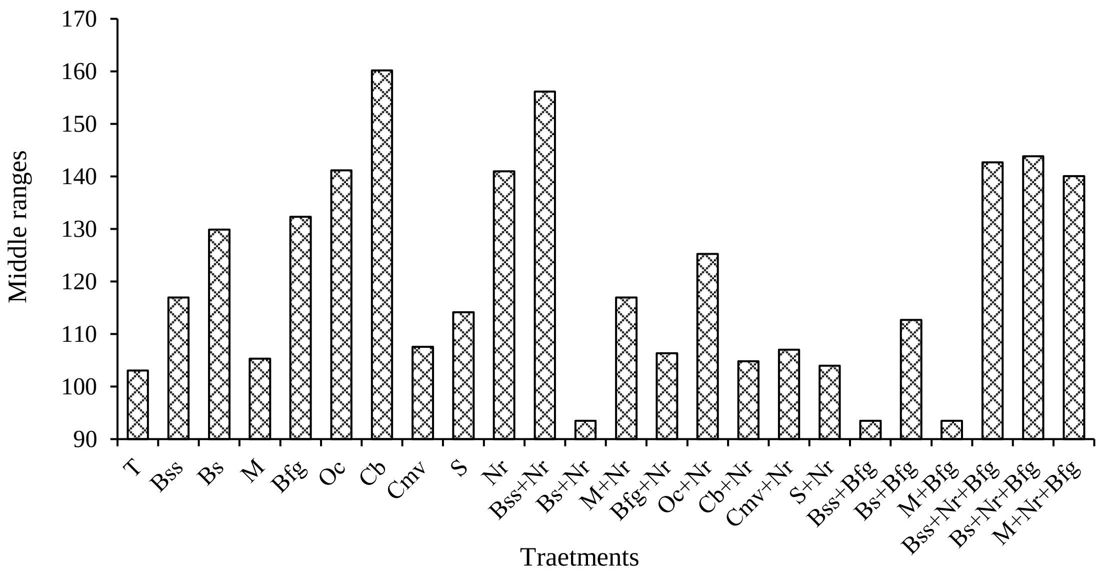

Figure 2. Biofungicide response to the incidence of *H. vastatrix* Berk. & Br. in seedlings of *C. arabica* L. var. Geisha at 150 dda. Low values of medium ranges equal a lower incidence.

According to Cacefo *et al*. (2016) when applying *B. subtilis* in the var. Icatu and Mundo Novo obtained a control of 24 and 17%, respectively. Likewise, Dorighello (2013) when using isolates of *B. subtilis* QST 713 and AP-3 in the control of soybean rust (*Phakopsora pachyrhizi*), observed a reduction in the germination of uredospores. Li *et al*. (2013) obtained a significant reduction in the incidence of *Puccinia striiformis* f. sp. *tritici* (Pst) (soybean rust) with the application of *B. subtilis* E1R-j under greenhouse conditions with two different formulations at 24 and 0 h before Pst inoculation.

Leonel and Barros (2013) obtained a lower incidence of *H. vastatrix* using a homeopathic complex, observing higher vegetative vigor. Likewise, Rissato *et al*. (2016) obtained the lowest incidence of white bean mold (*Sclerotinia sclerotiorum*) with homeopathic medicine applications. For their part, El-Sharkawy *et al*. (2015) used *Streptomyces viridosporus* in combination with *Trichoderma harzianum* for the control of greenhouse rust of wheat (*Puccinia triticina*), with which they obtained a decrease in the number of pustules per unit area (cm^2).

For the SVR and SDR there were no significant differences; however, it was numerically observed that the combinations of Bs + Nr; Bss + Bfg and M + Bfg generated greater control over the severity in both variables (Figures 3a and 3b). Cisneros-Rojas *et al*. (2017) reported greater growth and development of coffee seedlings applying *B. subtilis* with individual effect and in combination with the *Kocuria* sp., Cacefo *et al*. (2016) point out that *B. subtilis* stimulates the plant to activate its defense mechanisms for greater resistance, mitigating the harmful effects of pathogens. According to Pérez *et al*. (2015) the biocontroller effect of *Trichoderma* spp. it has a mode of action that regulates the development of phytopathogenic fungi.

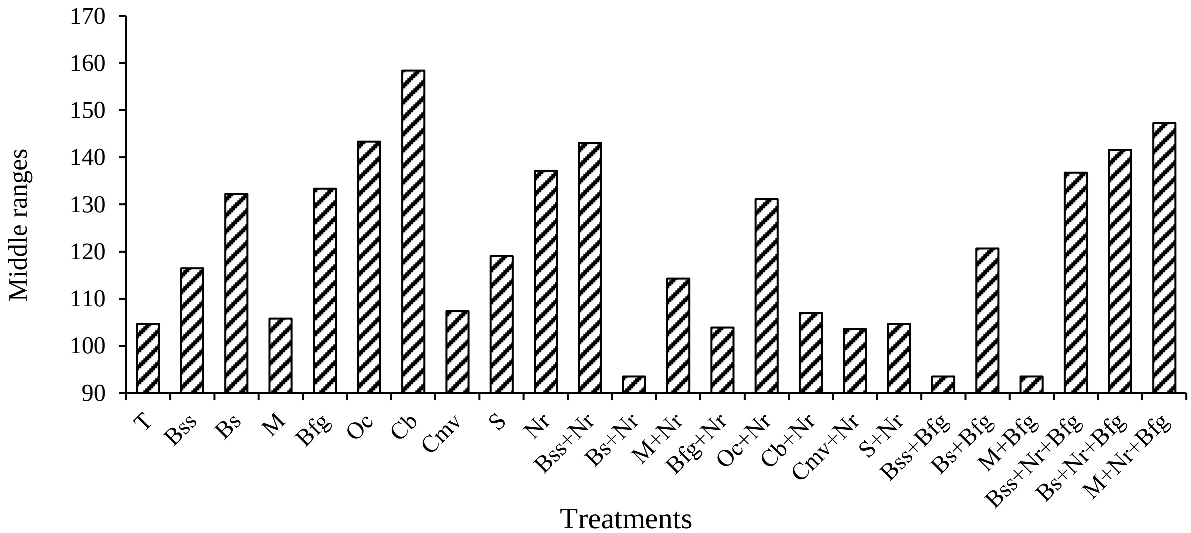

Figure 3a. Response of biofungicides on the visual severity of *H. vastatrix* in seedlings of *C. arabica* L. var. Geisha, at 150 dda. Low values media ranges equivalent to less severe.

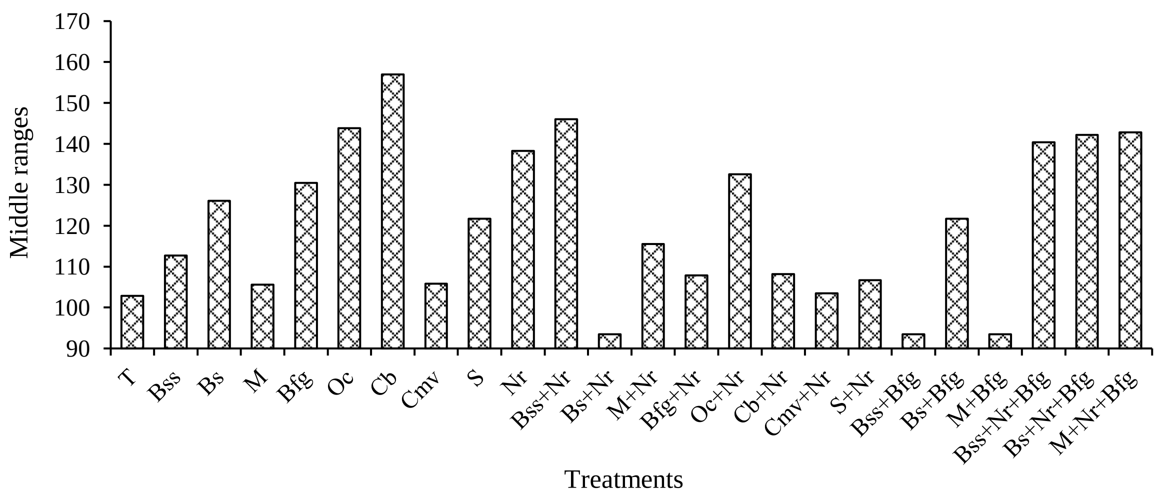

Figure 4. Response of biofungicides on digitized severity of *H. vastatrix* in seedlings of *C. arabica* L. var. Geisha, at 150 dda. Low values media ranges equivalent to less severe.

Infante *et al*. (2009) indicate that, during the infection process, *Trichoderma* spp., deactivates the enzymes of the phytopathogenic fungi. El-Sharkawy *et al*. (2015) found that *Trichoderma harzianum* and *Streptomyces viridosporus* had the best effects for controlling the severity of wheat leaf rust, compared to chemical fungicides. Evangelista-Martínez *et al*. (2015) mention that bacteria of the genus *Streptomyces* are producers of secondary metabolites and enzymes that act in the control of diseases caused by fungi in plants.

Conclusions

The logarithmic-diagrammatic scale facilitated the measurement of the severity in leaves in the field; however, although this activity requires time to evaluate each of the leaves with rust, since a thorough revision of the leaves is required when very small lesions appear and resemble the images proposed on the scale. The bacterium *B. subtilis* is used for the biological control of coffee rust, but its effects also influence the growth and development of seedlings. *B. subtilis* in combination with *Streptomyces* sp. and bat guano, the latter rich in nutrients that help strengthen seedlings.

Which is also reflected when applying the *Trichoderma harzianum* fungus that helps the seedling to inhibit rust and to capture nutrients found in the soil. *B. subtilis* has proven to be an important option in biological control, especially of foliar diseases. Bacteria belonging to the genus *Bacillus* have been used in the biological control of phytopathogenic fungi. The use of *B. subtilis* bacteria is an alternative for the biological control of coffee rust, and it has also been used for the development of successful coffee seedlings.

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