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Original article

Management of phytopathogenic fungi on Oryza sativa with the application of Trichoderma asperellum

Manejo de hongos fitopatógenos en *Oryza sativa* con la aplicación de *Trichoderma asperellum*

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ABSTRACT: Fungal diseases in rice crop (*Oryza sativa* Lin.) are considered among the main causes of low cereal yields. In this sense, numerous studies indicate that the applications of *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg strains to the soil in fields destined to the cultivation of rice, are promising for the control of numerous phytopathogenic fungi. The objective of the work was to evaluate the effect of the Ta 78 strain of *T. asperellum* against the most important diseases of rice cultivation under field conditions. For this, the strain of *T. asperellum* was sprayed in a terrace system during soil preparation, and a terrace was used as a production control. The severity and incidence of the main diseases caused by fungi were evaluated at various times and the number of plants, tillers and panicles per m^2 was evaluated. *R. solani*, *Helminthosporium* sp. and *Cercospora* sp. were the fungi that most affected rice crop. The incidence and severity of the diseases evaluated in the treatment with *T. asperellum*, decreased significantly with respect to the control treatment, regardless of the time of the evaluation. On the other hand, the number of tillers and spikes was significantly higher in the plot treated with biological control.

Key words: biological control, severity, incidence, yield.

RESUMEN: Las enfermedades fúngicas en el cultivo del arroz (*Oryza sativa* Lin.) están consideradas entre las principales causas de los bajos rendimientos del cereal. Numerosos estudios indican que las aplicaciones al suelo de cepas de *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg en campos destinados al cultivo del arroz, son promisorias para el control de numerosos hongos fitopatógenos. El objetivo del trabajo fue evaluar el efecto de la cepa *Ta. 78* de *T. asperellum* frente a las enfermedades más importantes del cultivo del arroz, en condiciones de campo. Para ello, se asperjó al suelo la cepa *Ta. 78* de *T. asperellum*, en un sistema de terrazas durante la preparación de suelo, y se utilizó una terraza sin asperjar como testigo de producción. Se evaluó la severidad y la incidencia de las principales enfermedades causadas por los hongos, así como el número de plantas, hijos y panículas por m^2. Los hongos que más afectaciones causaron al arroz fueron, *R. solani*, *Helminthosporium* sp. y *Cercospora* sp. La incidencia y la severidad de las enfermedades evaluadas en los tratamientos con *T. asperellum* disminuyó, significativamente, con respecto al tratamiento control, independientemente del momento de la evaluación. Por otro lado, el número de hijos y de espigas fue significativamente mayor en la parcela tratada con el control biológico.

Palabras clave: control biológico, incidencia, rendimiento, severidad.

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INTRODUCTION

Low yields in rice production in rice producing countries are closely related to the high incidence of fungal diseases, which can reduce yields by 20-40 % (1,2).

In Cuba, to control these diseases, producers use numerous fungicides at increasingly higher doses, without achieving the desired control, a situation that increases production costs and reduces profits. On the other hand, it is reported that the application of products based on Trichoderma spp. strains is promising for the control of numerous phytopathogenic fungi (3,4). These positive results are due, fundamentally, to the presence in the Trichoderma strains, of several mechanisms of action with direct and indirect effects on the target phytopathogenic microorganisms, such as: inactivation of infection systems, competition, antibiosis, mycoparasitism, besides stimulating the vegetal development and inducing defensive mechanisms in the plant (5-7). It is known that species of the genus Trichoderma are avirulent plant symbionts and there are records of their presence in diverse habitats. They are microorganisms that actively interact with plant roots, soil and foliar environments. They can colonize plant roots both externally and internally (7) and are therefore effective not only against a variety of plant pathogenic microorganisms, but also in improving overall plant performance.

The National Center for Animal and Plant Health (CENSA, according its acronyms in Spanish) has a strain with isolates of Trichoderma asperellum Samuels, Lieckfeldt & Nirenberg promising for the control of rice phytopathogenic fungi (8).

Based on this background, the objective of the work was to evaluate the incidence and severity of diseases in rice crop upon soil treatment with strain Ta. 78 strain of T. asperellum.

MATERIALS AND METHODS

The experiment was developed at the Scientific and Technological Base Unit, Los Palacios, Pinar del Río, Cuba; belonging to the National Institute of Agricultural Sciences (INCA), during the pre-spring (April), 2016, in a system of flat terraces, in rice soil classified as Hydromorphic Gley Nodular Petroferric Nodular (9).

Two terraces with an area of 0.6 ha were used.

The strain of *T. asperellum* (Ta. 78) used in the experiment was isolated, characterized and conserved at CENSA (10).

The strain was sprayed before the swamp (fangueo), at the dose recommended for the control of other phytopathogenic fungi in the rice crop (1kg ha^{-1}), with a titration of 10^9 conidia g^{-1} (5). For this purpose, 420 g of the product were suspended in 1600 L of water, 420 g of the product were strained with a cloth and applied in a terrace with a MATABI backpack, with a capacity of 20 L and another terrace was taken, without spraying, as a production control.

Direct sowing technology was used in clear water, at a sowing density of 120 kg ha^{-1} and the technical norms for rice cultivation were followed, with the exception that no fungicide was applied (10).

Diseases that appeared endemically were evaluated. Plants with typical symptoms of fungal diseases were analyzed in the Plant Mycology laboratory of UCTB (Scientific and Technological Base Unit, according its acronyms in English) Los Palacios, by observations under stereomicroscope (Novel NSZ-606) and optical microscope and compared with the symptoms and microscopic structures described in the literature (10).

Subsequently, the evaluations were carried out in each variant in 5 points (1 m^2/point) in the form of an English flag and the severity and percentage of incidence of the main diseases caused by fungi in the rice crop were determined (10).

Evaluation of severity

Disease symptoms were evaluated in plants 65 days after seed germination (dag) up to 115 dag with a frequency of 15 days. The evaluation scales used for each disease were those cited in the Standard Evaluation System of the International Rice Research Institute IRRI (11) and severity was determined using the formula (12):

$$S = [\sum(a.b)/NK]100$$

where:

S- Severity

$\sum(a.b)$ - Sum of the products of the number of organs or plants with symptoms (a) by their corresponding grade on the scale (b).

N- Total number of plants observed

K- Highest grade of the scale

The analysis of the severity of the diseases in the terrace treated with Trichoderma asperellum and the production control was carried out by means of the Test of Comparison of Means with Independent Samples, using the "Student's t" statistic.

Evaluation of disease incidence

At 80 days after germination (dag) of rice, 100 plants were counted diagonally across /point, the number of plants with disease symptoms was evaluated, and the percentage of disease incidence (13) was determined:

$$P = (a/100)N$$

where:

P- Percentage of incidence

a- Number of diseased plants

N- Total plants evaluated

The analysis of the incidence of diseases in the terrace treated with Trichoderma asperellum and the production control was carried out by means of the Test of Comparison of Means with Independent Samples using the "Student's t" statistic.

Table 1. Severity (%) of fungal diseases present on rice crop at different times after germination and in areas treated and untreated with *T. asperelum*

Disease	Moment (dag)	Production test	*Trichoderma asperellum*	*T student*
R. solani	65	24	14	0.001675 *
	80	28	5	0.001665 *
	95	34	10	0.001874 *
	110	50	28	0.000247 *
P. grisea	65	0	0	Ns
	80	0	0	Ns
	95	11	4	0.000623 *
	110	16	9	0.023542 *
Helmintosporium	65	18	12	0.031267 *
	80	30	21	1.67E-05 *
	95	35	27	0.004358 *
	110	48	43	0.000388 *
Cercospora	65	16	13	0.010655 *
	80	18	31	4.21E-06 *
	95	24	18	0.004290 *
	110	24	19	0.007951 *
S. oryzae	65	0	0	Ns
	80	0	2	Ns
	95	11	11	Ns
	110	10	9	Ns

Yield variables

At 110 dag, the number of plants, offspring and panicles in one m^2 was evaluated at five points in the area, taking into account the 1 m edge effect of the terrace. With the data of each of the yield variables evaluated in each variant, the statistical analysis was carried out by means of the Test of Comparison of Means with Independent Samples using the "Student's t" statistic.

RESULTS AND DISCUSSION

In Table 1 it is observed that as the days of Trichoderma application passed, the severity of the evaluated fungi increased. This could be due to the fact that the concentration of conidia of the biological control in the soil has diminished, due to the fact that it is an aerobic microorganism and that the conditions of waterlogging of the crop do not favor its permanence and development. Other authors propose a second application of biocontrol in the maturation phase of crops such as beans and soybeans for the control of fungi that affect late stages of crops (14,15).

The treatment with *T. asperellum*, in most cases, significantly reduced the severity of the phytopathogenic fungi evaluated with respect to the control treatment, independently of the time evaluated. No differences were observed between the treatments at 95 and 110 dag, when evaluating the fungus *P. grisea* and in all the evaluations the fungus *S. oryzae*, so the desired control of these fungi was not evidenced. This could be because these pathogens, mainly, manifest their symptoms in the aerial parts (panicle) of plants during the reproductive phase of the crop, so the action of the biocontroller is limited, since it is a soil fungus and was applied prior to planting. Similar results were obtained by other authors, with this same strain, when evaluating the severity of phytopathogenic fungi in other scenarios and different planting technologies (5).

The fungi that showed the greatest severity were *R. solani* and *Helminthosporium* sp. These results are in agreement with evaluations carried out in the last five years in rice-growing areas of the Los Palacios Grain Agroindustrial Company.

In studies carried out it was found a peptide antibiotic known as alamethicin, secreted by *Trichoderma*, to efficiently permeabilize the cells of the plants, this antibiotic allows a potential mechanism for the tolerance of the plants to *Trichoderma*, necessary for the mutualistic symbiosis, benefiting the growth of the plants and the resistance to the pathogens (16). Once the symbiosis is established, the interaction of Trichoderma with the phytopathogenic fungi occurs, which involves a sequence of complex events that include: recognition, growth towards the pathogen, excretion of exochitinases, release of oligomers from the host cell wall, production of fungitoxic endochitinases that diffuse and begin to affect the pathogen before contact (5,17,18).

Before the mycoparasitism, the antagonist adheres to lectins of the pathogen through carbohydrates of the cell wall, coils and forms structures similar to appressoria and secretes degrading enzymes of the cell wall (chitinases, glucanases, proteases, among others) and antibiotic peptabiols (19). They facilitate the entrance of the hyphae of Trichoderma to that of the phytopathogenic fungus and the cell wall content assimilation (20). It has been shown that the production of these hydrolytic enzymes does not depend on contact, but on a diffusible molecule produced

by the host (21). It is likely that rice phytopathogens that were not affected by Ta. 78 produce these metabolites in low concentrations or not at all, which would explain the result obtained.

Figure 1 shows that, except for the fungus *S. oryzae*, which did not affect the crop at 80 dag, a significantly lower distribution of phytopathogenic fungi was observed in the area treated with *T. asperellum* compared to the untreated area. The fungus *Cercospora* sp. had the most intense distribution, followed by Helmintosporium sp., *R. solani* and *P. grisea*. *Cercospora* and *Helmintosporium* sp. showed an intense and medium distribution, respectively. The fungi *R. solani* and *P. grisea* were distributed differently, and showed differential behavior among the treatments evaluated. In this sense, *R. solani* and *P. grisea* had a medium and light distribution in the untreated area and light and none in the area treated with the biological control, respectively.

The evaluations of the physiological variables (Figure. 2) showed that the treated plants had a significantly higher number of sons and spikes than the controls. Authors state that *Trichoderma*, besides being a biocontrol agent, has the capacity to stimulate the growth and development of the plants, so they can support more the attack of the pathogen, besides it induces defense mechanisms in the plants that increase their resistance to a wide range of phytopathogenic agents (5,22).

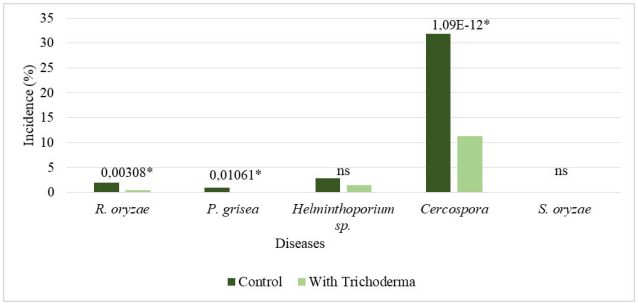

Figure 1. Incidence (%) of the most important rice crop diseases at 80 days after germination (dag) in areas treated with *T. asperelum* and untreated

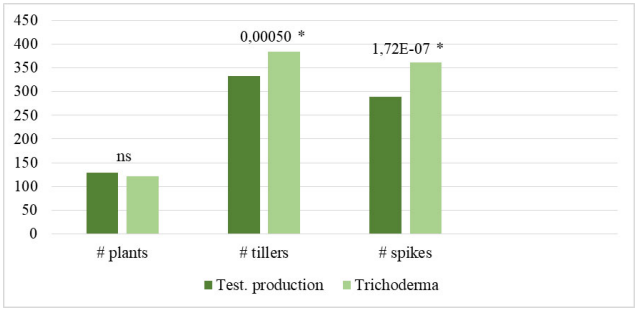

Figure 2. Yield components per m^2 in *T. asperelum*-treated and untreated plots

CONCLUSIONS

In general, *T. asperellum* strain Ta. 78 strain of T. asperellum significantly decreased the incidence and severity of most of the fungi evaluated, when compared to the control treatment, with the exception of *S. oryzae*. However, their regulatory activity decreased over time, an aspect that should be taken into account for their proper management. Similarly, the number of progeny and spikelets was significantly higher with the application of the biological control.

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