CARIBBEAN FOOD CROPS SOCIETY

48

Forty-eight Annual Meeting 2012

Playa del Carmen, Mexico
Vol. XLVIII

PROCEEDINGS

OF THE

48th ANNUAL MEETING

Caribbean Food Crops Society
48th Annual Meeting
May 20th – 26th 2012

Hotel Barceló Riviera Maya
Playa del Carmen, Mexico

"Education, Productivity, Rural Development, and Commercialization in the XXI Century"

Edited
by
Wanda I. Lugo and Wilfredo Colón

Published by the Caribbean Food Crops Society

ISSN 95-07-0410

Copies of this publication may be obtained from:

Secretariat, CFCS
P.O. Box 40108
San Juan, Puerto Rico 00940

or from:

CFCS Treasurer
Agricultural Experiment Station
Botanical Garden South
1193 Guayacán Street
San Juan, Puerto Rico 00926-1118

Proceedings of the Caribbean Food Crops Society. 48:147-152. 2012

INVESTIGATION OF THE EFFECTS OF MYCORRHIZAL FUNGI ON CADMIUM ACCUMULATION IN CACAO

Gideon Ramtahal[1], Ivan Chang Yen[1], Deodath Seegobin[4], Isaac Bekele[2], Frances Bekele[3], Lawrence Wilson[2], and Lisa Harrynanan[4], [1]Department of Chemistry, University of the West Indies, Trinidad & Tobago, [2]Department of Food Production, University of the West Indies, St. Augustine, Trinidad & Tobago, [3]Cocoa Research Unit, University of the West Indies, St. Augustine, Trinidad & Tobago, [4]Ministry of Food Production, Central Experiment Station, Centeno, Trinidad & Tobago

ABSTRACT: Currently, increasing emphasis is being placed on the contamination of cacao beans by heavy metals, particularly cadmium (Cd). Since the primary source of Cd contamination in cacao has been attributed to metal-contaminated soils, it is becoming crucial to develop strategies to minimize its uptake. Recent studies have shown that mycorrhiza could contribute to the immobilization of Cd in soils, thereby decreasing Cd toxicity to plants. A preliminary pot trial study was designed to determine whether mycorrhizal fungi in the form of bio-fertilizers could be used as a method of soil remediation, to inhibit Cd uptake by cacao plants. Roots of cuttings of a single variety and age of cacao were grown with and without a commercial bio-fertilizer, in a Cd-spiked, sterilized soil in pots, in randomized blocks in a greenhouse. The experiment was conducted over a period of four months, with replicates of plants being sacrificed and analyzed monthly, to determine the Cd contents of leaf and stem samples. Microscopic examinations were also done to detect mycorrhizal infection of roots of bio-fertilizer treated and control plants. Paired data analysis demonstrated that cacao plants treated with the mycorrhizal bio-fertilizer accumulated significantly higher ($p<0.05$) levels of Cd in both leaves and stems than non-mycorrhizal-treated plants for the period of the study. The higher Cd concentrations found for the mycorrhizal treatment may have resulted from an increase of Cd absorption into the plants by soil mycelia, known to spread several centimeters around the plant roots. This investigation, while preliminary, indicates that the variety of cacao plant used with the commercial mycorrhiza used accumulates significantly higher levels of Cd in their leaves and stems than non-mycorrhizal plants.

Keywords**:** Mycorrhiza, bio-fertilizer, cadmium, cacao

INTRODUCTION

There are increasing concerns over the intake of excessive Cd in contaminated food crops, which may be toxic to animals, including humans (Welch and Norvell, 1999). It can cause abdominal cramps, headaches, vomiting and diarrhea (ATSDR, 1993) and in severe cases, kidney tubule damage, anemia and severe loss of bone minerals (Reilly, 2002). The levels of heavy metals, particularly Cd in cacao beans, have been of concern for many years (BCCCA, 1996). Concentrations of Cd in cocoa powders and liquors made from cacao beans and used in chocolate production are gaining public significance (Mounicou et al., 2002, 2003; Dahiya et al., 2005; Jalbani et al., 2009). International legislative bodies, as well as chocolate manufacturing countries have introduced new Cd regulations for the protection of the health of their consumers (FSA, 2009; Ducos et al., 2010; ICCO, 2010).

An investigation by Lee and Low (1985) reported high levels of Cd in raw cacao beans. It was speculated that Cd present in cacao beans is due to uptake by cacao plants from Cd contaminated soils (Fauziah et al., 2001; Mounicou et al., 2003; Beckett, 2009). Such soil contamination can be treated using various technologies, including physical (Evanko and Dzombak, 1997) and chemical treatments (Mahabadi et al., 2007). However, low-cost and low-impact biological techniques such as the application of microorganisms and microbial products have been receiving increasing attention for soil remediation (Leyval et al., 2002; Doumett et al., 2008; Karimi et al., 2011). Soil microorganisms such as mycorrhizal fungi have been shown to restrict or enhance heavy metal uptake (Joner et al., 2000; Janoušková et al., 2005; Hildebrandt et al., 2007). Mycorrhizal hyphal mats act as barriers against metal transport across root cell walls, thereby reducing metal uptake by plants (Gaur and Adholeya, 2004; Andrade and Silveira, 2008). In addition to sequestration of heavy metals in the vacuole of the fungal cells, extracellular heavy metal chelation by secreted mycorrhizal exudates appear to provide barriers to heavy metal absorption by plant roots (Göhre and Paszkowski, 2006; Muthukumar and Bagyaraj, 2010). Recent studies have shown that mycorrhiza could also contribute to the immobilization of Cd in soils, thereby decreasing Cd toxicity to plants (Janoušková et al., 2005; 2006). As a result, it is possible that this approach can also apply to cacao plants, minimizing or preventing Cd uptake throughout its tissues.

Thus the main objective of this research is to determine whether mycorrhizal fungi could be used as an alternative method of soil remediation to inhibit Cd uptake in cacao.

MATERIALS AND METHODS

For this study a pot trial was designed to assess the effectiveness of mycorrhizal fungi in the form of bio-fertilizers on the minimization of Cd uptake in cocoa.

Rooted cacao cuttings of a single variety and stage of maturity (approx. one month) were obtained. At the start of the trial, five samples of cuttings were taken for analysis to determine their initial levels of Cd, in addition to the mycorrhizal colonization of roots of plants. A commercial mycorrhizal preparation (MycoApply® Micronized Endo bio-fertilizer, Oregon, USA) was used to supply mycorrhiza to the roots of cacao cuttings. Mycorrhizal non-inoculated (Control, Treatment A) and inoculated (Treatment B) rooted cacao cuttings were then planted in sterilized Cd-spiked soil (10 µg/g) in polyethylene bags.

The experiment was set up in a completely randomized design, with five replications per treatment for a period of four months in a naturally illuminated greenhouse, where plants were watered periodically. Each plant was placed on a heavy-duty polystyrene plate, to minimize cross-contamination from excess irrigation water on the floor. Each month, plants were sacrificed for analysis, where leaf and stem Cd levels were determined by flame atomic absorption spectrometry (FAAS) on a Varian SpectrAA 880 (Australia), and the mycorrhizal infection of bio-fertilizer-treated roots through a clearing and staining procedure, as outlined by Koske and Gemma (1989).

The differences in Cd levels between the non-inoculated and inoculated mycorrhizal treatments were evaluated using pair data analysis (p 0.05). Statistical software Minitab (version 16), 2010 was applied.

RESULTS AND DISCUSSION

Cadmium Accumulation in Mycorrhizal vs Non-Mycorrhizal-Treated Cacao Plants

Tables 1 and 2 illustrate the mean Cd concentration of leaves and stems per gram of plant dry weight (DW), respectively, for treatments A and B, during each month of the experimental period.

Table 1. Mean Leaf Cd Concentrations per gram of Plant DW for Treatments A and B over four months.

Treatment	Mean Cd (µg/g)/g in leaves (DW) ± SD for each month			
	1	2	3	4
A	0.86±0.13	5.45±0.62	12.15±0.65	13.16±1.20
B	1.43±0.32	8.92±1.49	15.40±0.82	16.41±1.72

Table 2. Mean Stem Cd Concentrations per gram of Plant DW for Treatments A and B over four months.

Treatment	Mean Cd (µg/g)/g Stem DW ± SD for each Month			
	1	2	3	4
A	2.06±0.39	6.73±0.19	9.36±0.54	7.37±0.56
B	3.77±0.86	12.29±0.25	12.30±2.34	9.57±0.41

Paired data analysis demonstrated that cacao plants treated with the mycorrhizal bio-fertilizer accumulated significantly higher ($p<0.05$) levels of Cd in both leaves and stems than non-mycorrhizal plants for the period of the investigation. These results are contrary to those reported to show decreases in Cd uptake by mycorrhiza (Janoušková et al., 2005; Janoušková et al., 2006). However, plants grown with mycorrhiza may show enhanced Cd uptake and root-to-shoot transport (Vogel-Mikus et al., 2005; Göhre and Paszkowski, 2006), as found in our preliminary study.

Even though this difference in Cd uptake can be attributed to the presence of mycorrhiza in the commercial mycorrhizal preparation, results from mycological analyses over the four-month study period showed that none of the roots sampled were infected. It is possible that low infection rates of the roots by the mycorrhiza may not have allowed their true effects to be realized within the four months of our trial. However, intra-radical mycorrhizal colonization may be reduced by high heavy metal levels in soils (Liao et al., 2003), and high soil Cd can reduce the mycorrhizal infections of roots in such soils (Tullio et al., 2003; Andrade and Silveira, 2008). It is thus possible that the soil Cd concentration used in this experiment may have had a similar affect on colonization of mycorrhiza in the roots of the cacao plant.

It must also be noted that the mycelia of mycorrhiza do not propagate only in the root cortex of the host plant, but also in the soil around the root (Neumann and George, 2009). Thus, the significantly higher Cd concentrations found for the mycorrhizal treatment may have resulted from

an increase of Cd absorption into the plants by soil mycelia, known to spread several centimeters around the plant roots (Gowariker et al., 2009).

CONCLUSION

This investigation, while preliminary, indicates that the variety of cacao plants treated with the commercial mycorrhiza accumulated significantly higher levels of Cd in the leaves and stems than non-treated plants. Consequently, mycorrhiza in the form of bio-fertilizers may not be effective treatment to minimize Cd accumulation in cacao plants. However, it is possible that other mycorriza and cacao plants may behave differently, as found in other plants and should be further investigated.

ACKNOWLEDGEMENTS

The authors are grateful for the La Reunion Cocoa Research Station, East Trinidad, for provision of the cacao plants and use of their greenhouse; the Bio-Fertilizer Laboratory of the Ministry of Food Production Land and Marine Affairs for facilitating the experiment; and the University of the West Indies, St. Augustine for infra-structural and administrative support for the project.

REFERENCES

Agency for Toxic *Substances and Disease Registry (ATSDR). 1993. Toxicological Profile for Cadmium*; U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia.

Andrade, S.A.L., and A.P.D. Silveria,. 2008. Mycorrhiza Influence on Maize Development under Cd Stress and P Supply. *Braz. J. Plant Physiol. 20*, 39-50.

Beckett S.T. 2009. Industrial Chocolate Manufacture and Use; 3rd ed.; Wiley-Blackwell: Iowa.

BCCCA (Biscuit, Cake, Chocolate and Confectionery Alliance). 1996. *Cocoa Beans: Chocolate Manufacturers' Quality Requirements*; 4th ed.; Association. Cocoa Research: UK

Dahiya, S., R. Karpe, A.G. Hegde, and R.M. Sharma. 2005. Lead, Cadmium and Nickel in Chocolates and Candies from Suburban Areas of Mumbai, India. *J. Food Compos. Anal. 18*, 517–522.

Doumett, S., L. Lamperi, L. Checchini, E. Azzarello, S. Mugnai, S. Mancuso, G. Petruzzelli, and M. Del Bubba. 2008. Heavy Metal Distribution between Contaminated Soil and Paulownia Tomentosa, in a Pilot-Scale Assisted Phytoremediation Study: Influence of Different Complexing Agents. *Chemosphere. 72* (10), 1481-1490.

Ducos S., M. Hamester, and M. Godula. 2010. ICP-MS for Detecting Heavy Metals in Foodstuffs: The Technology can Analyze 50 Samples in an Hour. http://www.foodquality.com/details/article/809781/ICPMS_for_Detecting_Heavy_Metals_in_Foodstuffs_.html?tzcheck=1

Evanko, C.R., and D.A. Dzombak. 1997. Remediation of Metal-Contaminated Soil and Groundwater; *GWRTAC Technology Evaluation Report, TE-97-01*, Ground-Water Remediation Technologies Analysis Center, Pittsburgh, PA.

Fauziah C.I., A.R. Zaharah, S. Zauyah, and A.R. Anuar. 2001. Proposed Soil Heavy Metal Reference Values to be used for Site Assessment. Proceedings of Brownfield 2001: *First National Conference on Contaminated Land, Petaling Jaya:* The Institution of Engineers, Malaysia.

FSA (Food Standards Agency). 2009. Chemical Contaminants Interested Parties Letter: Environmental Contaminants (Inorganic contaminants). http://www.food.gov.uk/multimedia/pdfs/envirocontam 0703.pdf

Göhre, V., and U. Paszkowski. 2006. Contribution of the Arbuscular Mycorrhizal Symbiosis to Heavy Metal Phytoremediation. *Planta 223* (6), 1115-1122.

Gowariker,V., V. N. Krishnamurthy, S. Gowariker, M. Dhanorkar, I. Paranjape and N. Borlaug. 2009. *The Fertilizer Encyclopedia*. Hoboken: John Wiley & Sons.

Hildebrandt, U., M. Regvar, and H. Bothe. 2007. Arbuscular Mycorrhiza and Heavy Metal Tolerance.*Phytochemistry 68* (1), 139-146.

ICCO. (International Cocoa Organization). 2010. What is the Situation Regarding Cadmium, Lead and Copper in Cocoa Beans? http://www.internationalcocoaorganisation.net/\ questions/cadmium.htm

Jalbani, N., T.G. Kazi, H.I. Afridi, and M.B. Arain. 2009. Determination of Toxic Metals in Different Brands of Chocolates and Candies, Marketed in Pakistan. *Pak. J. Anal. Environ. Chem. 10*, (1-2), 48-52.

Janoušková, M., D. Pavlíková, T. Macek, and M. Vosátka. 2005. Arbuscular Mycorrhiza Decreases Cadmium Phytoextraction by Transgenic Tobacco with Inserted Metallothionein. *Plant Soil. 272* (1), 29-40.

Janoušková, M., D. Pavlikova, and M. Vosatka. 2006. Potential Contribution of Arbuscular Mycorrhiza to Cadmium Immobilisation in Soil. *Chemosphere 65* (11), 1959-1965.

Joner, E., R. Briones, and C. Leyval. 2000. Metal-Binding Capacity of Arbuscular Mycorrhizal Mycelium. *Plant Soil.226* (2), 227-234.

Karimi, A., H. Khodaverdilou, M. Sepehri, and M.H. Rasouli-Sadaghiani. 2011.Arbuscular Mycorrhizal Fungi and Heavy Metal-Contaminated Soils. *Afr. J. Microbiol. Res. 5* (13), 1571-1576.

Koske, R. E., and Gemma, J. N. 1989. A Modified Procedure for Staining Roots to Detect VA mycorrhizae. *Mycol. Res. 92* (4), 486-488.

Lee C.K., and K.S. Low. 1985. Determination of Cadmium, Lead, Copper and Arsenic in Cocoa, Semi-Finished and Finished Chocolate Products. *Pertanika.* 8(2), 243-248.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of Heavy Metal Pollution on Mycorrhizal Colonization and Function: Physiological, Ecological and Applied Aspects. *Mycorrhiza. 7*, 139-153.

Liao J.P., X.G. Lin, Z.H. Cao, Y.Q. Shi, and M.H. Wong. 2003. Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. *Chemosphere* 50:847-853. 50(6):847-53.

Mahabadi, A. A., M.A. Hajabbasi, H. Khademi, and H. Kazemian. 2007. Soil Cadmium Stabilization using an Iranian Natural Zeolite. *Geoderma 137* (3-4), 388-393.

Mounicou, S., J. Szpunar, R. Lobinski, D. Andrey, and C.J. Blake. 2002. Bioavailability of Cadmium and Lead in Cocoa: Comparison of Extraction Procedures Prior to Size-Exclusion Fast-Flow Liquid Chromatography with Inductively Coupled Plasma Mass Spectrometric Detection (SEC-ICP-MS). *J. Anal. Atom. Spec. 17*, 880-886.

Mounicou, S., J. Szpunar, D. Andrey, C. Blake, and R. Lobinski. 2003. Concentrations and Bioavailability of Cadmium and Lead in Cocoa Powder and Related Products. *Food Addit.Contam. 20* (4), 343-52.

Muthukumar, T., and D.J. Bagyaraj. 2010. Use of Arbuscular Mycorrhizal Fungi in Phytoremediation of Heavy Metal Contaminated Soils. In *Proceedings of The National*

Academy of Sciences India Section Biological Sciences, Allahabad: National Academy of Science, India, pp 103-12.

Neuman, E. and E. George. 2009. The effect of arbuscular mycorrhizal root colonization on growth and nutrient uptake of two different cowpea (*Vigna unguiculata* [L.] Walp.) genotypes exposed to drought stress. *Emir. J. Food Agric.* 21 (2): 01-17

Reilly, C. 2002. Transition metals. In *Metal Contamination of Food: Its Significance for Food Quality and Human Health;* 3rd ed.; Blackwell Publishing: U.K., 8, pp 160-165.

Tullio, M., F. Pierandrei, A. Salerno and E. Rea. 2003. Tolerance to cadmium of vesicular arbuscular mycorrhizae spores isolated from a cadmium-polluted and unpolluted soil. *Biol Fertil. Soils.* 37, 211–214.

Vogel-Mikus K., D. Drobne and M. Regvar. 2005. Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environ. Pollut.* 133: 233-242.

Welch, R.M. and W.A. Norvell. 1999. Mechanisms of Cadmium Uptake, Translocation and Deposition in Plants. In *Cadmium in Soils and Plants;* Singh, B., McLaughlin, M. J., Eds.; Kluwer Academic: Netherlands, 6, pp 125-126.