Tropical and Subtropical Agroecosystems, 16 (2013): 391 – 398.

EFFECT OF DIESEL AND BIODIESEL ON THE GROWTH OF *Brachiaria decumbens* INOCULATED WITH ARBUSCULAR MYCORRHIZAL FUNGI

[EFECTO DEL DIESEL Y BIODIESEL EN EL CRECIMIENTO DE *Brachiaria decumbens* INOCULADA CON HONGOS MICORRÍZICOS ARBUSCULARES]

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SUMMARY

Arbuscular mycorrhizal fungi have been found to be associated with plants useful in soil phytoremediation. The aim of this study was to compare the effects of diesel and biodiesel in soil and sand on the growth of *Brachiaria decumbens* inoculated with mycorrhizae. Two experiments were carried out: one experiment in soil and another in sand. A two-level- factorial design with three factors was used (one on sterile and another on non-sterile soil, with and without mycorrhizae; and one with diesel and another with biodiesel). In sand, a two-factor design with two levels was used (with and without mycorrhizae and with diesel and biodiesel), both with three replications. NOVADIESEL, biodiesel and PEMEX diesel were use as contaminants, both at 7%. The fresh and dry weight of the plants and percentage of mycorrhizal colonization, were assessed 30 days after planting. In soil, biodiesel was more toxic and reduced the fresh and dry weights of plants, especially in non-sterile soil. Biodiesel yielded greater mycorrhizal colonization values that doubled those of the control. In sand, diesel was found to reduce three times the fresh and dry weights of plants, compared to the biodiesel. In sand diesel presented high values of mycorrhizal colonization in comparison with biodiesel. Plants inoculated with mycorrhizal fungi exhibited better development than non-inoculated plants, even in the presence of contaminants.

Key words: Soil contamination; fuel; symbiotic fungus; grass; phytoremediation.

RESUMEN

Se ha encontrado que los hongos micorrízicos arbusculares, asociados a plantas pueden ser útiles para la fitoremediación de suelos. El objetivo de este estudio fue comparar el efecto del diésel y el biodiesel en suelo y arena en el crecimiento de *Brachiaria decumbens* inoculada con hongos micorrízicos. Se llevaron a cabo dos experimentos: uno en suelo y otro en arena, se utilizó un diseño factorial con tres factores (en suelo estéril y sin esterilizar, con y sin micorriza y con diésel y biodiesel). En arena, se utilizó un diseño bifactorial con dos niveles (con y sin micorrízica con y sin biodiesel), ambos con tres replicas. Se utilizaron como contaminantes biodiesel NOVADISEL y diésel PEMEX al 7%. 30 días después de la siembra se evaluó el peso seco y fresco de las plantas y el porcentaje de colonización micorrízica. En suelo el biodiesel fue más toxico y redujo el peso fresco y seco de las plantas, especialmente en suelos no estériles. En el biodiesel se encontraron mayores porcentajes de colonización micorrízica que duplican a los encontrados en el testigo. En arena se encontró que el diésel reduce 3 veces el peso fresco y seco de las plantas comparado con el biodiesel, en arena el diésel presento altos valores de colonización micorrízica en comparación con el biodiesel. Las plantas inoculadas con los hongos micorrízicos, presentaron mejor desarrollo que las no inoculadas, aún en presencia de los contaminantes.

Palabras clave: Suelo contaminado; combustible; hongos simbióticos; pasto; fitorremediacion.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are considered one of the most common microorganisms, constituting an important functional component of plant-soil systems in almost all habitats and climates, and they have been shown to facilitate plant growth in soils contaminated with hydrocarbons. AMF have also been found to regularly associate with the roots of plants used in soil phytoremediation (Binet *et al*., 2001; Leyva *et al*., 2002; Vosátka *et al*., 2006).

AMF have been extensively studied as a potential tool in the recovery of contaminated sites by various agents; thus to date, several studies investigating the effects of diesel soil contamination have been conducted; however, no reports have assessed the effects of biodiesel. Most research work on the field of interaction of contaminant mycorrhizae has focused in heavy metals (Kirk *et al*., 2005; Graman *et al.,* 2003**;** Sudova *et al.,* 2007; Alarcón *et al.,* 2008; Usman and Hashem, 2009; Hernández-Ortega *et al*., 2012). Considering the current use of biodiesel as an energy alternative, it is critical to understand its effects on soil microflora and plants (Binet *et al*., 2001; Leyva *et al*., 2002; Xiaolin *et al*., 2002).

In this paper, we consider that due to the higher chemical reactivity of the fatty alkyl acid esters (biodiesel), these will degrade faster than those diesel hydrocarbons reagents. Nevertheless, our hypothesis is that in the short time, biodiesel will have a more toxic effect on plants and AMF will help to the survival of vegetables in their early growth stages. AMF will move the necessary resources so that plants will have an acceptable development, further more they will facilitate that degrader microorganisms of pollutants proliferate in the rhizosphere.

The goal of the present study was to compare the effects of diesel and biodiesel contamination in soil and sand on the growth of *Brachiaria decumbens* Stapf., a mycorrhizal plant that grows easily in the tropical areas of southern Mexico.

MATERIALS AND METHODS

Characterization of the soil

The soil used was taken at a depth of 20 cm and it was collected from coffee farms in Xalapa, Veracruz. The physicochemical properties of the soil were determined using the Mexican Official Standard 021 RECNAT 2000. The soil had a texture sandy-loam with the following properties: 49.20% sand, 21.80% clay and 29.00% silt; 6.56% organic matter, and a $pH_{water\ (1:2\ p/v)}$ of 5.79. The concentrations of soil nutrients were as follows: 28 ppm inorganic N, 13.6 ppm P *i*, 395 ppm K, 1561 ppm Ca, 262 ppm Mg, 29.7 ppm Fe, 3 ppm Zn, 1.5 ppm Cu, and 24.8 ppm Mn.

Contamination of the substrate

The contaminant used was Diesel which was taken at random to compare with biodiesel (made from residual oils). These contaminants or similar such as petroleum, biodiesel, diesel, born oil etc. have been used many times (Lapinskiene *et al*., 2006; Demello *et al*., 2007; Alarcón *et al*. 2008). The substrates (soil and sand) were dried, disaggregated and sieved to particle sizes between 2.00 and 2.36 mm to achieve a more homogeneous distribution of the contaminant and to ensure the occurrence of aerobic processes.

The portion of the soil used for the control was sterilized in an autoclave operating at a temperature of 121°C and a pressure of 15 kg/m^2 for 1 hr. The remaining portion was not sterilized. Both portions were used to compare the behaviour of the microorganisms in the biodegradation in soil contaminates.

The substrates were contaminated separately for the different experimental treatments: sterile soil plus diesel (SS+D), sterile soil plus biodiesel (SS+B), sterile soil minus diesel (SS-D), sterile soil minus biodiesel (SS-D), sand plus diesel (S+D), sand plus biodiesel (S+B). An intermediate value of 7%, as reported by Lapinskiene *et al*. (2006), was used as the concentration for both contaminants. Diesel (15.2 ml) and biodiesel (16.25 ml) were dissolved in 100 ml acetone and then used to saturate 195 g of each substrate. Each substrate was subsequently mixed with an electric mixer to homogenize the contaminants and it was allowed to dry in glass trays for one week. Glass containers were filled with 32.5 g of each substrate.

Experimental design and statistical analysis

Two experiments were performed. In the first one, a randomly factorial design soil substrate was used; it conveyed three factors: sterile [(SS+) and non-sterile (SS-)]; contaminants [biodiesel (B) and diesel (D)] and mycorrhizae with mycorrhizae [(M+) and without mycorrhizae (M-)], with 12 treatments and three repetitions each. The second substrate consisted of bank sand, with a randomly factorial design: mycorrhizae [with and without (M+, M-)], two types of contaminant [biodiesel (B) and diesel (D)], six treatments and three repetitions. The experiment was conducted in a growth chamber. Conditions during the course of the trial were as follows: 25°C average temperature, 80% relative humidity and a photoperiod of 12 hours light and 12 hours dark. Data were subjected to analysis of variance (ANOVA) with the following programs: STATGRAPHICS CENTURIUN XV 15.206. Followed by Fisher's least significant difference (LSD) test at $P < 0.05$.

Inoculation

Treatment with mycorrhiza was inoculated with 5 g of MTZ1-UV mycorrhizal complex, which consists of 12 AMF species (*Acaulospora morrowiae, A. spinosa, A. scrobiculata, Funneliformis mosseae, F. geosporus, Gigaspora rosea, Gi. decipiens, Glomus macrocarpum, Gl. aggregatum, Rhizophagus intraradices, Scutellospora pellucida* and *Claroideoglomus etunicatum*) with 75% mycorrhizal colonization, provided by the laboratory investigating beneficial organisms at the Universidad Veracruzana. *B. decumbens* seeds (3 g) were sown in each of the pots and watered with 10 ml of tap water every two days.

Evaluated Variables

The weight of fresh and dry leaves was evaluated (at 60°C for 72 hours) 30 days after planting for each treatment, and we also evaluated the percentage of root colonization by mycorrhizal fungi (Giovannetti and Mosse, 1980).

RESULTS

In sand, only were found significant differences in the biomass fresh and dry weights, the contaminant-mycorrhizal interaction (Table 1) was only observed due to the effect of the pollutant. In soil, 30 days after planting, statistically significant ($P<0.01$) differences were observed for the fresh and dry weights and the percentage of mycorrhizal colonization due to the effect of the contaminants (diesel and biodiesel). For the interaction between the contaminant, sterile soil and for the three factors evaluated, differences in the fresh and dry weights were only observed when comparing the control and contaminated treatments (Table 1). The higher values of biomass fresh and dry weights were in sand the contaminant and the lower was the interaction between contaminant and mycorrhiza. In soil the higher was the interaction between contaminated soil sterilization and the lower was the interaction between the three factors.

Plants inoculated with the AMF showed a significant difference in comparison to those which were not inoculated.

The fresh weight of plants inoculated with mycorrhizal fungi was significantly higher than those non-inoculated plants (Fig. 1a). However, differences in dry weight were not observed (Fig. 1b). Thus, these differences affecting only the fresh weights were likely due to the effect of the mycorrhiza and their interaction with the contaminant.

The presence of diesel in the sand caused a significant drop (Figs. 1a y b), and compared to the uncontaminated control, the presence of diesel in the sand caused a significant reduction three times ($P < 0.001$) in the fresh and dry weight of the plants (Figs. 1a and b). Similarly, biodiesel caused significant decreases ($P < 0.001$) in the fresh and dry weights of the plants (Figs. 1a and b). When plants were grown in diesel-contaminated sand, inoculation with mycorrhizal fungi did not have any effects on the dry and fresh weights of the plants. In contrast, inoculated plants growing in biodiesel-contaminated sand showed an increase a half time in fresh and dry weights compared to non-inoculated plants grown in contaminated sand (Figs. 1a and b).

Table 1. ANOVA of mycorrhizal root colonization and dry shoot weight of *B. decumbens* in soil and sand contaminated by diesel and biodiesel.

	Sand			Soil		
	DW	FW	Col	DW	FW	Col
Contaminant (C)	***	**	NS	**	***	*
Mycorrhiza (M)	NS	NS	-	NS	*	-
C-M	NS	**	-	NS	**	-
Sterilization (S)	-	-	-	NS	NS	NS
C-S	-	-	-	***	***	NS
S- M	-	-	-	NS	NS	-
C-M-S				*	*	_

*, P < 0.05; **, P< 0.01; ***, P <0.001; DW, dry weight; FW, fresh weight; Col, colonization; NS, no significative; -, not measured.

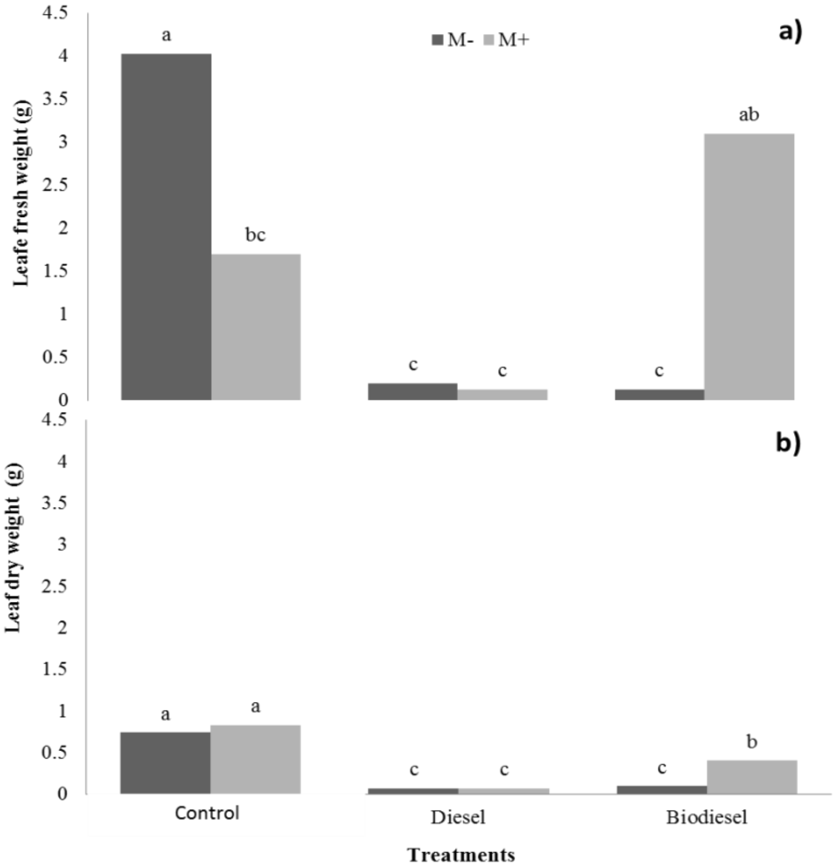

Figure 1. Leaves fresh a) and dry b) weights of *Brachiaria decumbens* inoculated and not by mycorrhizal fungi in sand with diesel, biodiesel and a control. Bars with equal letters are statistically similar (LSD, $P \leq 0.05$); n=3 M-, without mycorrhiza; M+, with mycorrhiza.

While a decreasing trend in mycorrhizal colonization was observed for sand contaminated with diesel compared to the control (Fig. 2b), an increasing trend was observed for biodiesel-contaminated sand relative to the control (Fig. 2b). The presence of diesel and biodiesel in the non-sterile soil diminished the weight of fresh and dry plants; nevertheless, biodiesel caused a higher reduction of weight in the plant leaf mass (Figs. 2 a y b).

Plants inoculated with AMF showed a reduction in fresh leaf weight; nevertheless, this was not observed in the dry weight (Figs. 2 a y b). Mycorrhizal inoculation did not have any effect on fresh or dry leaf mass, or in non-sterile contaminated soil with diesel or biodiesel (Figs. 2 a y b). However, in sterile soil mycorrhizal inoculation countered the reduction of fresh and dry leaf mass in plants only in those treatments with biodiesel. Soil sterilization did not affect mycorrhizal colonization However, only the presence of biodiesel increased colonization relative to the control and diesel (Fig. 3a).

DISCUSSION

The results of the present study demonstrate that biodiesel is more toxic to plants than diesel (Figure 1). This may be due to the fact that the soil particles stay impregnated by the biodiesel due to the facts that obstructs the pore spaces and hamper the adequate flux of water and nutriments, which at the same time may be the cause of the damage to the plants, such as has been reported by (Lapinskiené *et al.*, 2006).

On the other hand, despite the fact that biodiesel seems to be more toxic to plants, it has been reported that can be used as a biological solvent in soils contaminated with diesel, because of the transformational co-metabolic process in which microorganisms use a second substrate as a source of energy to degrade the first substrate (Pasqualino *et al*., 2006). It had been mentioned that biodiesel is more viscous (3.5-5.0) than diesel itself (2.4-4.5) and has a very low steam pressure (Fangrui and Hanna, 1999). Nonetheless, the degrading percentage of biodiesel is 80% in 28 days, while that of diesel is

45% in the same amount of time, but disappears more easily than biodiesel (Lapinskiené *et al.*, 2006; Pasqualino *et al.*, 2006).

Moreover, the limited growth of *B. decumbens* was observed at concentrations of 7% v/v. Even in the presence of diesel, plants developed better than those in soil contaminated with these observations could be due to various factors, such as the volatilization of the contaminant (diesel is more volatile than biodiesel) (Biodisol, 2005; Martínez, 2008), in turn diesel contains compounds of intermediate and low molecular weight in addition to organic compounds of higher molecular weight, while biodiesel (a mixture of methyl or ethyl esters of fatty acids) does not (Adams and Morales-Garcia, 2008). Furthermore, these compounds are characterized by their non polar nature (hydrophobic), which confers greater volatility to diesel (Adams and Morales-Garcia, 2008), and the concentration of diesel may have therefore declined. On the other hand, the lower density and viscosity of diesel (may have allowed it to leach rather than being retained in the area of the rhizosphere (Knothe and Steidley, 2005; Baroutian *et al*., 2010)

No effect of soil sterilization was observed possibly because the sampling time was very short (30 days). Alternatively, it may not have been possible to detect effects due to the presence of microbial complexes involved in the degradation of the pollutants, particularly for biodiesel. Several studies (Makareviciene and Janulis, 2003) have indicated that biodiesel is more readily degraded by microorganisms, tales como *Bacillus, Proteus, Pseudomonas, Citrobacter* and *Enterobacter* (Leahy and Colwell, 1990; Lutz *et al.*, 2006).

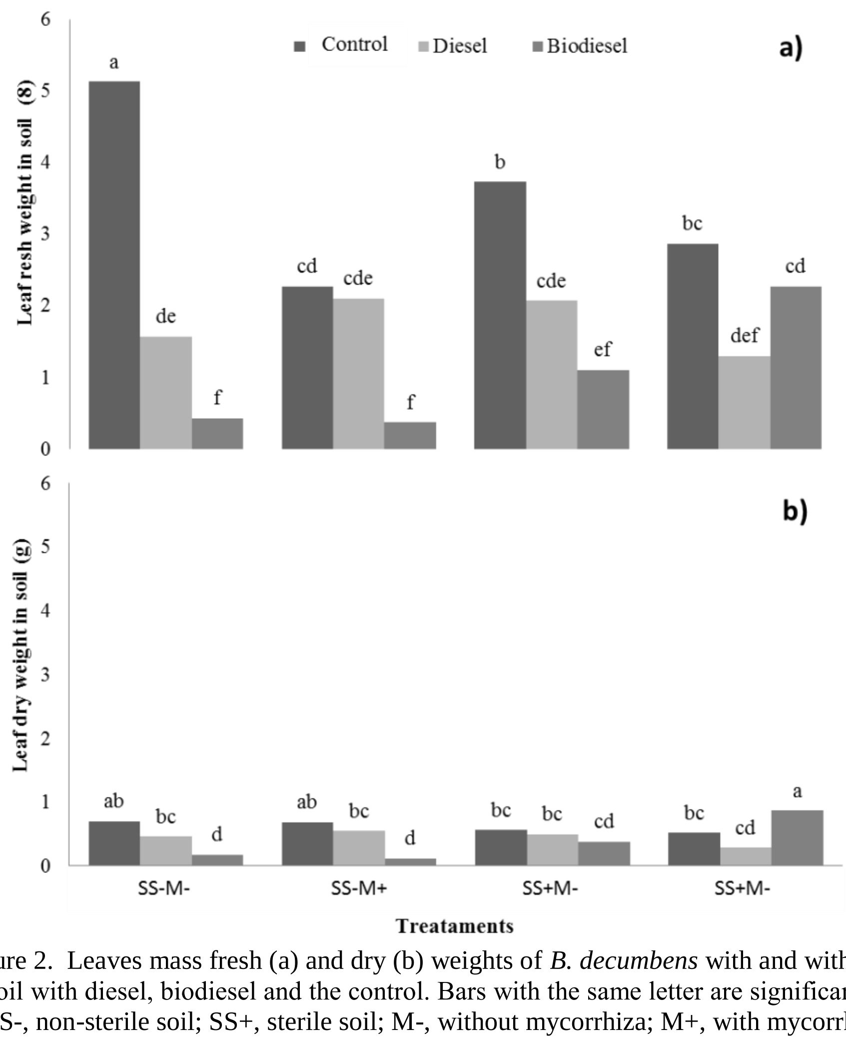

Figure 2. Leaves mass fresh (a) and dry (b) weights of *B. decumbens* with and without mycorrhizal fungi inoculation in soil with diesel, biodiesel and the control. Bars with the same letter are significantly different (LSD, $P \leq 0.05$); n = 3. SS-, non-sterile soil; SS+, sterile soil; M-, without mycorrhiza; M+, with mycorrhiza.

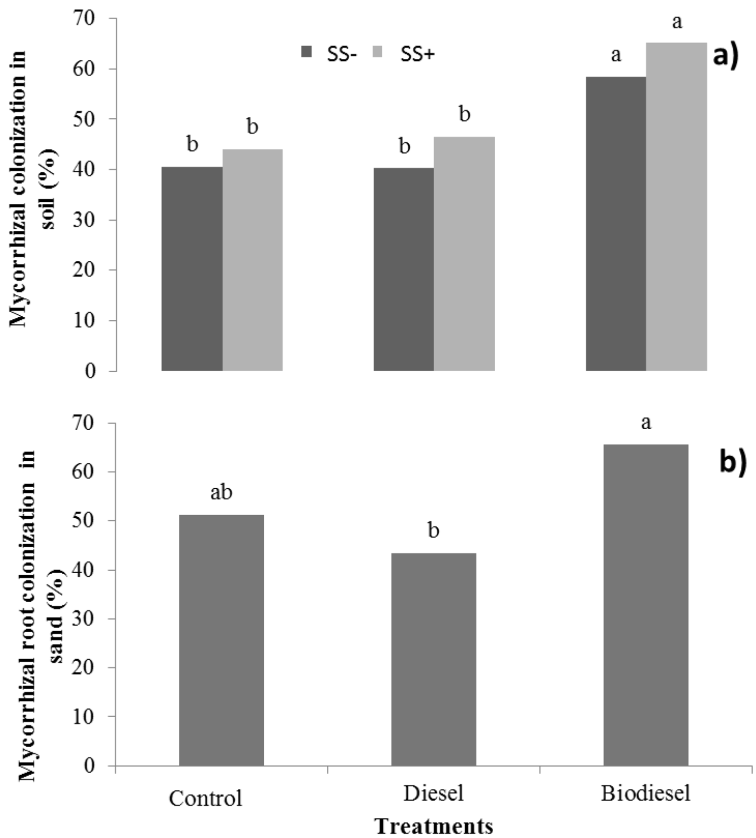

Figure 3. Mycorrhizal colonization (%) in sterile and non-sterile soil contaminated with diesel and biodiesel (a) and in sand contaminated with diesel and biodiesel (b). Bars with the same letter are significantly different (LSD, $P \leq 0.05$); n = 3. SS-, non-steril soil; SS+, steril soil.

A significant effect was observed when the interaction among the contaminant, soil sterilization and mycorrhizal inoculation was analysed. There was a clear tendency of clustering due to the effect of the pollutant. The greatest fresh and dry weights were observed with the controls, and biodiesel was found to have a more negative effect on plant growth than diesel. This result suggests that arbuscular mycorrhiza tolerates the presence of biodiesel in the ground, as shown in several studies investigating soils contaminated with hydrocarbons (Leyval *et al*., 2002, Jeffries *et al*., 2003 Volante *et al*., 2005). In unsterilized soil, however, it appears that native microflora do not permitted to establish the symbiosis, and the beneficial effect of AMF in promoting growth is suppressed, which is consistent with the findings reported by Hetrick and Wilson (1990). Similar trends were observed when sand was used as the substrate, and colonization increased when the substrate contained biodiesel. When diesel was used as the contaminant in sand, there was little difference in the percentage of colonization compared to the control. Similar results for biodiesel were obtained by Alarcón *et al*. (2008), who observed that colony-forming units (cfu) of filamentous fungi increased when the inoculated soil was contaminated with oil.

CONCLUSIONS

The presence of diesel in soil affected negatively mycorrhizal colonization and plant biomass. In contrast, biodiesel promoted greater mycorrhizal colonization but led to reduced plant development. In sand, diesel contamination resulted in higher values of mycorrhizal colonization compared to contamination with biodiesel. However, plant biomass was nearly three times higher in sand contaminated with biodiesel than with diesel. In the presence of the contaminants, plants inoculated with mycorrhiza developed better than non-inoculated plants. *Brachiaria decumbens* can be used as plant shape for the study of the interactions AFM and contaminants of the type of the hydrocarbons, since they present an excellent and quick response to the settling mycorrhizae (20-30 days); they have short life cycle; and its minimal pigmentation at the roots facilitates the observation of mycorrhizal fungal structures. Likewise, these plants have a relative degree of tolerance to the presence of fossil and non-fossil fuels, which allows for the completion of the plant life cycle. It will be critical to further investigate the extent of degradation of biodiesel and diesel in the rhizosphere of contaminated soils and the potential use of mycorrhiza as an alternative for soil remediation in response to fuel spills.

ACKNOWLEDGEMENTS

We would like to acknowledge the Faculty of Agricultural Sciences for lending their facilities, especially to the Laboratory of Beneficial Organisms. With special recognition to PhD. Samuel Cruz Sanchez and MSc. Jacob Bañuelos for their support at analysing the data and doing the statistical inform; to MSc. Ingrid Karina López Guzman for the analysis of samples and to PhD. Ulises Coffen, from the Bioprocess Solutions, for donating the biodiesel used for this experimentation.

REFERENCES

Adams, R. H. And Morales García, F. 2008. Concentración residual de hidrocarburos en el suelo del trópico y consideraciones para la salud pública y protección al ganado. Interciencia. 33:476-482.

Alarcón, A., Davies, Jr. F.T., Autenrieth, R. L., Zuberer, D. A. 2008. Arbuscular mycorrhiza and Petroleum-degrading microorganisms enhance pythoremediation of petroleum-contaminated soil. International Journal of Phytorremediation. 10:251-263.

Baroutian, S., Aroua, M. K., Raman A.A.A., and Sulaiman, N. M. N. 2010. Viscosities and Densities of Binary and Ternary Blends of Palm Oil + Palm Biodiesel + Diesel Fuel at Different Temperatures. Journal of Chemestry Engineering and Data. 55:504–507.

Binet, P., Portal, M., And Leyva, C. 2001. Application of GC-MS to the study of anthracene disappearance in the rhizosphere of ryegrass. Organic Geochemestry. 32:217-222.

Biodisol. 2005. biodiesel ¿Qué es el biodiesel?, http://www.biodisol.com/biodiesel-que-es-el-biodiesel-definicion-de-biodiesel-materias-primas-mas-comunes/, recuperado en el año 2010.

Demello, J.A., Carmichael, C.A., Peacock, E.E., Nelson, R.K., Arey, J.S., Reddy, C.M. 2007. Biodegradation and environmental behaviour of biodiesel mixtures in the sea: an initial study. Marine Pollution Bulletin. 54:894–904.

Fangrui, M., and Hanna, M.A, 1999. Biodiesel production: a review. Bioresource Tech. 70:1-15.

Giovannetti, M., and Mosse, B. 1980. An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. New Phytologist. 84:489–499.

Hernández-Ortega, H.A., Alarcón, A., Ferrera-Cerrato, R., Zavaleta-Mancera, H.A., López-Delgado, H.A., Mendoza-López, M. R. 2012. Arbuscular mycorrhizal fungi on growth, nutrient status, and total antioxidant activity of *Melilotus albus* during phytoremediation of a diesel-contaminated substrate. Journal of Environmental Management. 95:319–324.

Hetrick, B.A.D., and Wilson. G.W.T. 1990. Relationship of native and introduced mycorrhizal fungi to mycorrhizal dependence of Andropogon gerardii and Koeleria pyranidata. Mycologia. 82:779-782.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., Barea, J.M. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils. 37:1-16.

Kavanova, M., Grimaldi, A.A., Lattanzi, F.A. and Schnyder, H. 2006. Phosphorus nutrition and mycorrhizaeffects on grass leaf growth. P status-and size-mediated effects on growth zone kinematics. Plant Cell and Environment. 29:511-520.

Kirk, J.L., Moutoglis, P., Klironomos, J., Lee, H., and Trevors, J.T. 2005. Toxicity of diesel fuel to germination, growth and colonization of Glomus intraradices in soil and in vitro transformed carrot root cultures. Plant and Soil. 270:23-30.

Knothe, G., Steidle, K.R. 2005. Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components. Fuel. 84:1059–1065.

Lapinskiene, A., Martinkus, P. and Rébzdaite, V. 2006. Eco toxicological studies of diesel and biodiesel fuels in areated soil. Environmental pollution. 142:432-437.

Leahy, J.G., and Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews. 54(3):305-315.

Leyva, C., Joner, E.J., Del Val C. and Haselwandter, K. 2002. Potential of arbuscular mycorrhizal fungi for bioremediation. In: (eds.) Gianinazzi, S., Schüepp, H., Barea, J.M. Haselwandter, K. Mycorrizal Technology in Agriculture. pp. 175-186.

Lutz, G., Chavarría, M., Arias, M.L., and Mata-Segreda, J.F. 2006. Microbial degradation of palm (Elaeis guineensis) biodiesel. Revista de Biología Tropical. 54(1) 59-63.

Makareviciene, V., and Janulis, P. 2003. Environmental effect of rapeseed oil ethyl ester. Renewable Energy. 28:2395–2403.

Malgorzata Hawrot, H.P. 2011. The influence of diesel of fuel on soil microbial biomass. Polish Journal of Environment. 20(2):497-501.

Martínez, S. 2008, Carburantes diesel y el futuro http://www.silviamar.com/Spanish/Documentos/diesel.htm recuperado en el año del 2010.

Pasqualino, J.C., Montané, D., and Salvadó, J. 2006. Synergic effects of biodiesel in the biodegradability of fossil-derived fuels. Biomass and Bioenergy. 30(10):874–879.

Sudova, R., Pavlikova, D., Macek, T., and Vosatka, M. 2007. The effect of EDDS chelate and inoculation with the arbuscular mycorrhizal fungus Glomus intraradices on the efficacy of lead phytoextraction by two tobacco clones. Applied Soil Ecology. 35 (1):163-173.

Volante A., Lingua, G., Cesaro, P., Cresta, A., Puppo, M., Ariati L. and Berta, G. 2005. Influence of three species of arbuscular mycorrhizal fungi on the persistence of aromatic hydrocarbons in contaminated substrates. Mycorrhiza. 16:43–50.

Vosátka, M., Rydlová, J., Sudová, R., and Vohní, M. 2006. Mycorrhizal fungi as helping agents in phytoremediation of degraded and contaminated soils. Phytoremediation Rhizoremediation. 9A:237-257.

Xiaolin, L., Chen, B., Feng, G. and Christie, P. 2002. Role of arbuscular mycorrhizal fungi in alleviation of Zn phytotoxicity and mineral nutrition of host plants. Acts of the 17th World Congress of Soil Science, Thailand, Symposium No. 42, paper no. 1649.

Submitted July 28, 2012– Accepted November 13, 2012
Revised received August 22, 2013