Mechanisms of Phytoremediatory Effect of Ocimum basilicum L. and its Rhizosphere Exposed to Different Concentrations...

Conference Paper · January 2013

DOI: 10.3303/CET1334013

CITATIONS
2

READS
36

3 authors, including:

Sae Muñiz-Hernandez
Instituto Nacional de Cancerología - Mexico
29 PUBLICATIONS **255** CITATIONS
SEE PROFILE

Jesus Velazquez
Universidad Autónoma de Nayarit
21 PUBLICATIONS **125** CITATIONS
SEE PROFILE

CHEMICAL ENGINEERING TRANSACTIONS
VOL. 34, 2013
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Guest Editors: Neven Duić, Petar Sabev Varbanov

ISBN 978-88-95608-25-9; **ISSN** 1974-9791
DOI: 10.3303/CET1334013

Mechanisms of Phytoremediatory Effect of *Ocimum basilicum* L. and its Rhizosphere Exposed to Different Concentrations of the Organochlorine Pesticide Endosulfan

Maricela Ramirez-Sandoval[a], Saé Muñiz-Hernández[b], Jesús Bernardino Velázquez-Fernández*[,a]

[a]CEMIC03, Posgrado CBAP, Universidad Autónoma de Nayarit
[b]Subdirección de Investigación Básica, Instituto Nacional de Cancerología, Secretaría de Salud México (SS), Mexico City, Mexico
jesusbvf@gmail.com

Endosulfan is a toxic organochlorine pesticide, environmentally persistent, banned and restricted in many countries. For phytoremediation purposes, we have previously observed that the presence of *Ocimum basilicum* decreased the concentration of endosulfan in experimentally-polluted soil by 37% after 30 days. To study the possible mechanism, we evaluated whether endosulfan could affect 1) the activity of glutathione S transferase (GST) of O. basilicum and 2) microorganisms from rhizosphere. Young plants were added to experimentally-polluted soil with endosulfan. Rhizosphere microorganisms were exposed to several concentrations of endosulfan and cultured in Luria Bertani (LB) broth or agar, their growth was determined by triplicate either spectrophotometrically or by plate count. After exposure to the pesticide endosulfan in *O. basilicum* and its rhizosphere, three effects were observed: 1) In LB broth, optimal growth of microorganisms was observed at 72 and 48 h after exposure to endosulfan 2.4 and 3.4 mg/10 mL. 2) Optimal growth of microorganisms in LB agar was observed at 0.3 and 2.4 mg/10 mL. 3) GST was increased after exposure to these pesticides over its control. These observations suggest that phytostimulaton and phytotransformation could be involved as possible mechanisms of the phytoremediatory effect of *O. basilicum.*

1. Introduction

Organochlorine pesticides are considered persistent in the environment (Calva and Torres, 1998). They are toxic to animal species including man and can be stored in adipose tissue. Endosulfan, an organochlorine pesticide, is widely used for agricultural purposes (Harikrishnan and Usha, 2004). Nevertheless is freely sold to the public (González-Arias et al., 2010). However, given its environmental mobility, endosulfan reaches and affects non-target organisms (ATSDR, 2004). Phytoremediation is a bioremediation technology that uses plants and it has been shown to be useful for pesticide pollution (López-Martínez et al., 2005, EPA, 2012). The detoxification of organic compounds by phytoremediation, is carried out via the metabolism of plants or the rhizosphere - the narrow zone of soil around the roots of plants- (Walker et al., 2003). Rhizosphere and plant metabolism improves phytorremediation: plants stimulates, by its root exudates (López-Martínez et al., 2005), the growth of soil microorganisms capable of degrading xenobiotic organic compounds (Kuiper et al., 2001); thus, achieving the objective of phytoremediation: reducing the environmental effects of pollution. In the laboratory, it was observed that the presence of Ocimum basilicum L. decreased the concentration of endosulfan in soil by 37 % (Ramírez-Sandoval et al., 2011). The aim of the present work was to evaluate the effect of O. basilicum presence and Endosulfan on rhizosphere microbial community and the GST activity of the plant after exposition to endosulfan via experimentally polluted soil.

Please cite this article as: Ramirez-Sandoval M.,Muñiz-Hernández S.,Velázquez-Fernández J. B.,2013,Mechanisms of phytoremediatory effect of ocimum basilicum l. and its rhizosphere exposed to different concentrations of the organochlorine pesticide endosulfan, Chemical Engineering Transactions, 34, 73-78 DOI:10.3303/CET1334013

2. Material and methods

2.1 Exposure to endosulfan

O. basilicum L. plants were exposed to endosulfan as previously reported (Ramírez-Sandoval et al., 2011). Soil were added with different endosulfan concentrations: 10, 100 or 1.000 mg endosulfan/kg of soil. Endosulfan amount were calculated using the concentration of the active compound (35%) of the commercial product endosulfan (Thiodan ® BAYER). Young plants were used (50 days old with less than 8 cm in length) and grown in a greenhouse under normal growth conditions.

2.2 Isolation and quantification of microbiota in the rhizosphere of Ocimum basilicum L

This method is based on (Camacho et al., 2009). To identify bacteria associated with rhizosphere of O. basilicum L., soil samples were added with sterile water (by triplicate). After homogenization, samples were diluted 100-times and inoculated into Luria-Bertani (LB) broth (five replicates) with different concentrations of technical-grade endosulfan (0.3, 1, 1.7, 2.4, 3.4, 9, 17 and 34 mg endosulfan/mL of broth). Growth was evaluated at different times (24, 48, 72 and 96 h). Duplication time was calculated from equations for biomass increase in time described elsewhere. After culture in broth, 1 μL of each culture was placed in LB agar plates and colony forming units (CFU) were counted after 48 h.

2.3 Determination of GST activity

GST extraction from plant was performed according to (Scarponi et al., 2006) modified for O. basilicum L. Plants were previously exposed to 10, 100 and 1000 mg endosulfan / kg of soil.

The GST activity was measured in O. basilicum leaves, roots and stems. Enzyme activity was measured as the absorbance increase of the glutathione conjugate with 1-chloro-2,4-dinitrobenzene at 340 nm during three minutes. To calculate the enzyme activity, molecular extinction coefficient of product (9.600 M^{-1} cm^{-1}) (Habig and Jakoby, 1981) was used. Enzyme activity was expressed as nmoles of product / (min • mg total protein extract).

2.4 Statistics

The results were expressed as means ± standard deviation (SD). One-way ANOVA-Bonferroni was applied and a $P < 0.05$ was used for significance. The STATA program, version 8.0 (Stata Corp., College Station, TX) was used for all statistical calculations.

3. Results

3.1 Growth of microorganisms in culture media

When microorganisms from rhizosphere were placed in LB broth, the highest optical density was reached at 48 hours. Optical density values were lower before and after that time (Figure 1). Based on data, the duplication time was calculated to be 35 min.When the microbiota from the rhizosphere of *O. basilicum* L. was grown on agar with endosulfan, the highest optical density growth was observed at 72 and 48 hr for 2.4 and 3.4 mg endosulfan/mL of broth (Figure 2). Moreover, the shape and colour of colonies differ from those cultured in agar without endosulfan suggesting that other microorganisms grew in the presence of endosulfan. Consistently, after transference to agar plates and incubation during 48 hr, the maximum CFU number was achieved from those samples at 2.4 mg endosulfan/mL of medium (Figure 3). Interestingly, at least three types of colonies were observed. Identification of bacteria is still being carried out in our laboratory.

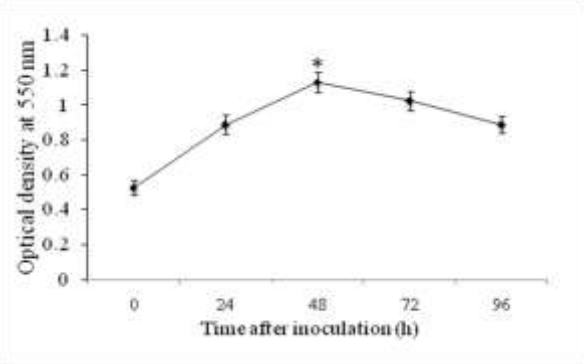

*Figure 1: Growth of microbiota isolated from rhizosphere of O. basilicum exposed to endosulfan. Microbiota was diluted 10^{-2} prior to inoculation in LB broth. * Statistically significant difference with time 0. (P<0.05, ANOVA-Bonferroni, n=3)*

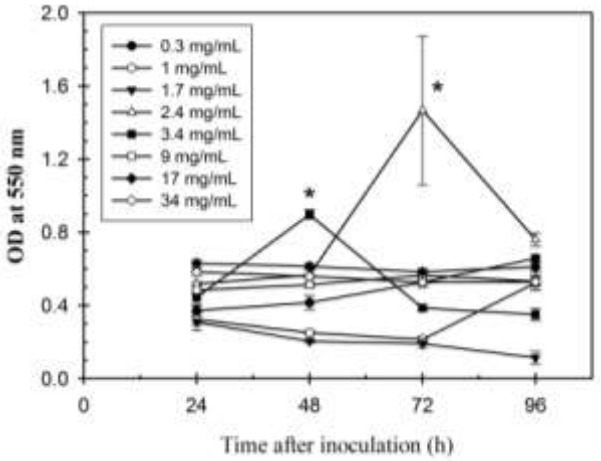

*Figure 2: Growth of bacteria from rhizosphere of O. basilicum exposed to endosulfan at different concentrations (as placed in figure) inoculated in LB broth. * P <0.05, ANOVA (n=3).*

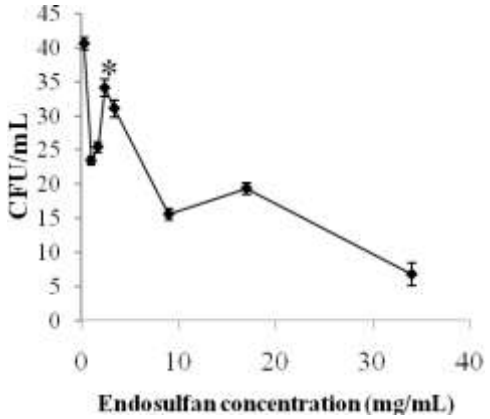

*Figure 3: Colony forming units (CFU) from rhizosphere of O. basilicum exposed to different concentrations of endosulfan after 48 hrs of growth in LB agar. * P < 0.05, ANOVA (n=2)*

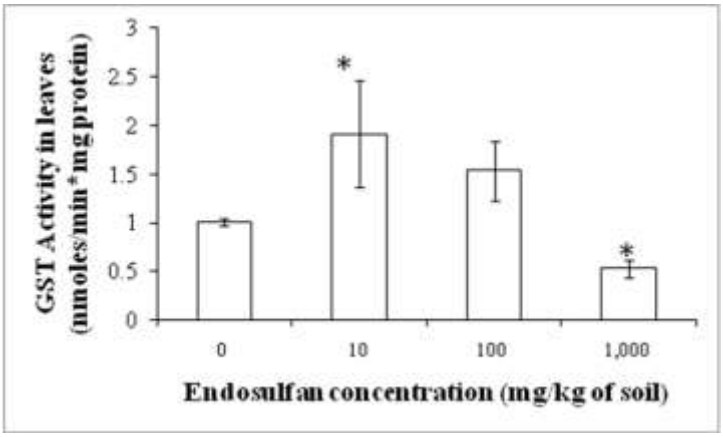

Figure 4: GST activity in leaves of O. basilicum exposed to endosulfan as measured P < 0.05 respect to control, ANOVA (n=3).*

3.2 GST activity

GST activity varies according with each part of the plant and endosulfan concentration in soil. In leaves and roots of plants, significant increases in GST activity were observed compared to control when exposed to endosulfan at 10 mg/kg of soil (Figures 4 and 5). Although, the GST activity decreased significantly in leaves from plants exposed to endosulfan 1,000 mg/kg of soil (Figure 4). No significant differences were found in GST activity from stems of *O. basilicum* (Figure 6).

4. Discussion

In the last decades several efforts have been made to remediate environmental pollution. The applied technologies have used some chemical or biological strategies. In CET series, a previous volume has been dedicated to remediation efforts (BOSICON, 2012). In the case of pesticide remediation, most of studies are focused on the use of microorganisms (Velázquez-Fernández et al., 2012). Although, in the case of agricultural fields or extensive areas, the use of plants, i.e. phytoremediation has shown several advantages such as sustainability, low cost and aesthetics among others. Because of this, EPA has recommended the use of phytoremediation (EPA, 2012). Nevertheless, studies on phytoremediation of insecticde pollutants are scarce.

It is generally accepted that phytoremediation is best when plant and rhizosphere collaborate (Kuiper et al., 2001) in remediating pollutant; having a pair able to biotransform endosulfan could improve our knowledge and capabilities for bioremediation. Moreover, it is advisable that any bioremediation strategy should be "tailored" for each zone, i.e., selection of the bioremediator organism should take into account several issues: 1) organism should be able to reduce pollutant concentration in soil, air or water or at least to decrease the undesirable effects of pollutants at times as short as possible, 2) organism should endure the pollutant concentration to which it will be exposed, 3) bioaugmentation are to be avoided: new organisms should not be introduced within the region, thus, the use of indigenous or domesticated organisms are preferable in order to alter the ecosystem as little as possible.

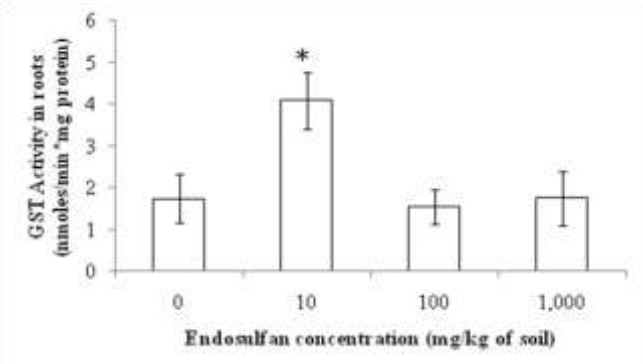

Figure 5. GST activity in roots of O. basilicum exposed to endosulfan as measured P < 0.05 respect to control, ANOVA (n=3).*

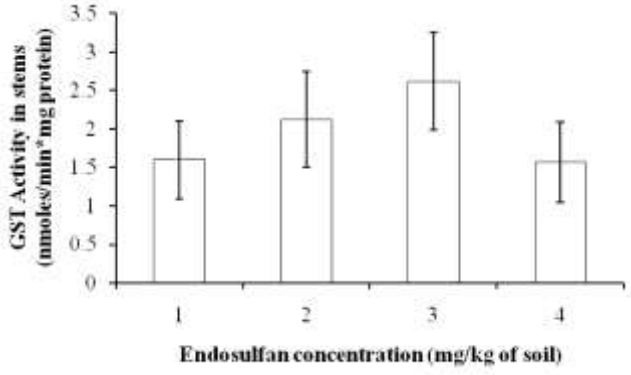

Figure 6. GST activity in stems of O. basilicum exposed to endosulfan as measured P < 0.05 respect to control, ANOVA (n=3)*

Endosulfan is still being commercialised in our zone (González-Arias et al., 2010) and it has been found in environment (Rojas, 1998). In addition, Endosulfan has been added to the list of persistent organic pollutants of Stockholm convention (Stockholm Convention, 2011). The aim of this work is to select plant-rhizosphere organism pair to bioremediate endosulfan.

Previously, O. basilicum has been shown to decrease the concentration of endosulfan by 37 % in experimentally polluted soil at 30 days (Ramírez-Sandoval et al., 2011). This phytoremediatory effect of O. basilicum could be explained by biotransformation of the plant itself or those microorganisms from rhizosphere that could be stimulated by plant root exudation. Whichever the mechanism is, it is worth, for bioremediation purposes, to isolate microorganisms able to biotransform pollutants. In this work, we describe the isolation of more than three microorganisms able to grow at high concentrations of

endosulfan (Figures 2 and 3). They and the plant can live and grow in endosulfan-polluted soil. To characterize the symbiosis between plant and rhizosphere are beyond the scope of the present work. Still, the results suggest that bacteria can well survive and use endosulfan at 2.4-3.4 mg/mL of culture media. The fact that concentrations lower than 2.4 mg/mL and greater than 3.4 mg/mL mg have lower growth could be explained by the combined effect of a) the cometabolism of the components of endosulfan and b) the toxic effects of the same components at higher concentrations. All this data suggest that rhizosphere of O. Basilicum may collaborate to bioremediate endosulfan. Identification of bacteria and identification of genes/enzymes are being carried out in our laboratory at present. Nevertheless, kinetic characterization and identification of parameters that could affect survival/growth of rhizosphere bacteria is needed in order to prospect bioremediation proficiency, i.e. if bacteria cannot endure or can survive only in the first hours of exposition, it will be necessary to innoculate them every now and then. Keeping a proper number of bacteria would enable us to bioremediate efficiently and perhaps shorten application times.

GST has been shown to be involved in plant defence against pollutants. Even more, conjugates of pollutants with glutathione could lead to their bioaccumulation in plant vacuoles (Dixon et al., 2002) and there is no doubt on participation of GST activity in plant defence to pollutants (Scarponi et al., 2006). Thus, to evaluate GST activity would shed light on plant response to GST. In the present work we found that GST activity responds differently in leaves, roots and stems. In stems, no variation with endosulfan concentration was observed (Figure 6). In leaves, an increase of 90 % was observed at 10 mg endosulfan/kg of soil, but a decrease of 50 % was observed at 1,000 mg endosulfan/kg of soil (Figure 4). In roots, an increase of 134 % was observed at 10 mg endosulfan/kg of soil. This variation in GST activity clearly suggests that each part of the plant responds differently to pollution. Furthermore, the increase observed in root GST activity is in agreement with the importance of root in plant defence as it has been pointed out by Schröder et al., (2007) in barley. The possibility that plant bioaccumulates endosulfan into plant vacuoles is being investigated in our lab nowadays.

5. Conclusion

It is important to have the certainty on tolerance of organisms which are candidate for bioremediation of pollutants. Also, to know the ability of plant itself or rhizosphere itself as biodegraders -organisms able to biotransform- pollutants could also help developing strategies to improve phytoremediation process. For bioremediation purposes, further research is needed to show biodegradation capabilities of plant and its rhizosphere taken together or separately. To study enzymatic biodegradation constitutes a helpful tool for research on bioremediation. Moreover, because of the adaptative responses, it is likely to find biodegraders among those resilient organisms. Thus, to know mechanisms of resilience would shed light on biodegradation process, and that could be a start point for further research on bioremediation.

6. References

Calva L.G., Torres M.R., 1998. Organochlorine pesticides (in Spanish), Lab. de ecosistemas costeros, Dpto. de Hidrobiología, D.C.B.S. UAM-I, Mexico.

Harikrishnan V.R., Usha S., 2004. Endosulfan, informative sheet and frequently asked questions. IPEN Pesticide Working Group Project-2004**.** Thanal. Kerala, India. <www.Thanal.Org>, Accesed 07/01/ 2010.

González-Arias C.A., Robledo-Marenco M.L., Medina-Díaz I.M., Velázquez-Fernández J.B., Girón-Pérez M.I., Quintanilla-Vega B., Ostrosky-Wegman P., Pérez-Herrera N.E., Rojas-García A.E., 2010. Pattern of Pesticides Use and Sale in Nayarit (in Spanish), Mexico. Rev. Int. Contam. Amb. 26(3), 221-228.

ATSDR (Agency for Toxic Substances and Disease Registry), 2004. Toxicological profile of Endosulfan, GA: Center for Disease control. 2004. <www.atsdr.cdc.gov/es>, Accesed 20/07/2010.

López-Martínez S., Gallegos-Martínez M.E., Pérez Flores L.J., Gutiérrez Rojas M., 2005. Mechanisms of phytoremediation in polluted soils with organic xenobiotic (in Spanish). *Rev Int Contam Amb.*, Vol. 21, No. 2, pp 91-100.

EPA (Environmental Protection Agency), 2012. Phytoremediation: a citizen's guide to phytoremediation. EPA 542-F-12-016. <www.epa.gov/tio/ download/citizens/a_citizens_guide_to_ phytoremediation.pdf>, Accesed 10/04/ 2013.

Walker T.S., Pal B.H., Grotewold E., Vivanco J.M., 2003. Root Exudation and Rhizosphere Biology. Plant Physiol. 132, 44-51.

Kuiper I., Bloemberg G.V., Lugtenberg J.J., 2001. Selection of a Plant-Bacterium Pair as a Novel Tool for Rhizostimulation of Polycyclic Aromatic Hydrocarbon-Degrading Bacteria. Mol. Plant-Microbe Interact., 14(10), 1197-1205.

Ramírez-Sandoval M., Melchor-Partida G.N., Muñiz-Hernández S., Girón-Pérez M.I., Rojas-García A.E., Medina-Díaz M., Robledo-Marenco M.L., Velázquez-Fernández J.B., 2011. Phytoremediatory effect and growth of two species of *Ocimum* in endosulfan polluted soil. J Hazard Material., 192, 388–392.

Camacho A., Giles M., Ortegón A., Palao M., Serrano B., Velázquez O., 2009. Techniques of Microbiological Analysis in Foods (in Spanish), 2ª ed. Facultad de Química, UNAM. Mexico.

Scarponi L., Quagliarini E., Del Buono D., 2006. Induction of wheat and maize glutathione *S*-transferase by some herbicide safeners and their effect on enzyme activity against butachlor and terbuthylazine. Pest. Manag. Sci., 62, 927–932.

Habig W. H., Jakoby W.B., 1981. Assays for differentiation of glutathione S- transferases, Methods Enzymol. 77, 398-405.

Rojas G.A.E., 1998. Contribution to environmental diagnose of estuaries from San Blas, Nayarit. *Grad. Thesis* UACQBF, Universidad Autónoma de Nayarit, México. (in Spanish).

Stockholm convention, 2011. Available on internet: chm.pops.int/Convention/ThePOPs/tabid/673/Default.aspx. Accesed 10 February , 2012.

Dixon D., Lapthorn A., Edwards R., 2002. Plant glutathione transferases. Genome Biol., 3(3), 1-10.

Schröder P., Scheer C.E., Diekmann F., Stampfl A., 2007. How plants cope with foreign compounds: Traslocation of xenobiotic glutathione conjugates in roots of barley (*Hordeum vulgare*). Env. Sci. Pollut. Res., 14(2), 114-122.

BOSICON, 2012. 3rd International Conference on Contaminated Sites Remediation. Edit. Merli C Chemical Engineering Transactions, 28.

Velázquez-Fernández J.B., Martínez-Rizo A.B., Ramírez-Sandoval M, Domíguez-Ojeda D., 2012. Biodegradation and Bioremediation of Organic Pesticides. Chapter 12. in Pesticides-Recent Trends in Pesticide Residue Assay. Intech. Croatia, 2012. DOI: dx.doi.org/10.5772/46845.