Trichoderma Harzianum: An Effective and Eco-friendly Biocontrol Agent for Degradation of Pesticide Endosulphan

Priya Ayyar and Shivaji Kamble
Research Scholar, Professor
Department of Botany, Shivaji University, Kolhapur, India

Abstract:

Pesticide uses are widely documented for controlling various pests that attenuate the agricultural productivity. However extensive use of pesticides is considered as one of the most important agent in polluting the environment. Microbial degradation could be one of the efficient and eco-friendly methods for removing the environmental contaminants. *Trichoderma* species are the fungi which belong to the family Hypocreaceae is one of the well-studied fungus know to control other genera fungal infection in plants caused by various pathogenic necrotrophs. Here in this paper we have documented how *Trichoderma harzianum* KRL-AG2 strain is capable of degrading pesticide endosulphan and this could practically be possible for suing in open field. This method of detoxification could be proved to be one of the efficient methods in bioremediation process.

Keywords: Pesticide, Biodegradation, Fungus, Endosulphan

I. Introduction:

Pesticides are the chemical substances that are designed to prevent, destroy, repel or control any pests from rodents, weeds, insects or microbes (Donaldson et al. 2002, Reigert and Roberts 1999, Tayade et al. 2013). Pesticides are low degradable and therefore known to be toxic substances. Endosulphan is one of the most toxic insecticides which is an organochlorine, mixture of α and β isomers in 70%, 30% ratio (Goebel 1982, Wan et al. 2005). Endosulphan has been extensively used for longer period of time on various crops vegetables, cereals, fruits and cotton (Saiyed et al. 2003, Sarah et al. 2012, Silambarasan and Abraham 2013). Endosulphan is recognised as the most hazardous pesticide by the United States Environmental Protection Agency (Nag and Raikwar 2008). The presence of endosulphan is observed in ground water, surface water, air and soil while traces of metabolites of endosulphan are reported in human and animal milk (Campoy et al. 2001). Increased use of pesticides has affected farmers and co-workers by affecting the skin and causing fatal deformities (Lu et al. 2010). Constant and long term use of endosulphan has reported to affect humans by causing chromosomal abnormalities, mental retardation and neurological disorders (Amizadeh and Askari Saryazd 2011). Congenital birth defects are also an impact of long exposure of pesticide (Siddique et al. 2003). Bioremediation method is seeking more attention compared to landfilling and incineration as it is eco-friendly and ensures complete degradation of toxic substances (Mulbry 1991). Microbial organism are found to have an ability to survive in soil that has pesticide spray, therefore such microorganism are isolated and used for degradation (Shivaramaiah and Kennedy 2006). Genus *Trichoderma* consist of about 40 species and it has an ability to grow towards the hypae of other fungi which coils and degrade the cell wall of plant pathogenic fungi therefore, is known as an effective biocontrol agent. Here in our study we used *T. harzianum* strain KRL-AG2 for the degradation of pesticide endosulphan.

II Material and Methods:

2.1 Chemicals:

Technical grade 35EC pure endosulphan was used which is manufactured in excel crop care limited (Mumbai). For the preparation of malt extract agar plates and broth malt extract base, peptone and agar were purchased from HiMedia labs, Mumbai. All other chemicals used were of analytical grade. Growing of *Trichoderma harzianum* strain was maintained on malt extract agar plates. A loopful of pure strain of *T. harzianum* was taken and inoculated on malt extract plates. Malt extract agar plates and broth were prepared according to the manufactures procedure and incubated for 2-3 days at 30 ± 2 °C. Colonies of *T. harzianum* produces green conidia and therefore are fuzzy in appearance.

2.2 Degradation of Endosulphan

Malt extract broth was prepared and pure endosulphan was taken (0.2%) and added into conical flask. A loop wire was sterilized in spirit lamp flame and after cooling for few seconds a loopful culture of pure fungus *T. harzianum* was taken and inoculated in the culture broth. All process was carried out aseptically and conical flasks were incubated for 3 days at 30 ± 2 °C on rotatory shaker at 120 rpm rotation. A flask containing endosulphan without fungus was treated as control.

2.3 Preparation of TLC plate

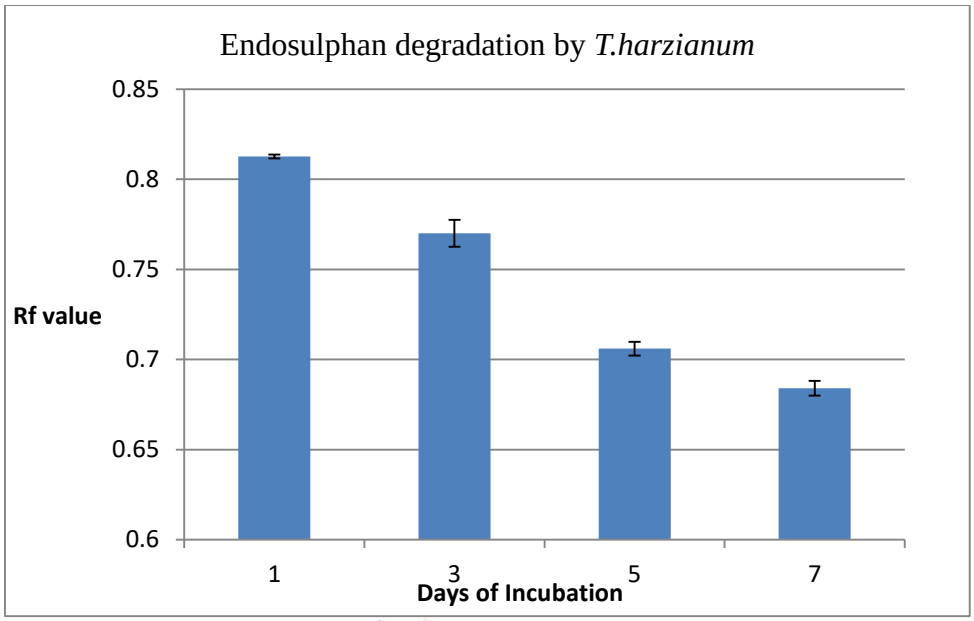
In order to analyse the endosulphan degradation the cell culture broth containing endosulphan was treated with saturated sodium chloride and then with ethyl acetate for two to three times (Awasthi et al, 2003). From the two layer formation, upper layer was collected in a beaker. Samples from each flask were collected separately and were used to analyze by TLC.

2.4 Qualitative analysis

Silica plate was prepared using 20 gm of silica gel G mixed with chloroform to make a slurry which was quickly applied to the TLC glass plate. Samples were applied with the help of capillary or a syringe. Methanol and chloroform was used as solvent system at a 95:5 ratio. The chamber was covered with glass plate, and after sufficient running of the solvent TLC plate was removed and air dried at room temperature. Spots were developed by keeping the plates in iodine chamber. To analyse the degradation of pesticide by fungus, Retention factor (Rf) values were observed and calculated.

III Results and Discussion:

T. harzianum strain was grown on malt extract agar plates at 30± 2 ^{0} C for 2-3 days. It grows rapidly and forms sticky clumps and is fuzzy in appearance.



Graph.1. Graphical representation of endosulphan degradation by *T. harzianum* 1st,3rd, 5th and 7th day of incubation. Data points represent mean ± s.e of at least three biological replicates. Student *t* test; *≤0.05, **≤0.01, showing significant difference in Rf values of control and Rf values of pesticide degradation by *T. harzianum*.

The culture was incubated on rotatory shaker at 30± 2^0 C for 7 days. The degradation was observed by thin layer chromatography. Pesticide degradation was recorded at different time points such as 1st, 3rd, 5th and on 7th day. Samples and control Rf values were analysed. It was observed that the organochlorine pesticide was readily degraded from the 1st day of incubation and the degradation was enhanced as the incubation time period was increased. The media appeared turbid compared to the other days of incubation showing the presence of the long hypal fungal growth on the 7th day. It clearly states that the degradation was more as the day span were increased. Graphical representation of pesticide degradation is shown in graph.1.The average Rf value of control was 0.86, 0.85, 0.80 and 0.86 while the average Rf value for the samples were 0.81, 0.77, 0.71 and 0.68 for 1st, 3rd, 5th and 7th day respectively. The Rf values for samples were found to be lower and decreased as the time duration was progressed compared to the Rf value of control. Experiment was carried out in three biological replicates. The statistical analysis displays a significant difference in the control and the samples containing fungal strain. This clearly states the strain of *T. harzianum* was growing and capable of degrading endosulphan.

IV Discussion:

Trichoderma harzianum is a well-known biocontrol agent. Usage of *T. harzianum* could be proved as an additional advantage over the use of chemical fertilizers and pesticides. Previous literature has shown it protects numerous vegetable crop plants against various plant fungal diseases. Bioremediation is a process in which biological organisms are used to degrade any hazardous pesticides. It is established as a well-documented method over physicochemical methods and usage of fungus for degrading chemical toxins is eco-friendly and cost effective.

In this study we used the strain KRL-AG2 of *T. harzianum* culture to study the degradation of an extensively used detrimental pesticide endosulphan. Biomass fungal culture was maintained consisting of endosulphan for a period of seven days and our results concluded *T. harzianum* growth was denser at increased day span and endosulphan could be readily degraded by *T. harzianum*. It might lead to several breakdowns into metabolites of endosulphan. This study could lead to an important key in

process of bioremediation. Further detecting the metabolites of endosulphan after degradation would be of interest while the mechanism of the microbes and their enzymes while the degradation process could lead to a vital impact on the knowledge of pesticides and environmental studies.

Acknowledgment:

Authors are thankful to the Department of Biotechnology, Shivaji University, Kolhapur for providing financial assistance through the project.

References:

Amizadeh M, Askari Saryazd G. 2011. Effects of endosulfan on human health. Webmed Cent Toxicology 2:2617.

Campoy C, Jimenez M, Olea-Serrano M, Moreno-Frias M, Canabate F, Olea N, Bayes R, Molina-Font J. 2001. Analysis of organochlorine pesticides in human milk: preliminary results. Early Human Development 65: 183–190.

Donaldson D, Kiely T, Grube A. 2002. Pesticide's industry sales and usage 1998-1999 market estimates. US Environmental Protection Agency; Washington (DC): Report No. EPA-733-R-702-700I.

Goebel H. 1982. Chemical and physical properties of endosulfan and its degradation products. Residue Review 83: 8–28.

Lu JL, Cosca KZ, Del Mundo J. 2010. Trends of pesticide exposure and related cases in the Philippines. Journal of rural medicine : JRM 5: 153-164.

Mulbry W. 1991. Degradation of pesticides by micro-organisms and the potential for genetic manipulation 10: 334-346.

Nag S, Raikwar M. 2008. Organochlorine pesticide residues in bovine milk. Bulletin of Environmental Contamination and Toxicology 80: 5–9.

Reigert R, Roberts R. 1999. Recognition and management of pesticide poisonings. United States Environmental Protection Agency 5: Washington DC.

Saiyed H, et al. 2003. Effect of endosulfan on male reproductive development. . Environmental Health Perspectives 111: 1958-1962.

Sarah S, Krishnamurthy V, Mahmood R. 2012. Degradation of Endosulfan by Mixed Bacterial Cultures Enriched from Endosulfan Contaminated Soils of Southern India. International Journal of Bioscience, Biochemistry and Bioinformatics 2: 31-35.

Shivaramaiah H, Kennedy I. 2006. Biodegradation of Endosulfan by a Soil Bacterium Journal of Environmental Science and Health 41: 895–905.

Siddique T, Okeke B, Arshad M, Frankenberger WJ. 2003. Enrichment and isolation of endosulfan-degrading microorganisms. J Environ Qual 32: 47-54.

Silambarasan S, Abraham J. 2013. Mycoremediation of endosulfan and its metabolites in aqueous medium and soil by Botryosphaeria laricina JAS6 and Aspergillus tamarii JAS9. PloS one 8: e77170.

Tayade S, Patel ZP, Mutkule DS, Kakde AM. 2013. Pesticide Contamination in Food. IOSR Journal of Agriculture and Veterinary Science 6: 2319-2372.

Wan M, Kuo J, Buday C, Schroede G, Van Aggelen G. 2005. Toxicity of α-, β-, (α + β)-endosulfan and their formulated and degradation products to Daphniamagna, Hyalella azteca, Oncorhynchus mykiss, Oncorhynchus kisutch, and biological implications in streams. Environ Toxicol Chem 24: 1146-1154.