Scientific article

Fungal inoculation effect on post-harvest sugarcane residue decomposition under field conditions

Descomposición fúngica de residuos agrícolas de la cosecha en verde de caña de azúcar en condiciones de campo

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Abstract

Fungal inoculation effect on post-harvest sugarcane residue (SCR) decomposition in field conditions was studied. In vitro interaction between selected fungi was determined by direct confrontation. Mycelial growth response to different concentrations of herbicides and urea was also assessed. Fungal combinations did not show inhibitory effects. *Bjerkandera* sp. Y-HHM2 and *Myrothecium* sp. S-3.20 growth decreased in 2,4-D agar plates. However, *Pleurotus* sp. Y- RN3 showed a stimulation in 10 ppm 2,4-D agar plates. The biggest growth in ametrine supplemented cultures was observed in *Pleurotus* sp. Y-RN3. Evaluation of increasing urea concentrations on mycelial growth showed that 5,000 ppm significantly inhibits mycelial growth. The field assay under rainfed condition showed that post-harvest SCR decomposition rate significantly increases in Y-HHM2, Y-RN3 and Y-HHM2/Y-RN3/S-3.20 treatments. This is the first report on the capability of *Bjerkandera* sp. Y-HHM2 and *Pleurotus* sp. Y-RN3 to accelerate post-harvest SCR decomposition in rainfed conditions at field scale, suggesting that these fungi might be useful tools in the sugarcane crop system.

Keywords: Fungal strains; Soil organic matter; Decomposition rate; Rainfed condition.

Resumen

Se estudió el efecto de la inoculación de hongos en la descomposición del residuo de caña de azúcar (SCR) en condiciones de campo. La interacción *in vitro* entre hongos seleccionados y la descomposición del residuo de cosecha en verde de caña de azúcar se determinó mediante confrontación directa. También se evaluó la respuesta del crecimiento micelial a diferentes concentraciones de herbicidas y urea. Las combinaciones de hongos no mostraron efectos inhibitorios. El crecimiento de *Bjerkandera* sp. Y-HHM2 y *Myrothecium* sp. S-3.20 disminuyó en las placas de agar con 2,4-D. Sin embargo, *Pleurotus* sp. Y- RN3 mostró una estimulación del crecimiento en placas de agar con 10 ppm de 2,4-D. El mayor crecimiento en los cultivos suplementados con ametrina se observó en *Pleurotus* sp. Y-RN3. La evaluación de las concentraciones crecientes de urea mostró que 5.000 ppm inhiben significativamente el crecimiento micelial. El ensayo de campo en condiciones de secano mostró que la tasa de descomposición del residuo de cosecha aumenta significativamente en los tratamientos Y-HHM2, Y-RN3 e Y-HHM2 / Y-RN3 / S-3.20. Este es el primer reporte sobre la capacidad de *Bjerkandera* sp. Y-HHM2 y *Pleurotus* sp. Y-RN3 para acelerar la descomposición del residuo de cosecha en verde en condiciones de secano a escala de campo, lo que sugiere que estos hongos podrían ser herramientas útiles en el sistema de cultivo de caña de azúcar.

Palabras clave: Cepas fúngicas; Materia orgánica; Tasa de descomposición; Secano.

Introduction

Soil microbial communities play a key role in maintaining soil fertility and productivity (Paul, 2007). The improvement of soil properties by microbial inoculation of post-harvest sugarcane residue (SCR) has been reported by Johnson *et al*. (2007), who conducted greenhouse degradation experiments of post-harvest SCR using bacteria (*Cellulomonas* and *Corynebacterium*) and fungi

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(*Phanerochaete* and *Cerioporiopsis*) acting alone and in consortium. Also, Beary *et al*. (2002) used a commercial bacterial and fungi consortium to accelerate the post-harvest SCR decomposition. In addition, Boopathy *et al*. (2001) evaluated molasses supplementation to accelerate post-harvest SCR decomposition by indigenous microbial population and Sanomiya *et al*. (2006) studied vinasse and nitrogen fertilizers supplementation on SCR mineralization.

Although sugarcane residue burning is a frequent practice in the tropics and subtropics zones, there has been efforts to establish the theoretical background for the use of post-harvest SCR inoculation with native fungi that may contribute to decomposition and incorporation of organic matter in soil, improve its physical and chemical fertility and develop an important role in reducing greenhouse gas emissions.

The main objective of this work was to evaluate the effect of fungal inoculation in post-harvest SCR decomposition in greenhouse and field conditions. *In vitro* fungal interactions by direct confrontation, *in vitro* mycelial growth in presence of herbicides and urea (commonly used in the management of sugarcane culture), and fungal SCR decomposition under field conditions, were assessed.

Materials and methods

Fungal isolates, culture conditions and inocula production

Two basidiomycetes, *Bjerkandera* sp. Y-HHM2 (KF578081) and *Pleurotus* sp. Y-RN3 (KF578085), and an ascomycetes *Myrothecium* sp. S-3.20 (KF578084), previously isolated and verified as lignocellulolytic fungi (Maza *et al*., 2014) were used in this study. Stock cultures were kept on modified malt extract agar (MEA) slants containing in g/L: malt extract, 20.0; yeast extract, 1.0; agar, 17.0; pH 6.0 at 4 °C.

For *in vitro* assays, the inoculum consisted of one agar plug (5 mm diameter) removed from the growing edge of an 8 day old colony.

For field assay, 30 days solid fungal inoculants of *Bjerkandera* sp. Y-HHM2 and *Pleurotus* sp. Y-RN3 (1 x 10^5 colony-forming units/g (CFU/g) of residue) and *Myrothecium* sp. S-3.20 (1 x 10^8 CFU/g of residue) were applied. Five grams of post-harvest SCR were dried and cut to 20-30 mm pieces, amended with 0.3 % molasses solution to obtain moisture content of about 60 % (w/w) and sterilized at 121 °C for 20 min. Then, SCR was inoculated with three agar plugs and incubated at 30 ± 1.5 °C for 30 days.

Effect of herbicides and urea on in vitro fungal growth

Herbicides supplementation effect, 2,4-D (2,4-dichlorophenoxyacetic acid) 58.4 % and ametrine (N_2-ethyl-N_4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine) 50 %, on fungal development were assessed on MEA. MEA plates without herbicides were prepared and used as control (Westerhuis *et al*., 2007). The medium with products was prepared by adding the herbicide to sterilized (121 °C, 20 min) medium and mixed thoroughly before pouring into the Petri-dishes. Serial dilutions were made to obtain desired concentrations of 0.1, 1.0, 10 and 100 ppm; also, the commercial dose was evaluated in each case (2,4-D 58.4 %: 1.5 l/ha; ametrine 50 %: 2.0 l/ha and urea: 180 kg/ha). Additionally, this assay was conducted to explore the sensitivity of fungal isolates to different concentrations of urea [CO $(NH_2)_2$; 46 % de N_2]: 100; 1,000; 2,500; 3,500 and 5,000 ppm. Agar plugs were aseptically transferred to the centre of plates and incubated for 10 days at 30 ± 1.5 °C in continuous darkness. A complete randomized design with four replicates was used. Fungal growth was established by measuring two perpendicular diameters on a daily basis and results were expressed as a relative percentage of mycelial growth of each of the isolates in comparison with the control.

Dual culture inhibition assays

To evaluate the *in vitro* interactions in mixed culture, all possible combinations on MEA dual culturing method were evaluated (Kausar *et al*., 2010). MEA plates were inoculated with 5 mm plugs of two different fungal strains, cut from the growing edge of the colonies and placed 50 mm in front of each other on the same plate. A single culture served as the control. All the plates were incubated in darkness at 30 ± 1.5 °C. A complete randomized design was used (n = 6). Fungal growth was established by measuring two perpendicular diameters on a daily basis. The mean value of the two diameters was used in modelling. Fungal radial growth inhibition was calculated (Anees *et al*., 2010).

Field assay

A field at Estación Experimental Agroindustrial Obispo Colombres (EEAOC), located in Overa Pozo (26° 50' 0.04''S; 64° 52' 13.88''W), Cruz Alta, Tucumán province, Argentina was utilized. The field was planted with LCP 85-384 age range ratoon 2. The plots were harvested without burning using a combine harvester, maintaining post-harvest SCR coverage on the ground and kept under conventional agronomic management practices: post-emergence herbicides were used for weed control [2,4-D 58.4 % (1.5 l/ha) and ametrine 50 % (2.0 l/ha)], as well as urea for fertilization (90 kg/ha N_2). During the experiment, total rainfall was 660.9 mm and mean temperature was 24.6 °C (EEAOC, 2018). Each experimental plot consisted of five rows of 4 m in length, spaced at 1.4 m.

The soil is classified as an Entic Hapludox which is silty loam (Soil Survey Staff, 1999) at the depth of 0-30 cm. Physical and chemical soil characteristics at the beginning of the trial were the following: sand, 26.8 %; silt, 52.45 %; clay, 20.75 %; bulk density, 1.01 g/cm^3; pH (1:2.5), 6.85 and organic carbon, 16 g/kg (Sosa *et al*., 2014).

The assay was conducted during four months (November 2014 - March 2015) with a randomised complete block design with three replicates and five treatments: (1) control without fungal inoculation; (2) *Bjerkandera* sp. Y-HHM2; (3) *Pleurotus* sp. Y-RN3; (4) *Myrothecium* sp. S-3.20 and (5) co-culture of fungal isolates in irrigated (subsurface drip irrigation was installed 5 to 10 cm deep with a 1.0 l/h flow rate) and rainfed conditions. The fungal strains were applied as solid inoculant; the dose of solid inoculum used was 500 kg/ha and 125 kg/ha sterile SCR as vehicle to facilitate uniform dispersion of the inoculum (Yadav *et al*., 2009).

Soil analysis

Soil samples (0-15 cm deep) were collected, air dried, grinded and sieved (2 mm). Soil organic carbon (SOC) was analysed by the wet-digestion method of Walkley and Black (Nelson and Sommers, 1982) and total N content by Kjeldahl (Bremmer and Mulvaney, 1982).

Fungal counts were monitored in malt extract-Rose Bengal agar (MEA-RB) with streptomycin (250 mg/l) (Boopathy *et al.*, 2001). Basal soil respiration was determined by placing 20 g of soil into 250 ml glass beakers and incubating in the dark at 25 °C along with 30 ml of 0.1 N NaOH. The CO_2-C evolved was measured after 10 days by titration (Anderson, 1982). Fluorescein diacetate (FDA) hydrolysis was measured according to the method of Schnürer and Rosswall (1982) and the fluorescein released was quantified by spectrophotometry.

Post-harvest sugarcane residue analysis

At each plot, post-harvest SCR was sampled using 1.00 m^2 quadrants. The mass of SCR was measured by weighing the residue in each quadrant (Digonzelli *et al*., 2011). Post-harvest SCR samples were oven dried at 65 °C to constant weight, grinded and analysed for total C by dry combustion (Kalra, 1998) and total N content was determined by Kjeldhal distillation method (Bremmer and Mulvaney, 1982). Post-harvest SCR decomposition rate (DR) was calculated according to Shindoi *et al*., (2012) by the equation (1):

(1) $DR = (M_0 - M_t)/t$

where, M_t is the weight of post-harvest SCR at each sample period (g); M_0 is the initial mass (g) and t is the time (days).

Cellulose (CEL) and lignin (LIG) contents in post-harvest SCR were determined with QPT 25_42 (ANKOM, 2000).

Statistical analysis

Colony diameters were adjusted to the modified Gompertz model by Zwietering *et al*. (1990) using InfoStat (Di Rienzo *et al*., 2016). Growth parameters were estimated from the model (2):

(2) $D(t) = D_0 + D_{max} \times \exp(-\exp((\mu \times \exp(1)/D_{max})(\lambda - t) + 1))$

where $D(t)$ is the diameter of the fungal colony at time t (mm), D_0 is the agar plug diameter (mm) of inoculation, D_{max} is the maximum colony diameter (mm), μ is the specific growth rate (mm/d) and λ is the lag phase (d).

Data was subjected to analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) to compare the means (Di Rienzo *et al*., 2016).

Results

Effects of herbicides and urea on in vitro fungal growth

A slight decrease in *Bjerkandera* sp. Y-HHM2 growth was observed in the presence of 2,4-D (between 90 and 95 % relative to the control) and

Myrothecium sp. S-3.20 (above 95 %). However, *Pleurotus* sp. Y- RN3 presented stimulation with a maximum of 105.46 % for 10 ppm (Figure 1a). *Bjerkandera* sp. HHM2 relative growth was lower at a higher dose of the herbicide, on the other hand, in the other fungi evaluated that behaviour had not been seen.

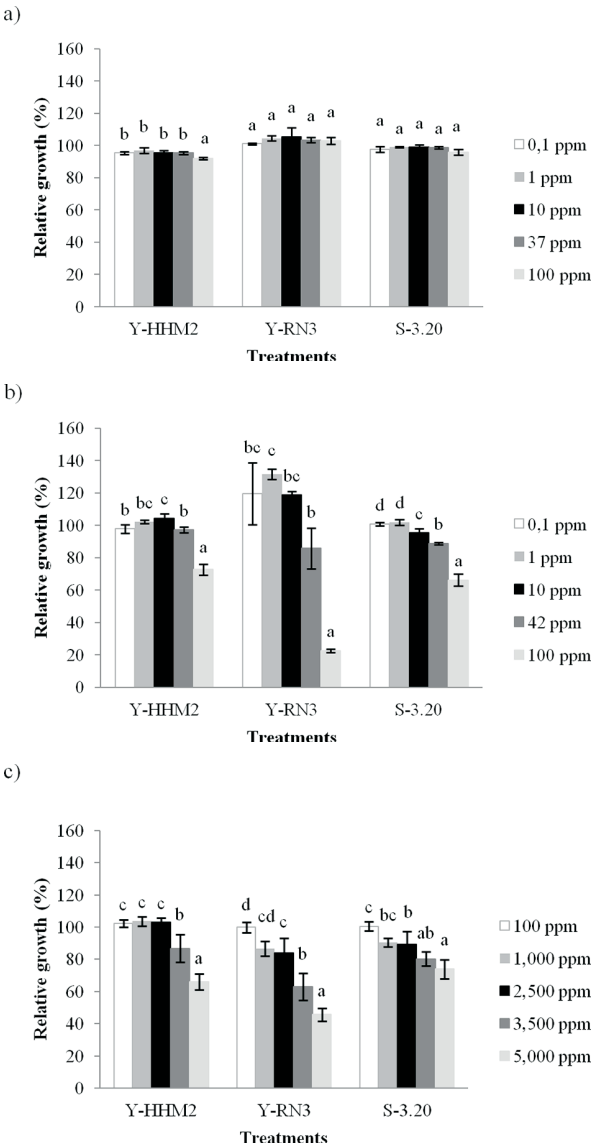

Figure 1. Relative fungal growth in comparison with the control, expressed as percentage (mean ± standard deviation) of *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3 and *Myrothecium* sp. S-3.20 on malt extract agar (MEA) plates supplemented with different 2,4-D 58.4 % (a), ametrine 50 % (b) and urea (c) concentrations. Means followed by the same letter are not significantly different according to Tukey's test, $p < 0.05$.

Pleurotus sp. Y-RN3 cultures supplemented with 0.1; 1.0 and 10 ppm of ametrine (50 %) showed the highest growth. *Myrothecium* sp. S-3.20 and *Bjerkandera* sp. Y-HHM2 showed a mycelial growth similar to control at 0.1, 1.0, 10 and 42 ppm. *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y- RN3 and *Myrothecium* sp. S-3.20 showed sensitivity to high concentrations of the herbicide (Figure 1b).

By evaluating increasing concentrations of urea on mycelial growth (Figure 1c) it was shown that 5,000 ppm significantly inhibited the mycelial growth of the fungal isolates with respect to control. Fungal strains showed different degrees of sensitivity to urea, a slight decline in *Pleurotus* sp. Y-RN3 and *Myrothecium* sp. S-3.20 growth was observed at 100, 1,000 and 2,500 ppm. On the other hand, fungal colony development of *Bjerkandera* sp. Y-HHM2 was stimulated.

Dual culture inhibition assays

In order to assess the feasibility of using mixed cultures for post-harvest SCR degradation, it was determined whether there were antagonistic effects among fungal isolates studied. Growth inhibition was observed between *Pleurotus* sp. Y-RN3 and *Myrothecium* sp. S-3.20 (growth inhibition % was less than 10 %). Three parameters (μ, D_{max} and λ) were calculated to characterize fungal growth dynamics using the equation of modified Gompertz model (Table 1).

Field assay

In relation to SOC content, the irrigated plots did not show significant differences compared to control at 120 days after inoculation ($F = 1.86$; d.f. error = 8; $p = 0.2106$). The SOC content remained relatively constant during the study. In rainfed conditions, SOC increased from initial values (14.30 g/kg) in all treatments, although these differences were not statistically significant (Table 2). Over the course of the experiment, TN content did not show significant differences compared to control ($F = 1.68$; d.f. error = 8; $p = 0.2467$ to irrigated plots and $F = 1.83$; d.f. error = 8; $p = 0.2169$ to rainfed plots) in the soil treated with different fungal isolates.

In order to evaluate fungal inoculation effect in soil microbial population, soil respiration and fluorescein released were measured. No appreciable change in soil respiration could be detected with fungal treatments in irrigated condition at 60 and 120 days after inoculation, with values between 200 and 400 mg CO2-C/ kg soil/10 d. A non significant increase in soil respiration was observed in rainfed condition at 60 days after inoculation in *Myrothecium* sp. S-3.20 and Y-HHM2/Y-

Table 1. Estimated parameters [D_{max}: maximum colony diameter (mm); μ: growth rate (mm/d); λ: lag phase (d) and D_0: diameter of inoculation agar plugs (mm)] through modelling of colony diameter by Gompertz modified model for all combinations of fungal strains in dual cultures.

Fungal isolates	Treatments	D_{max} ± SE	μ ± SE	λ ± SE	MS Error
Y-HHM2/S-3.20	Y-HHM2	72.97 ± 15.95	13.70 ± 0.54	1.22 ± 0.11	2.43
	S-3.20	0.32 ± 0.17 *	0.09 ± 0.01 *	-0.87 ± 1.73 *	0.0005
	Y-HHM2 /S-3.20	91.38 ± 29.99	12.99 ± 1.00	1.16 ± 0.16 *	1.63
	S-3.20/Y-HHM2	0.34 ± 0.10 *	0.08 ± 0.01 *	-0.81 ± 1.08 *	0.0002
Y-RN3/S-3.20	Y-RN3	60.10 ± 0.10	9.45 ± 0.40	2.10 ± 0.16	3.68
	S-3.20	84.79 ± 16.00	6.65 ± 0.19	0.41 ± 0.50	2.23
	Y-RN3/S-3.20	1.15 ± 0.05 *	0.23 ± 0.01 *	-0.06 ± 0.24	0.0009
	S-3.20/Y-RN3	55.08 ± 4.29	6.91 ± 0.28	0.76 ± 0.30	2.27
Y-HHM2/Y-RN3	Y-HHM2	95.17 ± 35.59	10.13 ± 0.38	-1.45 ± 1.25	0.76
	Y-RN3	0.53 ± 0.56 *	0.11 ± 0.03 *	-1.38 ± 3.84 *	0.0001
	Y-HHM2/Y-RN3	90.81 ± 50.73	9.70 ± 0.56	-1.62 ± 2.13	1.28
	Y-RN3/Y-HHM2	0.71 ± 1.32 *	0.13 ± 0.12 *	-2.43 ± 7.19*	0.0007

*Transformed variable: $\log_{10}$ diameter; SE: standard error of the estimated parameters.

Table 2. Soil organic carbon (SOC) and total nitrogen (TN) contents, expressed as g/kg soil, in *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3, *Myrothecium* sp. S-3.20 and the three fungal isolates (Y-HHM2/Y-RN3/S-3.20) treatments at 120 days after inoculation in irrigated and rainfed conditions in field assay.

Treatments	SOC (g/kg)		TN (g/kg)	
	Irrigated condition	Rainfed condition	Irrigated condition	Rainfed condition
Control	16.90 ± 0.96 a	17.39 ± 0.47 a	1.05 ± 0.03 a	1.09 ± 0.08 a
Y-HHM2	18.63 ± 1.01 a	15.48 ± 0.55 a	1.12 ± 0.05 a	1.14 ± 0.04 a
Y-RN3	15.43 ± 1.40 a	15.35 ± 1.25 a	1.05 ± 0.03 a	1.03 ± 0.04 a
S-3.20	16.10 ± 1.94 a	16.57 ± 0.24 a	1.09 ± 0.04 a	1.17 ± 0.03 a
Y-HHM2/Y-RN3/S-3.20	15.78 ± 1.04 a	15.76 ± 1.08 a	1.01 ± 0.03 a	1.15 ± 0.09 a

* Means ± standard error followed by the same letter are not significantly different according to Tukey's test, $p > 0.05$.

Table 3. Soil respiration, expressed as mg CO2-C/kg soil/10 d, in *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3, *Myrothecium* sp. S-3.20 and the three fungal isolates (Y-HHM2/Y-RN3/S-3.20) treatments at 60 and120 days after inoculation (DAI) in irrigated and rainfed conditions in field assay.

Treatments	Irrigated condition		Rainfed condition	
	60 DAI	120 DAI	60 DAI	120 DAI
Control	246.37 ± 21.15 a	309.63 ± 32.52 a	189.98 ± 33.62 ab	212.64 ± 33.57 a
Y-HHM2	246.37 ± 42.30 a	315.57 ± 78.34 a	140.63 ± 21.15 a	290.98 ± 39.30 a
Y-RN3	172.36 ± 30.52 a	402.89 ± 11.19 a	214.65 ± 36.63 ab	212.64 ± 22.38 a
S-3.20	334.49 ± 49.35 a	354.40 ± 36.74 a	299.24 ± 10.57 b	220.10 ± 35.59 a
Y-HHM2/Y-RN3/S-3.20	304.53 ± 37.01 a	283.52 ± 31.87 a	283.38 ± 37.01 ab	235.02 ± 33.57 a

Means ± standard error followed by the same letter are not significantly different according to Tukey's test, $p > 0.05$.

Table 4. Fungal inoculation effects on fluorescein release, expressed as mg/kg soil/2 h, during FDA hydrolytic activity in *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3, *Myrothecium* sp. S-3.20 and the three fungal isolates (Y-HHM2/Y-RN3/S-3.20) treatments at 60 and 120 days after inoculation (DAI) in irrigated and rainfed conditions in field assay.

Treatments	Irrigated condition		Rainfed condition	
	60 DAI	120 DAI	60 DAI	120 DAI
Control	55.11 ± 6.40 a	46.69 ± 1.92 a	62.01 ± 5.27 a	60.48 ± 3.27 a
Y-HHM2	59.71 ± 3.51 a	50.13 ± 4.66 a	70.44 ± 7.10 a	54.54 ± 3.04 a
Y-RN3	57.80 ± 2.51 a	44.77 ± 3.04 a	55.50 ± 1.38 a	50.90 ± 1.67 a
S-3.20	47.45 ± 6.27 a	52.43 ± 3.27 a	60.10 ± 4.42 a	56.65 ± 1.38 a
Y-HHM2/Y-RN3/S-3.20	49.37 ± 3.69 a	42.47 ± 2.39 a	61.25 ± 1.67 a	54.73 ± 1.67 a

Means ± standard error followed by the same letter are not significantly different according to Tukey's test, $p > 0.05$.

RN3/S-3.20 treatments (Table 3).

Bjerkandera sp. Y-HHM2 treatment presented a not significant increase of fluorescein released relative to abiotic control at 60 days (59.71 and 55.11 mg/kg soil/2 h, respectively) and 120 days (50.13 and 46.69 mg/kg soil/2 h, respectively) after inoculation in irrigated condition. Also, in rainfed condition, Y-HHM2 showed no significant increase ($F = 2.28$; d.f. error = 8; $p = 0.1491$) compared to abiotic control (70.44 mg/kg soil/2 h to fungal strain and 62.01 mg/kg soil/2 h to abiotic control) at 60 days after inoculation (Table 4).

Figure 2 shows OM in post-harvest SCR at 120 days after inoculation. There were no statistically significant differences in OM concentrations for irrigation and rainfed conditions, (F = 1.34; d.f. error = 6; p = 0.3553 and F = 2.85; d.f. error = 6; p = 0.1213 respectively). Regarding the effects of the fungal treatments on the OM, the SCR under Y-HHM2 effect increased 30.5 % and 69.6 % compared to abiotic control in irrigation and rainfed conditions, respectively. The highest increase for *Pleurotus* sp. Y-RN3 treatment was observed in rainfed conditions (53.3 %). Organic matter percentages presented a not significant increase of around 30.0 % in the case of SCR treated with S-3.20 and Y-HHM2/Y-RN3/S-3.20.

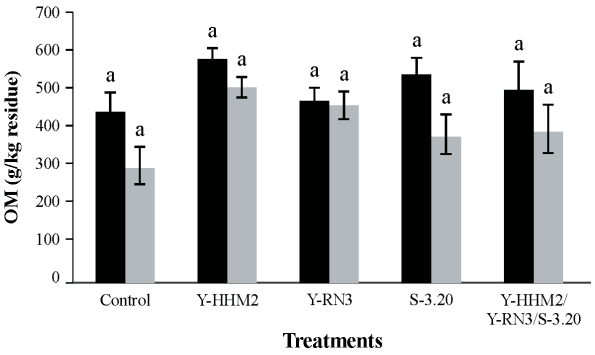

Figure 2. Organic matter (OM), expressed as g/kg, in post-harvest sugarcane residue at 120 days after inoculation with *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3, *Myrothecium* sp. S-3.20 and the three fungal isolates in irrigated and rainfed conditions in field assay. Means ± standard error followed by the same letter are not significantly different according to Tukey's test, p> 0.05. ■ Irrigated condition ■ Rainfed condition.

Post-harvest SCR initial TN content was 3.18 and 4.40 g/kg for irrigation and rainfed conditions, respectively.

Nitrogen concentration increased from the initial values in all post-harvest SCR where residues were inoculated with fungal strains until 120 days after inoculation.

In the case of irrigated plots, this increase was not significant (F = 1.40; d.f. error = 10; p = 0.3037). *Bjerkandera* Y-HHM2 and *Myrothecium* S-3.20 treatments in rainfed conditions significantly increased TN (F = 13.64; d.f. error = 8; p = 0.0012) compared to abiotic control (Figure 3).

In the irrigated conditions, cellulose contents were higher in inoculated SCR (Y-HHM2: 26.49 %; Y-RN3: 22.66 %; S-3.20: 25.60 %; Y-HHM2/Y-RN3/S-3.20: 29.52 %) than in the abiotic control (21.29 %). A similar result was obtained in rainfed conditions. Lignin contents were diminished by fungal treatment. The effect was considerable in Y-HHM2/Y-RN3/S-3.20 treatment in irrigation condition with a diminution of 30 % (abiotic control: 29.78 %; Y-HHM2/Y-RN3/S-3.20: 20.92 %).

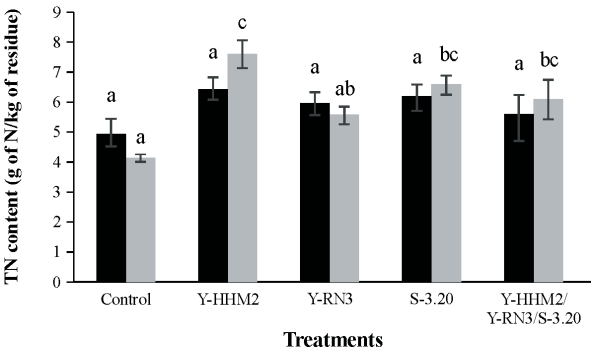

Figure 3. Total nitrogen (TN) content, expressed as g of N/kg residue, in post-harvest sugarcane residue at 120 days after inoculation with *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3, *Myrothecium* sp. S-3.20 and the three fungal isolates in irrigated and rainfed conditions in field assay. Means ± standard error followed by the same letter are not significantly different according to Tukey's test, p> 0.05. ■ Irrigated condition ■ Rainfed condition.

Table 5 presents the amount of post-harvest SCR at the beginning and end of the assay. Final residue amounts were significantly lower (F = 21.44; d.f. error = 5; p = 0.0024) in presence of Y-HHM2, Y-RN3 and Y-HHM2/Y-RN3/S-3.20 compared to abiotic control in rainfed condition. There was no significant difference in post-harvest SCR decomposition rate of residues inoculated with different fungal strains in irrigated condition at 60 and 120 days after inoculation (F = 0.51; d.f. error = 5; p = 0.7304 and F = 1.26; d.f. error = 5; p = 0.3937, respectively). In the present study, post-harvest SCR decomposition rate showed a significant increase (F = 13.20; d.f. error 4; p = 0.0142 and F = 21.44; d.f. error = 5; 0.0024 at 60 and 120 days after inoculation, respectively) in Y-HHM2, Y-RN3 and Y-HHM2/Y-RN3/S-3.20 treatments in rainfed condition.

Discussion

The effect of 2,4-D on fungal growth suggests that acts as metabolizable substrate for fungi, depending on the concentration used. The results are in line with the findings of Zain *et al*. (2013) for *Penicillium* sp., *Mucor* sp. and *Aspergillus* sp. in the presence of different herbicides, obtaining higher percentages of mycelial growth inhibition by increasing chemical doses. Westerhuis *et al*. (2007) found an *in vitro* inhibitory effect of glyphosate on *Sclerotium rolfsii*. According to these results, the effect of herbicides on fungal growth depends

Table 5. Initial, final and decomposed amounts, expressed as mg/ha, of post-harvest sugarcane residue and decomposition rate (DR), expressed as g/m²/d, in sugarcane plots inoculated with *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3, *Myrothecium* sp. S-3.20 and the three fungal isolates (Y-HHM2/Y-RN3/S-3.20) at 60 and 120 days after inoculation (DAI) in field assay.

	Control	Y-HHM2	Y-RN3	S-3.20	Y-HHM2/Y-RN3/S-3.20
Irrigated condition					
Initial amount (mg/ha)	24.76 ± 0.02				
Final amount (mg/ha)	4.00 ± 0.70 a	4.78 ± 0.46 a	5.02 ± 0.37 a	4.07 ± 0.73 a	5.25 ± 1.08 a
Amount decomposed (mg/ha)	20.76	19.98	19.74	20.69	19.51
Decomposition (%)	83.84	80.69	79.73	83.56	78.80
DR at 60 DAI (g/m²/d)	25.49 ± 1.03 a	25.01 ± 0.86 a	25.37 ± 0.79 a	25.07 ± 0.09 a	23.50 ± 0.30 a
DR at 120 DAI (g/m²/d)	16.85 ± 0.62 a	16.66 ± 0.39 a	16.46 ± 0.31 a	16.63 ± 0.03 a	15.45 ± 0.68 a
Rainfed condition					
Initial amount (mg/ha)	11.13 ± 0.21				
Final amount (mg/ha)	7.20 ± 0.66 b	4.51 ± 0.07 a	3.83 ± 0.10 a	7.63 ± 1.08 b	4.54 ± 0.46 a
Amount decomposed (mg/ha)	3.93	6.62	7.30	3.50	6.59
Decomposition (%)	35.31	59.48	65.59	31.45	59.21
DR at 60 DAI (g/m²/d)	4.26 ± 0.35 a	8.23 ± 0.11 abc	8.78 ± 1.33 bc	5.40 ± 0.69 ab	10.53 ± 0.06 c
DR at 120 DAI (g/m²/d)	3.27 ± 0.55 a	5.52 ± 0.05 b	6.08 ± 0.08 b	2.91 ± 0.90 a	5.49 ± 0.39 b

* Means ± standard error followed by the same letter are not significantly different according to Tukey's test, $p > 0.05$.

on the dose and type of chemical used, as well as the fungi evaluated. Veverka *et al*. (2007) reported that different degrees of sensitivity to urea could be due to the presence of NH_3 which causes an increase of the medium pH.

Antagonism effect on mixed fungal cultures employed in lignocellulosic residues breakdown has been investigated by several authors (Velázquez-Cedeño *et al*., 2004; Chi *et al.*, 2007; Lluyemi and Hanafi, 2009; Kausar *et al*., 2010). Antagonistic mechanisms include direct competition for space or nutrients, antibiotic and antifungal extracellular enzymes production and direct parasitism. These mechanisms are not mutually exclusive but can act synergistically (Howell, 2003). The results did not show antagonistic effects between selected fungal isolates, suggesting that it is possible to grow these fungal isolates together with the aim of increasing post-harvest SCR degradation. These results agree with those obtained by Lluyemi and Hanafi (2009), who developed mixed cultures of *Aspergillus niger* and *Trichoderma* sp. in order to increase hydrolytic enzyme production. Kausar *et al*. (2010) showed fungal antagonism as a defence strategy against mycelial growth potential competitors. However, lignocellulosic degradation can be improved by mixed cultures activity. For example, Chi *et al*. (2007) showed laccase and manganese peroxidase production increased in mixed cultures of *Pleurotus ostreatus* and *Ceriporiopsis subvermispora*.

Fungal SCR decomposition under field conditions produced an increase in SOC content compared to initial content. Similar results were published by Boopathy *et al*. (2001), who observed an increase in SOC (from 1.7 % to 2.7 % over a period of 225 days) in molasses supplemented soil. Also, the commercial consortium composed by *Ceriporiopsis subvermispora*, *Cellulomonas* sp. and *Azospirillum brasilense* increased SOC content from 1.4 to 1.7 % in 103 days (Beary *et al*., 2002). These increases in SOC content would indicate incorporation of plant material to the soil organic fraction and thus the breakdown of SCR by fungi. Low N concentration in SCR causes that soil microorganisms to turn to the soil solution for N. However, in this study, SOC and TN contents in soils were not significantly different to control, suggesting that fungal consortia involving post-harvest SCR degradation did not contribute to short term soil fertility.

Kennedy and Arceneaux (2006) observed an increase in post-harvest SCR decomposition rate, expressed as soil respiration, when the residue was reduced in particle size and/or soil incorporated. In this study, the residue was left undisturbed and therefore its specific surface for contact with inoculated fungi and native microflora was minor. Nitrogen concentration increased significantly in *Bjerkandera* sp. Y-HHM2 and *Myrothecium* sp. S-3.20 from the initial values in rainfed conditions at 120 days after inoculation. This increase in N concentration of SCR could be due to its temporary immobilization by soil microorganisms (Digonzelli *et al*., 2011; Basanta *et al*., 2003). On the other hand, N is released slowly than C during decomposition, so an increase in N concentration in SCR is observed (Digonzelli *et al*., 2013).

Cellulose contents were higher in inoculated SCR than in the abiotic control. According to Machuca and Ferraz (2001), cellulosic structure removal requires lignin degradation with consequent hydrolytic enzyme diffusion in plant cell walls. On the other hand, lignin contents were diminished by fungal treatment. Several researchers relate these results to stress response caused by ligninolytic fungi and soil microorganisms interactions that induced laccase production (Baldrian *et al*., 2000; Baldrian, 2004; Velázquez-Cedeno *et al*., 2004). In a previous work, Kausar *et al*. (2010) observed a decrease of cellulose and lignin contents during composting of rice straw using a lignocellulolytic fungal consortium as inoculant.

Conclusions

Bjerkandera sp. Y-HHM2, *Pleurotus* sp. Y-RN3 and *Myrothecium* sp. S-3.20 consortium may have facilitated breakdown of post-harvest SCR, which would increase microbial activity and therefore modify SCR for OM incorporation, preventing its accumulation on soil surface and burning risk. *Bjerkandera* sp. Y-HHM2, *Pleurotus* sp. Y-RN3 and *Myrothecium* sp. S-3.20 cultures did not evidence *in vitro* sensitivity to 2,4-D 58.4 % and ametrine 50 %. A negative correlation between urea concentration and fungi growth was presented.

To our knowledge, this is the first report on the capability of *Bjerkandera* sp. Y-HHM2 and *Pleurotus* sp. Y-RN3 to accelerate post-harvest SCR decomposition in rainfed conditions at field scale, suggesting that these fungi might be useful tools in the sugarcane crop system to facilitate harvesting and the subsequent ratoon cane establishment and improve soil properties. Further research is needed to optimize the conditions for longer experience of post-harvest SCR decomposition in field.

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