



# ARBUSCULAR MYCORRHIZA SYMBIOSIS REDUCES THE RHIZOCTONIA ROOT ROT AND ALTERS THE PHENOLIC PROFILE IN COMMON BEAN

## La simbiosis micorrízica arbuscular reduce la pudrición de raíz por rhizoctonia y altera el perfil de fenoles en frijol

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### ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have the potential to reduce the deleterious effect of soil-borne pathogens, but their ability for pathogen biocontrol may vary depending on the genotype of the plant, the pathogen, and the AMF interaction. Thus, the aim of this work was to evaluate the effect of the Mexican biofertilizer Rizofermic-UV based on a mix of AMF formulation against the common bean root rot caused by the pathogenic fungus *Rhizoctonia solani* Kühn 1858 (Teleomorph: *Thanatephorus cucumeris*). Additionally, the total phenolic content, individual phenolic acid (caffeic, ferulic, o-cumaric, p-cumaric, sinapic, and vanillic), and the flavonoid (catechin, kaempferol, quercetin, and rutin) profiles were analyzed. Our results show that the AMF biofertilization reduces the disease severity up to 68 %, and this was accompanied by a boost in total phenolic content in dual inoculation. Furthermore, a variation in the individual phenolic profiles caused by both AMF interaction and pathogen treatment alone were observed. In dual inoculations, vanillic acid was significantly different among treatments, suggesting it may contribute to the enhanced resistance of mycorrhizal roots to soil-borne pathogens. Further work is required to elucidate the exact role of these compounds in the bioprotection of arbuscular mycorrhizal to plant pathogens.

**Keywords:** Fungus, defense, plant disease.

### RESUMEN

Los hongos micorrízicos arbusculares (AMF) tienen el potencial de reducir el efecto negativo de los patógenos en plantas, pero su capacidad para el biocontrol puede variar dependiendo del genotipo de la planta, el patógeno y AMF. Por lo tanto, el objetivo de este trabajo fue evaluar el efecto del biofertilizante mexicano Rizofermic-UV basado en una mezcla diversos AMF contra la pudrición de la raíz del frijol causada por el hongo patógeno *Rhizoctonia solani* Kühn 1858 (Teleomorfa: *Thanatephorus cucumeris*). Se evaluó también el contenido de fenoles totales, el de ácidos fenólicos individuales (caféico, ferúlico, o-cumárico, p-cumárico, sinápico y vanílico) y los perfiles de flavonoides (catequina, kaempferol, quercetina y rutina). Nuestros resultados muestran que la biofertilización reduce la severidad hasta un 68 %, esto es acompañado por un aumento en el contenido de fenoles totales en la inoculación doble. Además, se observó una variación en los perfiles fenólicos individuales en la interacción con los AMF y en el tratamiento que contenía solo el

patógeno. En las inoculaciones duales, el ácido vanílico fue significativamente diferente entre los tratamientos, lo que sugiere que puede contribuir a una mayor resistencia de las raíces micorrizadas a los patógenos transmitidos por el suelo. Se requiere trabajo adicional para dilucidar el papel exacto de estos compuestos en la bioprotección de las micorrizas arbusculares contra los patógenos de las plantas.

**Palabras Clave:** Hongo, defensa, enfermedad de planta

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L. 1973) is one of the most important leguminous food crops in Mexico, where it is considered the center of origin, and worldwide, due to its significant content of carbohydrates, proteins, lipids, vitamins, and minerals (Chavez-Servia et al., 2016; Mecha et al., 2018). Similarly to other crops, the common bean is affected by the presence of plant pathogens that limit its production, such as the ubiquitous soil-borne fungus *Rhizoctonia solani* Kühn 1858 (Teleomorph: *Thanatephorus cucumeris*). *R. solani* is a plant pathogen with a broad host range (Valentín Torres et al., 2016) that causes the rot of hypocotyl and roots in the common bean, causing a devastating disease known as damping-off. In addition, an anastomosis variant of this pathogen (AG4 HI) can cause the foliage web blight disease in tropical areas (Valentín Torres et al., 2016; Martins et al., 2018). Current practices for pathogen control rely on chemical treatments, although this strategy is not always desirable for their negative effects in the environment and human health (Abdel-Fattah et al., 2011). It is necessary to improve the sustainability of crop production systems, including the common bean. Thus, biological control represents an alternative method to reduce the use of agrochemicals in crop protection (Mayo-Prieto et al., 2019).

Beneficial microorganisms can activate defense responses in plants, through the induction of gene expression and the production of secondary metabolites (Köhl et al., 2019). Phenolic compounds are secondary metabolites induced in plants by different factors, and include molecules with antimicrobial activity, or involved in signaling pathways, which play important roles in plant-microorganism interactions (Mandal et al., 2010). It has been reported that symbiosis can change the content of phenolic compounds in plants, e.g., a formulation of a mixture of Egyptian mycorrhizal fungi increased the total phenol content in the common bean, and significantly reduced both disease severity and disease incidence in *R. solani* (Abdel-Fattah et al., 2011). Also, it has been reported that total soluble phenol and flavonoid content was boosted in common bean roots after arbuscular mycorrhizal fungi (AMF) colonization, suggesting that the plant immune system was strengthened, and this was manifested as a reduction in the incidence and severity of infection by the pathogen *Fusarium solani* (Mart.) Sacc., 1881 (Eke et al., 2016). Inoculation of tomato plants with the AMF *Rhizophagus irregularis* (Blaszk., Wubet, Renker and Buscot) C. Walker and A. Schüßler 2010 (formerly *Glomus*

*intraradices*) increased total phenol content and reduced the root-knot nematode infection (Sharma and Sharma, 2017). In addition, individual phenolic acid concentrations (caffeic, chlorogenic and ferulic) have been reported in *R. irregularis* and *Funneliformis mosseae* (T.H. Nicolson and Gerd.) C. Walker and A. Schüßler 2010 (formerly *G. mosseae*) interactions in tomato root plants (López-Ráez et al., 2010). On the other hand, genes involved in the phenylpropanoid biosynthesis, such as PAL1 and HQT, are up-regulated by the mycorrhizal colonization of *Tomato mosaic virus* infected tomato plants (Aseel et al., 2019). However, no reports on the individual phenolic profile in the common bean co-inoculated with AMF and pathogens have been published. The aim of this work was to analyze the effect of the Mexican biofertilizer Rizofermic-UV, which is formulated as a mix of multi-taxa arbuscular mycorrhizal fungi, on the infection of the common bean by the soil-borne pathogen *R. solani*, as well as the total and individual phenolic (phenolic acids and flavonoids) compound content profile during the infection.

## MATERIALS AND METHODS

### Common bean seeds

Common bean seeds (variety Azufrasin) were provided by the CEVAF (Valle del Fuerte Experimental Field Station from Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias) from the harvest of January 2016. This variety was developed from crosses between three lines (Azufrado Pimono-78/Canario-72)//AZPA-5) in CEVAF, Sinaloa, Mexico.

### Biofertilizer

Rizofermic-UV is a biofertilizer formulated as a consortium of 12 arbuscular mycorrhizal fungi [*Acaulospora morrowiae* Spain and N.C. Schenck 1984, *Acaulospora scrobiculata* Trappe, *Acaulospora spinosa* Walker and Trappe 1981, *Claroideoglossum etunicatum* (W.N. Becker and Gerd.) C. Walker and A. Schüßler 2010, *Funneliformis geosporus* (T.H. Nicolson and Gerd.) C. Walker and A. Schüßler 2010, *F. mosseae*, *Gigaspora decipiens* I.R. Hall and L.K. Abbott 1984, *Gigaspora rosea* T.H. Nicolson and N.C. Schenck 1979, *Glomus aggregatum* N.C. Schenck and G.S. Sm 1982, *Glomus macrocarpum* Tul. and C. Tul. 1845, *Rhizophagus intraradices* (N.C. Schenck and G.S. Sm.) C. Walker and A. Schüßler 2010, and *Scutellospora pellucida* (Nicol. and N.C. Schenck) C. Walker and F.E. Sanders 1986], produced

in Mexico by the Laboratory of beneficial microorganisms of the Universidad Veracruzana.

### Pathogenic fungal inoculum

*Rhizoctonia solani* strain was kindly donated by Quintin Armando Ayala Armenta, Professor at the Universidad Autónoma de Sinaloa-Faculty of Agronomy. The inoculum was prepared by growing the fungus in bottles containing sterilized rice grain medium at  $25 \pm 2$  °C for 15 days.

### Planting, AMF inoculation, and pathogen infection

Common bean seeds were surface-sterilized by immersion in ethanol for two minutes followed by five minutes in 0.5 % sodium hypochlorite, rinsed four times with distilled water and sown in a pot (250 mL) containing a mix of sterile vermiculite and fine-sand (3:1). Half of the pots received 1 g of Rizofermic-UV inoculum per pot (according to the manufacturer's recommendation) at the moment of sowing, and as a soil drench two weeks after sowing (Abdel-Fattah *et al.*, 2011). Plants were fertilized twice a week with irrigation based on the modified Hoagland solution (Miller and Kitt, 1992), which contains one tenth (20 µM) of the regular potassium phosphate strength, for mycorrhizal and non-mycorrhizal sets. The experiments were carried out under greenhouse conditions to analyze the effects of Rizofermic-UV inoculation, on an eight h light (25 °C)/16 h dark (19 °C) photoperiod until harvest. Four weeks after planting, 1 g of infected rice seeds with *R. solani* was incorporated to the plant substrate to half of the mycorrhizal and non-mycorrhizal sets of plants. The inoculum was set surrounding the stem of the plantlets (Abdel-Fattah *et al.*, 2011). The four treatments: 1) No biofertilizer with no *R. solani* (Ctrl); 2) Biofertilizer with no *R. solani* (BF); 3) Biofertilizer with *R. solani* (BF-Rs); 4) No biofertilizer with *R. solani* (Rs) were arranged in a completely randomized design.

### Harvest, growth parameters and diseases assessment

One week after pathogen inoculation and five weeks after planting, sixteen plants from each treatment were carefully unrooted, and their roots washed using tap water. The disease incidence and severity as well as the growth parameters were registered at the harvest time. The disease incidence was determined by counting the number of plants exhibiting *R. solani* rot symptoms and expressed as a percentage of the total number of the plants assessed. The severity rate on roots and hypocotyls was evaluated based on a 1 to 4 disease scale: 0 = no damage; 1 = minor discoloration of the hypocotyl; 2 = discoloration plus small necrotic lesions (<1 mm in diameter) on the stem, hypocotyl, or roots; 3 = discoloration with large necrotic lesions ( $\geq 1$  mm in diameter) on the stem, hypocotyl, or roots; and 4 = death of the seedling

(Carling *et al.*, 1999). Disease severity was further calculated using the formula  $DS (\%) = \frac{\sum(ab) \times 100}{AK}$ , where  $a$  = number of diseased plants having the same degree of infection;  $b$  = degree of infection;  $A$  = total number of examined plants and  $K$  = highest degree of infection (Eke *et al.*, 2016).

### Root colonization

For the estimation of AMF root colonization, during harvest time, one portion of the root system from each plant was taken and washed with distilled water to remove substrate particles; immersed in 10 % (w/v) KOH; and heated for several 30 s intervals, until clarified. Subsequently, roots were rinsed twice with distilled water, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added. Then, roots were incubated at room temperature for 3-5 min. The hydrogen peroxide was removed, and the roots were rinsed three times with tap water, then stained with trypan blue (0.05 %) (Phillips and Hayman, 1970), and heated in the microwave for 30 s. The percentage of colonization was determined for each plant by the gridline intersection method, as previously described (Giovannetti and Mosse, 1980). Non-mycorrhizal control plants were also analyzed for colonization. No mycorrhiza-related structures or external hyphae were detected in non-inoculated roots at any point.

### Total phenolic content estimation

Phenolic extracts were prepared from 0.20 g of macerated fresh roots from *P. vulgaris*, from a pool of four plants, four pools for each treatment; sixteen plants in total were considered for each treatment. Samples were macerated in methanol (80 %) and incubated for 3 h at room temperature. The methanol was thoroughly evaporated at 40 °C using a rotary evaporator, and the aqueous phase was adjusted to 1 mL. Total phenolic content of the phenolic extracts was determined by the Folin-Ciocalteu assay, adapted to a 96-well microplate format (Nurmi *et al.*, 1996). Briefly, 140 µL of distilled water, 10 µL of extract (or 80 % ethanol as blanks, and gallic acid or (+)-catechin as standard), 10 µL of freshly prepared Folin-Ciocalteu reagent, and 40 µL of 7.5 % Na<sub>2</sub>CO<sub>3</sub> were mixed in a microplate well. The absorbance was monitored at 760 nm, and the results were expressed in mg of gallic acid equivalents (GAE) and (+)-catechin equivalents (CE) per g of bean root tissue.

### Phenolic profile analysis by HPLC

The phenolic profile was determined from the 80 % methanol extracts (filtered with 0.2 µm PTFE) using a HPLC system Dionex UltiMate DAD-3000 (Thermo Fisher Scientific, New York, NY), equipped with a titanium quaternary pump, an autosampler, a photodiode array detector (DAD-3000), and Chromeleon 7.0 Software; following what was

**Table 1.** Effect of biofertilizer alone and in dual inoculation with *Rhizoctonia solani* in common bean growth parameters.

Treatment	Length (cm)				Stem diameter (cm)		Fresh weight (g)				Disease	
	Plant		Root		Median (mean)	rank	Root		Shoot		Incidence (%)	Severity Scale 0-4 (%)
Median (mean)	rank	Median (mean)	rank	Median (mean)			rank	Median (mean)	rank	Median (mean)		
Ctrl	57 (55.45)	21.68 <sup>b</sup>	17 (17.63)	34.34 <sup>ab</sup>	3.26 (3.24)	46.72 <sup>a</sup>	1.10 (1.13)	38.6 <sup>ab</sup>	3.50 (3.86)	39.0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
BF	70 (70.73)	36.79 <sup>a</sup>	19 (19.81)	45 <sup>a</sup>	3.00 (3.01)	37.00 <sup>ab</sup>	1.20 (1.35)	44.5 <sup>a</sup>	4.15 (4.08)	42.6 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
Rs	43.75 (43.29)	7.75 <sup>c</sup>	9.25 (10.14)	12.09 <sup>c</sup>	2.03 (1.80)	10.25 <sup>c</sup>	0.10 (0.19)	9.34 <sup>c</sup>	0.70 (0.67)	8.56 <sup>b</sup>	100 ± 0 <sup>b</sup>	4.0 ± 0(100) <sup>c</sup>
BF - Rs	60 (56.22)	24.11 <sup>b</sup>	16.5 (16.05)	30.58 <sup>b</sup>	2.85 (2.74)	27.21 <sup>b</sup>	0.80 (0.83)	29.1 <sup>b</sup>	3.55 (3.25)	32.2 <sup>a</sup>	100 ± 0 <sup>b</sup>	1.5 ± 0.5(32) <sup>b</sup>

Ctrl= Control root, BF= biofertilized root, BF-Rs= biofertilized root combined with the pathogen *Rhizoctonia solani*, Rs= *Rhizoctonia solani* infected root. Different letters indicate significant statistical differences between treatments in each series, ( $p = 0.05$ ),  $n = 16$ .

reported by Sandoval-Castro et al. (2017). The separation was performed with an Aclaim® 120 A C18 column (250 x 4.6 mm i.d., 5 µm particle size) (Thermo Fisher, New York, NY). The volume of injection was 10 µL and the mobile phase consisted of two solvent systems: A=water acidified with acetic acid (pH 2.8), and B= acetonitrile at room temperature. The gradient elution was: 95 % A to 5% B from 0 to 2.5 min; 10 to 12 % B from 2.5 to 6 min; 12 to 23 % B from 6 to 18 min; 23 to 35 % of B from 18 to 24 min; 35 to 95 % B from 24 to 30 min; and returning to the initial conditions (95 % A and 5 % B) at 40 min. The flow rate was 0.3 mL/min, and the fixed wavelengths were 260, 280, 300, 342, and 350 nm. The phenolic compounds were identified and quantified using phenolic standards (Sigma-Aldrich; St. Louis, MO, USA).

### Statistical analysis

Two independent experiments were conducted for all analyses, using sixteen plants from each treatment. All data were subjected to normality tests; colonization rates were compared using Student's t-test (for two independent variables); the growth parameters were analyzed with the Kruskal-Wallis and Mann Whitney U tests; and ANOVA and a multiple ranges test (Tukey,  $p \leq 0.05$ ) were performed for phenolic assays using the Minitab v18 statistic program.

## RESULTS

### Colonization by the biofertilizer counteracts damage by *R. solani* infection

Percentages of mycorrhizal colonization in BF and BF-Rs treatments were  $17.5 \pm 5.6$  and  $14.12 \pm 4.5$  respectively, and no significant differences were found between

them according to the Student's t-test. Non-inoculated treatments had no visible AMF colonization. Typical root and hypocotyl rot symptoms were observed in the pathogen treatment (Rs), and interestingly, the biofertilizer (BF) treatment counteracted the pathogen deleterious effects, by maintaining growth at the uninfected control level (Ctrl) (Table 1). Despite the similarity in disease incidence in the Rs treatment compared to the AMF co-inoculation with the pathogen (BF-Rs), the severity of the disease was reduced up to 68 % in this last treatment (Table 1).

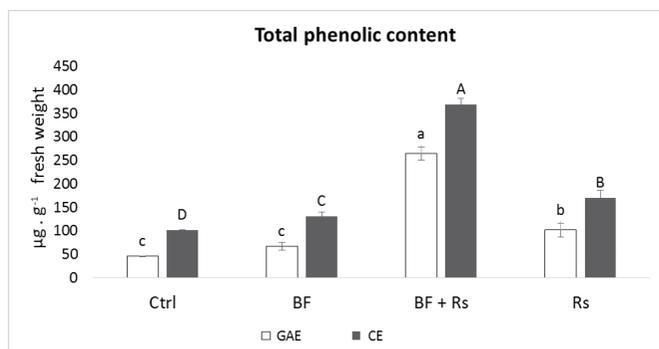
### Total phenolic content increased with mycorrhiza- pathogen interactions

Total phenolic content was measured, using both gallic acid, and catechin, as standards. Both sets of data are shown in Fig. 1. In the assay using gallic acid as a standard, no significant differences were found between Ctrl and BF treatments. However, in the assay using catechin as a standard, total phenolic content was statistically higher in BF than Ctrl. These data may indicate a slight, but important, difference in these compounds between Ctrl and BF treatments. On the other hand, a significant increase was found in the pathogen treatment (Rs) compared to the control. The highest increase in total phenolic content was observed in the co-inoculation treatment (BF-Rs), which was at least five-fold of gallic acid equivalents and three-fold of catechin equivalents (Fig. 1).

### Mycorrhiza and pathogen treatments change the phenolic compound profile

A set of phenolic acids (Fig. 2a-2f) and flavonoids (Fig. 3a-3d) were evaluated in root extracts of plants subjected to the different treatments. Except for rutin, which did not

show any difference compared to the control, the content of all the other phenolic compounds analyzed increased both in BF and Rs treatments, compared to the control. Individual analyses for each compound showed a high catechin concentration in the mycorrhiza treatment (BF) (Fig. 2a); while for caffeic acid, *o*-cumarinic acid, kaempferol and quercetin, the highest accumulation was observed in the pathogen infection treatment (Rs) (Figs. 2a, 2c and 3b, 3c). Finally, vanillic acid was the only compound that showed a significant increase in its content in the co-inoculation treatment (BF-Rs) (Fig. 3f).



**Fig. 1** Total phenolic compounds extracted from fresh root of common bean var. Azufrasin obtained from mycorrhiza-colonized and non-colonized plants grown under greenhouse conditions. Values are expressed as µg equivalents of gallic acid (GAE) or µg equivalents of catechin (CE) per g of root. Ctrl = control root, BF = biofertilized root, BF - Rs = biofertilized root combined with the pathogen *Rhizoctonia solani*, Rs = *Rhizoctonia solani* infected root. Different letters indicate significant statistical differences between treatments in each series, ( $p = 0.05$ );  $n = 16$ .

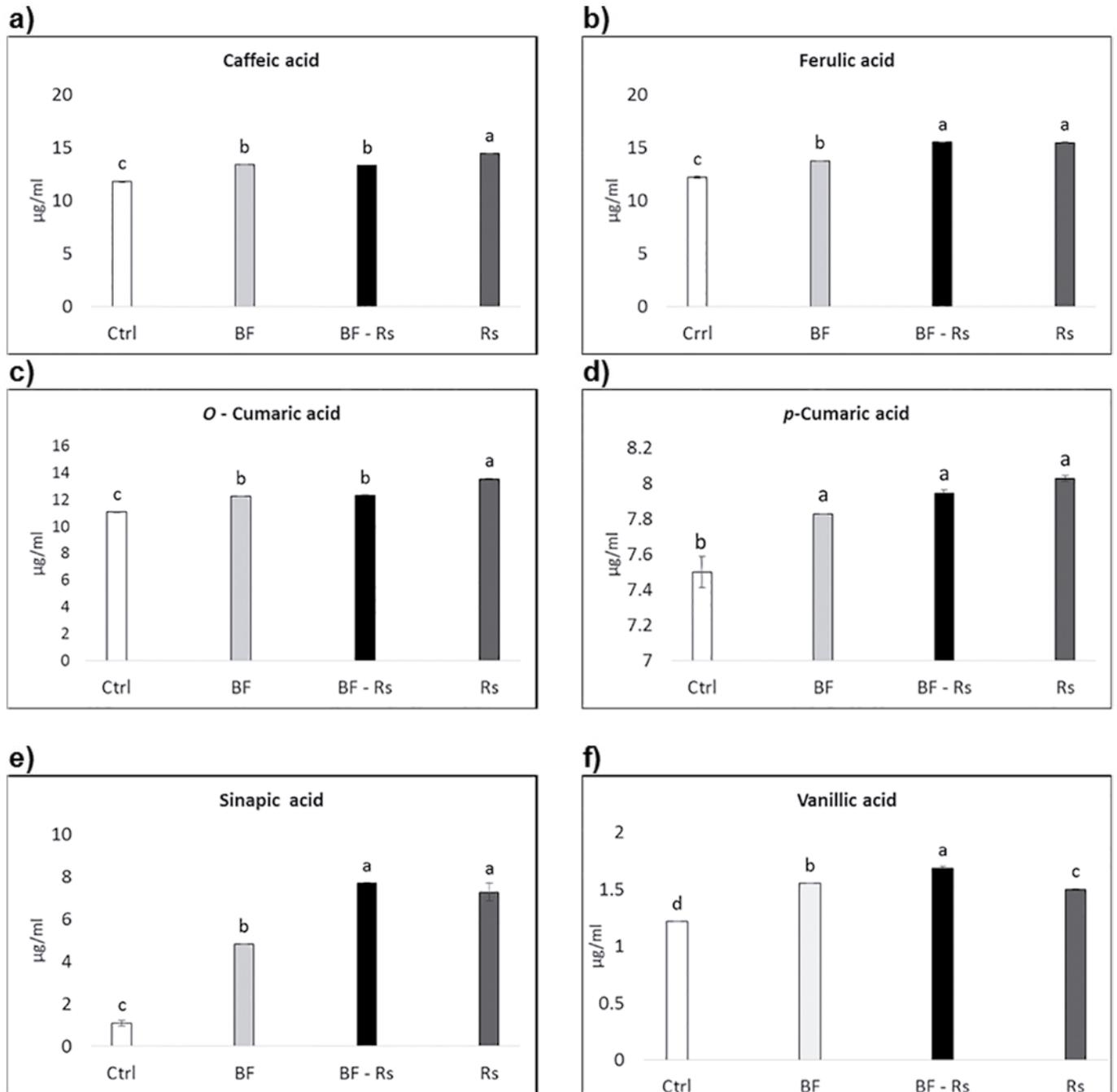
## DISCUSSION

The use of natural resources, such as soil microorganisms, for their application in sustainable agricultural practices is currently gaining importance, even more when those microorganisms can improve plant nutrient uptake and help to cope with pathogen invasion. AMF are capable of increasing plant nutrient uptake and enhance the plant's defense against pathogens. Mycorrhiza-induced protection against pathogens has been widely reported in different plant species and pathogens, including the protection of the common bean against the shoot pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary 1884 (Mora-Romero *et al.*, 2015), and the rot root diseases caused by *R. solani* (Abdel-Fattah *et al.*, 2011) and *F. solani* (Al-Askar and Rashad, 2010; Eke *et al.*, 2016). However, the defense response may vary according to the genotype of the plant species (Mora-Romero *et al.*, 2015), pathogen (Hao *et al.*, 2019), and AMF interaction (Pozo and Azcon-Aguilar, 2007; Eke *et al.*, 2016). Abdel-Fattah *et al.* (2011) reported the enhancement of common bean seedling growth treated with an Egyptian mix of mycorrhizal fungi (containing equal proportions of *G. mosseae*, *G. intraradices*, *G. clarum*, and *Gigaspora gigantea*) against the pathogen *R. solani*, compared to uninfected

plants. In this work, we tested a consortium of a Mexican AMF mix named Rizofermic-UV, which includes 12 species (*A. morrowiae*, *A. scrobiculata*, *A. spinosa*, *C. etunicatum*, *F. geosporus*, *F. mosseae*, *G. decipiens*, *G. rosea*, *G. aggregatum*, *G. macrocarpum*, *R. intraradices*, and *S. pellucida*) in common bean plants.

When plants were co-inoculated with the AMF consortium and the pathogen *R. solani*, despite the low rate of root colonization, a reduction of disease severity, up to 68 % was consistently observed (Table 1). Mora-Romero *et al.* (2015) also reported low-rate colonization (around 20 %) in mycorrhiza enhanced defense against the shoot pathogen *S. sclerotiorum* in common bean, suggesting that not full establishment of AMF is required in common bean to the induction of defense.

Regarding total phenolic content, the highest concentration was observed in plants infected with *R. solani* (Rs) compared to the control (Fig. 1). This is consistent with the previously reported fact that phenolic compounds have antimicrobial properties (Hammerschmidt, 2005; Mandal *et al.*, 2010). On the other hand, with the BF treatment, total phenolic content showed a significantly higher concentration compared to the control. Beneficial microorganisms such as arbuscular mycorrhizal fungi, are known to induce a defense priming mechanism, which is a physiological state that prepares plants to react in a stronger and faster manner to a subsequent pathogen attack (Pozo and Azcon-Aguilar, 2007). The priming responses are believed to be discrete in magnitude, but consistent (Conrath, 2011). Thus, the slight increase in phenolic compounds in BF plants might be reflecting the onset of a defense priming state. Interestingly, the co-inoculation of the AMF consortium and the pathogen (BF-Rs treatment) showed a very strong accumulation of total phenolic compounds in roots compared to the control (Fig. 1). This suggests that the putative priming response triggered by mycorrhizal colonization and the response against *R. solani* interacted in a synergistic way, inducing a significant accumulation of total phenolic compounds (Fig. 1), which paralleled a decrease in disease severity (Table 1). Phenolic compounds could be participating as direct defense molecules, or as players in signaling pathways (Hammerschmidt, 2005; Mandal *et al.*, 2010), which are influenced both by pathogens, as well as beneficial soil microorganisms like AMF (Al-Askar and Rashad, 2010; Abdel-Fattah *et al.*, 2011; Eke *et al.*, 2016; Sharma and Sharma, 2017). Changes in total phenolic compounds and flavonoids in the common bean interacting with different AMF and pathogens have been reported previously, e.g., enhanced biosynthesis and accumulation of total phenolic compounds were observed in AMF mix and *R. solani* co-inoculation (Abdel-Fattah *et al.*, 2011), as well as in *F. solani* infection (Al-Askar and Rashad, 2010; Eke *et al.*, 2016) and in the *R. irregularis* co-inoculated with the root-knot (Sharma and Sharma, 2017). Our results, considering the discrete phenolic compound accumulation induced by mycorrhiza inoculation (BF), and the boost in the tripartite interaction (BF-Rs), are consistent with these reports (Fig. 1).



**Fig. 2** Phenolic acid profile obtained by HPLC from ethanolic extracts of common bean var. Azufrasin roots. Ctrl = control root, BF= biofertilized root, BF - Rs = biofertilized root combined with the pathogen *Rhizoctonia solani*, Rs = *Rhizoctonia solani* infected root. Different letters indicate significant statistical differences between treatments, ( $p = 0.05$ );  $n = 16$ .

Several reports have been published on individual phenolic accumulation in mycorrhizal plants (Toussaint *et al.*, 2007; Schliemann *et al.*, 2008; López-Ráez *et al.*, 2010). However, to the best of our knowledge, this is the first report on individual phenolic compounds in the tripartite interaction, plant-mycorrhizal fungi-pathogen. In this study we report the profile of a small group of phenolic acids (Fig. 2a-f) and flavonoids (Fig. 3a-d), most of which increased their concentration in common bean roots by AMF colonization. The flavonoid catechin showed a significant increase in roots inoculated with the biofertilizer, even more than in the pathogen treatment (Fig. 3a). Previously, catechin accumulation has been reported in very specific root areas in ectomycorrhizal colonized roots (Weiss *et al.*, 1997). Whether this pattern of accumulation also occurs in the arbuscular mycorrhizal interaction remains to be investigated. However, the consistent high accumulation of catechin suggests a possible functional role for this metabolite in fungal symbiosis (Weiss *et al.*, 1997). On the other hand, (+)-catechin accumulation has also been reported as a response to a fungal pathogen attack (Chakraborty and Saha, 1994). It is interesting, though, that the accumulation of (+)-catechin decreased in the dual inoculation treatment compared to the AMF inoculation alone. This suggests that the role of this compound is different in beneficial and pathogenic interactions.

Regarding individual phenolic metabolite content, such as the levels of caffeic acid, our results contrast with previous studies, e.g., our results show that caffeic acid content increased in the common bean by AMF inoculation (Fig. 2a), whereas previous reports show a decrease in AMF-colonized roots (Toussaint *et al.*, 2007; López-Ráez *et al.*, 2010).

In the context of the interaction between the plants and the pathogen *R. solani*, we observed a slightly significant accumulation of the phenolic compounds caffeic and *o*-cumaric acid, and of the flavonoids kaempferol and quercetin, with pathogen infection compared to AMF inoculation alone (Fig. 2a, 2c and 3b, 3c).

Caffeic acid and *o*-cumaric acid, both classified as hydroxycinnamic acids, are present in the cell walls. Caffeic acid has been related to biocide activity and plant pathogen resistance against *Alternaria alternata* (Fr.) Keissl. 1912 (Ruelas *et al.*, 2006). *O*-cumaric acid is a biosynthetic precursor of salicylic acid, a signaling molecule that plays a role in host defense, and it has been reported to induce the expression of defense-related genes in plants (Montesano *et al.*, 2005; Yang *et al.*, 2008). The flavonoids kaempferol and quercetin act as deterrents against the nematodes *Radopholus similis* (Cobb) Thorne 1949 and *Meloidogyne incognita* (Kofoid and White) Chitwood 1949, and have shown anti-pathogenic activity (Mierziak *et al.*, 2014). This suggests that these molecules, caffeic acid, *o*-cumaric acid, kaempferol and quercetin, are activated mostly by the pathogen-plant interaction.

The induction of ferulic acid in mycorrhizal roots has been previously reported (López-Ráez *et al.*, 2010; Grandmaison

*et al.*, 1993). Consistently, in this study we observed a significant increase in ferulic acid with biofertilizer treatment alone compared to controls (Fig. 2b). Ferulic acid may be induced by fungal elicitors and jasmonates, suggesting that high levels of this compound may be involved in the onset of the priming state and contribute to the enhanced resistance of mycorrhizal roots to soil-borne pathogens (López-Ráez *et al.*, 2010). High levels of ferulic acid during the pathogen infection were also observed, more than in the AMF treatment alone (Fig. 2b).

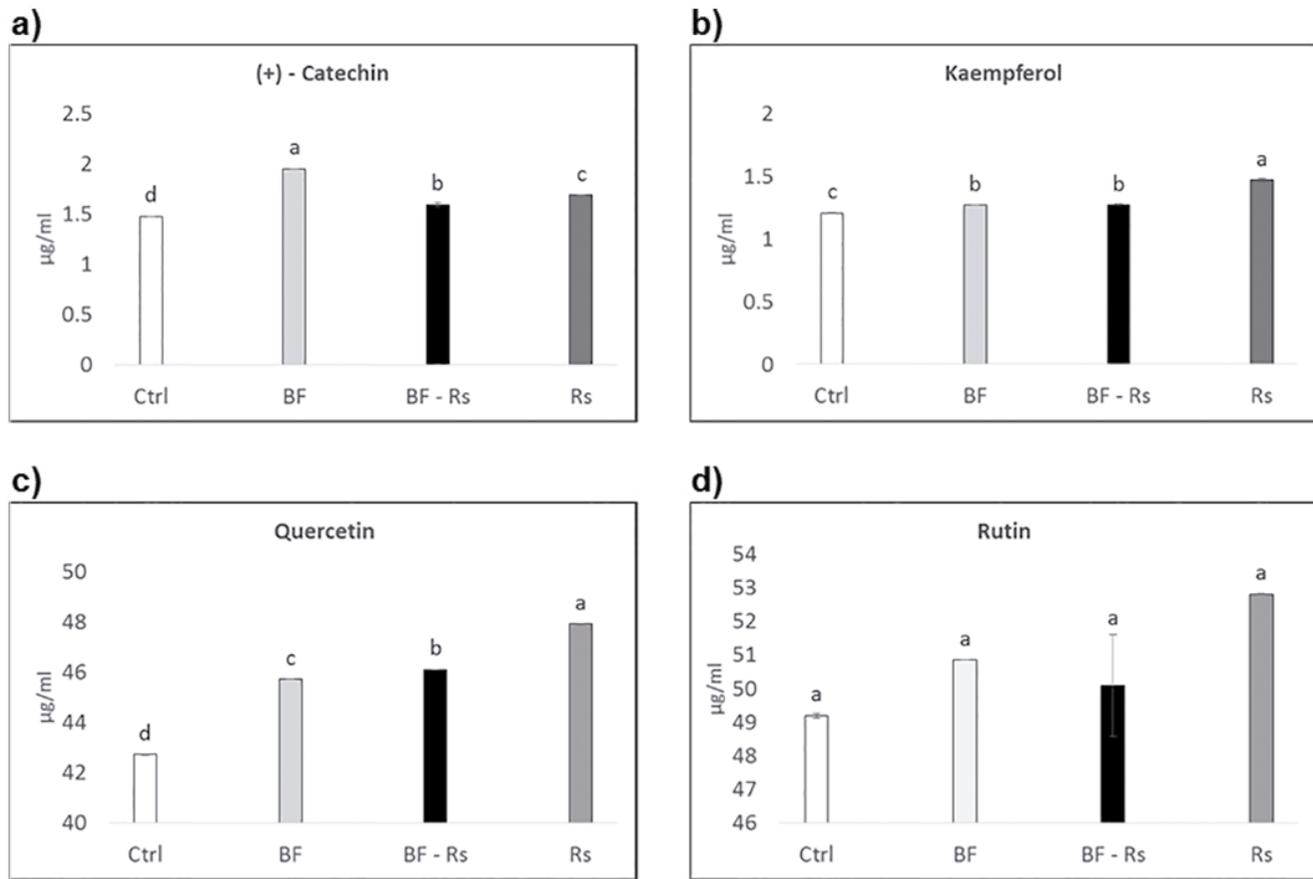
Sinapic acid is a common precursor of soluble secondary metabolites and has been detected in mycorrhiza-colonized onions roots (Grandmaison *et al.*, 1993), and involved in seed germination and plant development, affecting ABA catabolism (Bi *et al.*, 2017). Sinapic acid esters play a role in the defense response against pathogens (Konig *et al.*, 2014). In the present work, a dramatic variation of sinapic acid was observed in roots after AMF inoculation, but the accumulation was even greater in the treatment with the pathogen and in the dual inoculation (Fig. 2e). This might be an indication that this compound has a major role in plant-microbe interactions.

Significant increase of vanillic acid content was observed in dual inoculations (Fig. 2f), even more than in AMF or pathogen treatment alone. Its precursor, vanillin, has been detected in ectomycorrhiza (Weiss *et al.*, 1997). Also, the concentration of vanillic acid was shown to increase as part of the defense system during a pathogenic attack in tomato fruits (Ruelas *et al.*, 2006). Thus, the increase of vanillic acid levels may contribute to the enhanced resistance of mycorrhizal roots to soil-borne pathogens, although this remains to be functionally confirmed.

The role of phenolic compounds in mycorrhiza-induced resistance is controversial, although accumulation of these compounds has been reported in AMF colonized roots, as well as in plant-pathogen interactions. Here, we also observed changes in total and individual profiles of these compounds, both in AMF, as well as in pathogen inoculation. The contribution of other phenolic compounds not analyzed in this work cannot be ruled out, and additional work is required to elucidate their role in these plant-microbe interactions.

## CONCLUSION

Biofertilization based on a mix of arbuscular mycorrhizal fungi does not increase the root and shoot biomass but contributes to the reduction of the severity of the *R. solani* infection in the common bean var. Azufrasin plants. The enhanced defense against the pathogen observed in mycorrhizal treatment was accompanied by variations in total and individual phenolic compounds in the plants. This study provides evidence for the existence of a mycorrhiza-induced resistance mechanism in plants and suggests a possible participation of phenolic compounds.



**Fig. 3** Flavonoid profile obtained by HPLC, from ethanolic extracts of Azufrasin common bean roots. Ctrl = control root, BF= biofertilized root, BF + Rs = biofertilized root combined with the pathogen *Rhizoctonia solani*, Rs = *Rhizoctonia solani* infected root. Different letters indicate significant statistical differences between treatments, ( $p = 0.05$ );  $n = 16$ .

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## DISCLOSURE OF INTEREST

The authors declare that there is no conflict of interest.

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