



Biocontrol of coffee yellow rust (*Hemileia vastatrix* Berk. & Br.) with *Trichoderma* sp. endophyte strains

Biocontrol de la roya amarilla del cafeto (*Hemileia vastatrix* Berk. & Br.) con cepas de *Trichoderma* sp. endófito

 Giovana Mamani-Huayhua¹,  Betsabe Leon-Ttacca^{2*},
 Luis Alfredo Palao-Iturregui¹,  Yuri Rodrigo Borja-Loza³

¹Facultad de Ciencias Agrarias, Escuela Profesional de Ingeniería Agronómica, Universidad Nacional del Altiplano, av. Floral 1153, Puno-Perú, 21001, Puno, Perú.

²Escuela Profesional de Agronómica, Universidad Nacional de Cañete, Lima, Perú.

³Escuela de Postgrado, Universidad Nacional del Altiplano, Puno, Perú.

ABSTRACT: Yellow rust, caused by the fungus *Hemileia vastatrix* Berk. & Br., is one of the main diseases that limits commercial production and significantly reduces yields of coffee (*Coffea arabica* L.); fungicides are commonly used to control it, but the use of endophytic microorganisms such as the fungus *Trichoderma* is a promising alternative. Therefore, the biocontrol capacity of five endophytic *Trichoderma* sp. strains (TE1, TE2, TE3, TE4, TE5) on yellow rust and their effect on coffee seedling growth was determined at the nursery level. Conidial suspensions (1×10^7 cfu mL⁻¹) of strains were sprayed on soil and foliage. Plant height (PH); stem diameter (SD); length of main root (LMR); number of leaves (NL); disease incidence and severity; and area under the disease progress curve (AUDPC) were evaluated. The TE-1 treatment was the most efficient in reducing disease incidence (35.8 %) and severity (8.95 %). It also improved plant growth parameters in PH (12.70 cm), SD (2.5 mm), NL (7.6 units), LMR (11.38 cm), as well as AUDPC (56.625 units), compared to the control with values of 96.67, 39.50 %, 10.02 cm, 1.96 mm, 5.06 cm, 4 units and 365.00 units in the evaluated variables, respectively. This strain could be used to improve plant development and protect against coffee yellow rust.

Key words: biological control, coffee, fungi, antagonism, pucciniales.

RESUMEN: La Roya amarilla, causada por el hongo *Hemileia vastatrix* Berk. & Br., es una de las principales enfermedades que limita la producción comercial y reduce significativamente los rendimientos del cafeto (*Coffea arabica* L.); los fungicidas son comúnmente empleados para controlarla, pero la utilización de microorganismos endófitos como el hongo *Trichoderma* es una alternativa prometedora. Por ello, la capacidad de biocontrol de cinco cepas de *Trichoderma* sp. endófito (TE1, TE2, TE3, TE4, TE5) en la roya amarilla y su efecto en el crecimiento de plántulas de café fue determinada a nivel de vivero. Las suspensiones de conidias (1×10^7 ufc mL⁻¹) de las cepas fueron rociadas al suelo y follaje. Se evaluó la altura de planta (AP); el diámetro de tallo (DT); la longitud de raíz principal (LRP); el número de hojas (NH); la incidencia y severidad de la enfermedad y el área bajo la curva del progreso de la enfermedad (AUDPC). El tratamiento TE-1 fue el más eficiente en reducir la incidencia (35,8 %) y la severidad (8,95 %) de la enfermedad, también mejoró los parámetros de crecimiento de las plantas en AP (12,70 cm), DT (2,5 mm), NH (7,6 unidades), LRP (11,38 cm), así como el AUDPC (56,625 unidades), en comparación con el testigo con valores de 96,67 %, 39,50 %, 10,02 cm, 1,96 mm, 5,06 cm, 4 unidades y 365,00 unidades en las variables evaluadas, respectivamente. Esta cepa podría ser utilizada para mejorar el desarrollo de las plantas y proteger contra la roya amarilla del cafeto.

Palabras clave: control biológico, café, hongos, antagonismo, pucciniales.

*Author for correspondence: bleon@undc.edu.pe

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INTRODUCTION

Coffee (*Coffea arabica* L.) is Peru's main agricultural export product, with great economic and social importance that generates sources of income for producers in San Juan del Oro district, Sandia province and Puno Region, with climatic conditions that favor organic coffee production. Year 2014 was the most critical one for national production. At the end of 2012 the "yellow rust" disease affected coffee's leaves of the tree causing severe defoliation and added to the lack of renewal of old coffee plantations, brought as a consequence the decline of national production with a production of 209 182 tons (1). At world level, this disease is the main pathological problem in coffee cultivation, it can cause yield losses of up to 35 % and have a polycyclic epidemiological impact in subsequent years (2). Two approaches have been proposed to avoid losses caused by the disease. The first consists of fungicide use, which is a very costly alternative and potentially harmful to the environment. The second, through the development of improved varieties, which requires knowledge of resistance sources and pathogen diversity (3). However, the solution may have been developing for centuries in a natural way, from the interactions between organisms, such is the case of endophytic fungi that live inside the tissues of living plants without causing disease symptoms, providing ecological benefits to their host and diverse antagonistic mechanisms against pests, becoming an alternative for the biological control of diseases (4,5).

Likewise, these antagonistic endophytic fungi influence plant growth, generating resistance to biotic and abiotic stress, reflecting in plant vigor and with potential protection against the attack of pathogens; being the genus *Trichoderma* one of the endophytes widely studied in biological control (6-8). These fungi produce secondary metabolites and some antifungal and antibacterial compounds that inhibit the growth of other microorganisms, including plant pathogens, since they produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds of the pathogen cell wall such as chitin, protein, cellulose and hemicellulose (5,9). In addition, the use of *Trichoderma* in plant disease control increases crop production for the benefit of sustainable agriculture (10).

This situation led to carry out the present research work, with the aim of determining the biocontrol capacity of *Trichoderma* endophyte strains towards yellow rust (*Hemileia vastatrix* Berk. & Br.) and its effect on the growth of coffee (*Coffea arabica* L.) seedlings.

MATERIALS AND METHODS

Location

The experiment was conducted in the phytopathology laboratory of the Universidad Nacional del Altiplano Puno (UNAP) and in a nursery located in San Juan del Oro district, Sandía province, Region Puno- Peru at an altitude of 1,298 m, 14° 14' 03" S and 69° 9' 29" W.

Coffee (*Coffea arabica* L.) seedling production

Previously, coffee seeds, Caturra variety, were germinated in beds with solarized fine sand. After 80 days, they were transplanted in 2 L polyethylene bags with solarized substrate (agricultural soil and ash) and irrigated at field capacity for four months.

Provenance and multiplication of *Trichoderma* strains

Five endophytic fungi strains of the genus *Trichoderma* sp. were provided by the Phytopathology laboratory of the Universidad Nacional del Altiplano-Puno. These were isolated from leaves (strains TE1 and TE2) and stems (strains TE3, TE4 and TE5) of coffee plants var. Catimor from San Juan del Oro district in Papa dextrose Agar medium and conserved in a 20 % glycerin solution at -5 °C. For multiplication, strains were reactivated in Petri plates with PDA medium, half a plate with the fungus was deposited in polypropylene bags with solid substrate, sterilized with pre-cooked barley, incubated at 25 °C and removed for 15 days until drying, these were harvested and conserved in sealed polypropylene bags at 5 °C (11,12).

Trichoderma application

Previously, the spore count of the barley substrate was carried out by serial dilutions, and then a spore suspension of 1×10^7 cfu mL⁻¹ was obtained in sterile distilled water. Three applications were made to the substrate and one to the foliage in coffee stands. The first application was at time of transplanting the seedlings, and then the second and third applications were made every thirty days, spraying the substrate with 200 mL of the suspension (8). Thirty days after the third application, foliar spraying made growth parameters were evaluated in plants three months after transplanting, then a fourth application.

Inoculation of *Hemileia vastatrix* Berk. & Br

For the inoculum, leaves with presence of yellow rust pustules were collected from coffee cultivation fields of Caturra variety, pieces of leaves (0.5 cm²) with disease signs were obtained, these were submerged in sterile distilled water with tween 80 to detach the uredospores from the tissue and were standardized to a concentration of 2×10^4 uredospores mL⁻¹ (13,14). Inoculation was carried out by spray inoculation directed to the foliage of 94-day-old coffee seedlings after transplanting (dat). After 24 hours, *Trichoderma* sp. endophyte strains were applied at a concentration of 1×10^7 cfu mL⁻¹.

Parameter evaluations

At three months of age after replanting, five plants were evaluated for each treatment; for plant height (cm), measurements were taken from the plant collar to the stem apex. For stem diameter (mm) at a height of 3 cm from the ground; for main root length (cm) from plant collar to the main root cap; and for the number of leaves (unit), the total number of leaves per plant was counted.

After 23 days of pathogen inoculation, five evaluations were made every five days. For incidence, the number of leaves with the presence of the disease and the number of leaves observed per coffee plant posture were evaluated. For severity, two leaves per plant were evaluated with the help of a scale (15), with these evaluations the AUDPC (area under the disease progress curve) was determined.

Statistical analysis

To evaluate the effect of *Trichoderma* sp endophyte strains on the growth of coffee seedlings and the biocontrol capacity towards yellow rust (*Hemileia vastatrix* Berk. & Br.), a Completely Randomized Design was used, with five strains of *Trichoderma* and two controls (sick and healthy) making a total of seven treatments with five repetitions. The data expressed in percentage were transformed to log, with whose transformation the normality and the homogeneity of variances were confirmed, then the analysis of variance (ANOVA) and Duncan contrast tests were carried out, with a confidence level of 95 % and a margin of error of 5 %, with the use of a statistical software InfoStat, version 2008.

RESULTS

Effect of native *Trichoderma* sp endophytic strains on the growth of coffee (*Coffea arabica* L.) seedlings

Nine weeks after the transplanting of the coffee seedlings, the height of the plant and the main root increased significantly, in the presence of all the strains of endophytic *Trichoderma*, in comparison with the control (without *Trichoderma* application). However, in the stem diameter, the treatment TE3 did not show significant difference, in relation to the control without application, as well as in treatments TE4 and TE5 in number of leaves. It is necessary to emphasize that in these treatments strains from coffee stems were used. However, treatments TE1 and TE2 are strains from coffee leaves that significantly increased plant height, main root and number of leaves. Meanwhile, in stem diameter, treatment TE1 had the best effect, followed by TE5, TE2 and TE4 with no significant difference with respect to the control (Table 1). The treatment applied with strain TE1 showed the greatest increase in plant height, stem

diameter, main root and number of leaves with 21.10, 21.60, 55.54 and 47.34 %, respectively, and compared to the control.

Biocontrol capacity of *Trichoderma* sp. on yellow rust (*Hemileia vastatrix* Berk. & Br.)

In five evaluations of yellow rust incidence in coffee seedlings, at the nursery level, there were significant differences among treatments ($p \leq 0.05$). At 117 days after transplanting (dat), disease symptoms were observed, such as chlorotic spots on the upper side of the leaves without the presence of pustules. In this first evaluation the diseased control presented a high incidence, followed by the treatments with *Trichoderma* applications TE3, TE4 and TE5; however, in the treatments TE1 and TE2 presented low incidence. In the second, third and fourth evaluation of incidence, treatments TE3, TE4 and TE5 did not show significant differences with the diseased control. However, in the fifth evaluation, treatments TE4 and TE5 did show significant differences with the diseased control. Therefore, for all the evaluations, TE1 and TE2 were the best bio-controllers because they presented lower incidence percentages, unlike treatments TE3, TE4 and TE5, which were not efficient in reducing the disease (Table 2).

With respect to the severity (%), in the five evaluations all the treatments that received *Trichoderma* sp. applications reduced the disease significantly with respect to the diseased control (C-dis) that was the most affected. The coffee stands treated with treatments TE1 and TE2 were the least affected by the disease with lower severity values of 10.30 % up to the 5th evaluation (Table 3). Similarly, these treatments had the lowest AUDPC values of 56.63 and 63.38, respectively, with respect to the other treatments and the diseased control with 365 units (Figure 1).

DISCUSSION

In the investigation, there was a positive effect on coffee growth (*Coffea arabica* L.) seedlings at nursery level with the application of endophytic *Trichoderma* sp strains from leaves and stems of coffee plants. Similar results were reported by other authors, who point out the increase in plant size and the number of leaves in coffee seedlings at nursery level, with applications of *Trichoderma* sp. endophyte coming from coffee plants of Catimor variety (16).

Table 1. Growth of coffee (*Coffea arabica* L.) seedlings treated with *Trichoderma* sp. endophyte strains, under nursery conditions in San Juan del Oro district.

Treatments (Strains)	Plant height (cm)	Stem diameter (mm)	Main root (cm)	Leaf (unit)
TE1	12.70 a	2.50 a	11.38 a	7.60 a
TE2	12.10 ab	2.27 b	9.50 b	7.20 ab
TE3	10.94 c	2.12 bc	6.58 c	4.80 bc
TE4	11.42 bc	2.16 b	6.48 d	5.20 cd
TE5	11.64 b	2.25 b	8.10 d	6.00 cd
Contrl	10.02 d	1.96 c	5.06 e	4.00 d
CV	5.35 %	6.028 %	8.23 %	10.64 %
R2	0.710	0.651	0.929	0.652

According to Duncan's test, means with different letters indicate significant differences ($p \leq 0.05$) Each treatment had five replicates. Control: without *Trichoderma* application

Tabla 2. Incidence of yellow rust (*Hemileia vastatrix* Berk. & Br.) in coffee seedlings treated with *Trichoderma* sp. endophyte strains under nursery conditions in San Juan del Oro district.

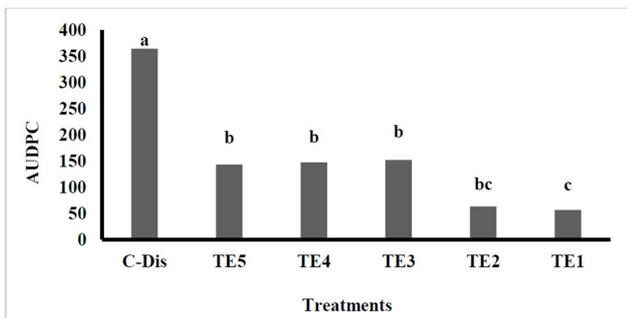
Treatments	Incidence evaluations (%)				
	1 st (117 dat)	2 nd (122 dat)	3 rd (127 dat)	4 th (132 dat)	5 th (137 dat)
TE1	3.33 b	5 bc	17.5 c	24.99 b	35.8 c
TE2	3.33 bc	10 b	22.5 bc	27.50 b	42.5 c
TE3	10 b	28.33 a	40.83 a	61.67 a	73.33 ab
TE4	12.5 b	31.67 a	43.33 a	56.67 a	70.00 b
TE5	11.67 b	26.67 a	35.83 ab	53.33 a	64.17 b
C-dis.	36.67 a	50 a	65.00 a	86.66 a	96.67 a
C-hea	0 c	0 c	0 d	0 c	0 d

According to Duncan's test, means with different letters indicate significant differences ($p \leq 0.05$). Each treatment had five replicates. C- dis: disease control (with pathogen inoculation) C-hea: healthy control (without pathogen inoculation). dat: days after transplanting.

Tabla 3. Percentage of severity (%) of yellow rust (*Hemileia vastatrix* Berk. & Br.) of coffee seedlings treated with *Trichoderma* sp. strains under nursery conditions.

Treatments	Number of severity assessments dat				
	1 st (117 dat)	2 nd (122 dat)	3 rd (127 dat)	4 th (132 dat)	5 th (137 dat)
TE1	0.3 cd	1.2 c	1.2 c	4.1 d	8.95 c
TE2	0.3 cd	1.2 c	1.5 c	4.7 cd	10.3 c
TE3	1.5 bc	2.1 b	4.7 b	11.25 bc	23.3 b
TE4	1.2 bc	2.7 b	5 b	10.25 bc	22 b
TE5	1.2 bc	2.7 b	6 b	12.25 b	14.3 bc
C-dis.	3 a	10 a	15.3 a	26.5 a	39.5 a
C-hea	0 d	0 d	0 c	0 e	0 d

According to Duncan's test, means with different letters indicate significant differences ($p \leq 0.05$). Mean severity data for five plants per treatment. C- dis: disease control (with pathogen inoculation). C-hea: healthy control (without pathogen inoculation). dat: days after transplanting.



Different letters indicate significant differences ($p \leq 0.05$)

Figura 1. Area under the disease progress curve (AUDPC) of yellow rust (*Hemileia vastatrix* Berk. & Br.) of coffee seedlings treated with *Trichoderma* sp. strains under nursery conditions in San Juan del Oro.

On the other hand, with the *T. harzianum* applications in coffee seedlings, a greater root length, plant height, stem diameter and number of leaves were obtained (17,18). Similarly, in other crops such as cocoa, plant growth was promoted at the pot level, the number of leaves, plant height, shoots and root dry matter increased significantly (19), yield improved in the cultivation of quinine and grapevine (20,21) and the number of leaves increased in rice (12). Possibly this is due to the capacity of these *Trichoderma* strains to acidify the rhizosphere, releasing organic acids and chelating metabolites that sequester cations and redox activity; these mechanisms make minerals soluble (22). On the other hand, it is known that *Trichoderma* species are

potential root colonizers, produce auxins, cytokinins and ethylene that are involved in the growth and protection of plants against pathogen attack (23,24), and induce resistance to diseases in a variety of plant species (6).

Likewise, the native *Trichoderma* sp. endophyte strains used in this research had the capacity to control the pathogen *H. vastatrix*, reducing the incidence, severity and AUDPC of the disease in coffee seedlings, since they were treated preventively with the strains at the root system level he and after the inoculation of the pathogen to the foliage.

On the other hand, strains from leaves were the ones that presented the best effect in reducing the disease, because these endophytes can produce an antifungal compound or a substance that can induce the defense mechanisms of the plant against the pathogen (6). In the same way, provoke systemic resistance induced by pathways dependent on jasmonic acid/ethylene and trigger defense responses in the plant because of the establishment of some *Trichoderma* strains in the rhizosphere (25).

Other mechanisms used by *Trichoderma* are the physical competition for space and nutrients; production of secondary metabolites with antibiotic or antifungal activity; mycoparasitism secreting hydrolytic enzymes like chitinases and glucanases that degrade the cellular wall of the phytopathogenic fungi (26-28). This agrees with the reported by other authors, who confirm antagonism of *Trichoderma* strains on *Hemileia vastatrix* Berk. & Br. because of the *Trichoderma* colonization on the pathogen and by competition for space and nutrients on coffee leaves with symptoms of the disease (29);

likewise, these antagonists induce resistance in the plant to infections by the pathogen (6). Likewise, they affirm mycoparasitism of *Trichoderma* strains on *Phytophthora megakarya*, where these antagonists significantly reduced the pathogen effects on plant leaves (9). At the same time, in preliminary studies, they indicate that strains of *Trichoderma endophytes* that were isolated from *Coffea* flowers in Africa inhibit the germination of uredospores of *H. vastatrix* and reduce the severity of the disease. It is considered as a potential biological controller for the development of biofungicides, because *Trichoderma* sp. possesses different characteristics such as protecting plants against root pathogens (30). For all the mechanisms of action that the *Trichoderma* strains possess and also for being coffee endophyte, the results obtained in the investigation are explained, about that all the *Trichoderma* endophyte strains had effect in the control of yellow rust in coffee plants of four months of age.

CONCLUSION

It was demonstrated that with applications of *Trichoderma* endophyte strains to the soil and foliage in coffee seedlings, the incidence and severity of yellow rust (*H. vastatrix*), and the growth parameters of the seedlings at nursery level are improved. The strain TE1, coming from coffee leaves is the one with the best response in the growth parameters (plant height, stem diameter, main root length and number of leaves); also with lower percentage of incidence, severity and AUDPC, in comparison with the control without *Trichoderma* application.

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