Int. J. Environ. Res.1 (1):1-4, Winter 2007
ISSN: 1735-6865
Graduate Faculty of Environment
University of Tehran

Potential Hazards of Gasoline Additives in Altering Soil Environment in Favor of Harmful Microorganisms

Shahidi Bonjar, G. H.*

Department of Plant Pathology and Biotechnology, College of Agricultural Sciences, Bahonar University of Kerman, Kerman, Iran

Received 24 Aug 2005; Revised 21 Oct 2006; Accepted 7 Nov 2006

ABSTRACT: This is the first report about adverse inhibitory effect of V-Guard and E-Guard gasoline additives against soil beneficial *Streptomyces*. V-Guard and E-Guard gasoline additives are anti valve seat recession agents used in unleaded gas for old car engines in Iran. They contaminate soil and groundwater by fuel leaks and spills. *Streptomyces* are of the major contributors to the biological buffering of soil environment by exerting antagonistic activity against wide range of bacteria and fungi. In order to elucidate antimicrobial activity of these additives, they were tested against fifteen soil isolates of *Streptomyces* and two plant pathogens including *Erwinia carotovora* and *Fusarium solani*. The additives did not reveal any growth inhibitory activity against *E. carotovora* and *F. solani*, but showed strong inhibitory effect against *Streptomyces* isolates. The Minimum inhibitory concentration (MIC) against *Streptomyces* isolates was 1/800 of the original concentrations of the additives. Fuel leaks and spills have the potential to suppress or eliminate the *Streptomyces* role in the soil environment or adversely alter the balance of soil micro flora. This change eventually would lead to domination of microorganisms with adverse effects on the soil environment.

Key words: V-Guard, E-Guard, Gasoline additive, Antagonism, Soil micro flora, Soil contamination

*Corresponding author E-mail: shahidi@mail.uk.ac.ir

INTRODUCTION

V-Guard and E-Guard gasoline additives are anti valve seat recession agents used in unleaded gas for old car engines in Iran in recent years. Gasoline spills during transportation, overflows during refills in gas stations or leakage from above or underground gas-storage tanks contaminate soil and groundwater. The main constituent of these additives is named BENADIT K/RAN and manufactured by Lang Chemie, Mauerbach/Wien. According to the manufacturer (Lang, 2001), it is composed of oil soluble organic potassium compound in organic solvent. It's organic potassium compound is more than 50 wt%, it's aromatic solvent is less than 50% and it's potassium content is 3 wt%. It irritates eyes and skin and is harmful to human causing lung damage if swallowed. For toxicological information, the manufacturer expresses that "For the formulated product no experimental evaluated data are available". For ecological, disposal and transport information, they only provide that "For precaution reasons take care that no uncontrolled release of product into the environment will occur", "Disposal in accordance with local regulations for hazardous waste" and "Environmentally hazardous ubstance", respectively. Finally, they express that "Customers must satisfy themselves that the product is suitable for a particular purpose". From this information, it is not clear if it is safe for the soil environment.

Another gasoline additive is Methyl tert-butyl ether (MTBE). Many oil companies to enhance combustion efficiency of automobiles and reduce air pollution add this additive to gasoline. It is the most commonly used oxygenate because of its low cost, high-octane level, and ease of blending with gasoline (Johnson, *et al.,* 2000). Due to its water solubility, high mobility and low biodegradability, it leaches in soil subsurface at the speed of groundwater. Amending gasoline with MTBE has made a widespread contamination of groundwater, surface waters in coastal environments and at low levels in well water in USA (Hoffert, 1998; Reuter, *et al.,* 1998; Brown, *et al.,* 2000; Johnson, *et al.,* 2000; Bennett, 2001; An, *et al.,* 2002). The carcinogenic effect of MTBE has also been

observed in animals and furthermore, its metabolites have shown mutagen city effects in the Ames bacterial assay (Caprino and Togna, 1998; Williams-Hill, *et al.,* 1999). Although current public concern about MTBE contamination, its ban and phase out or its substitution with ethanol is widely discussed in the USA media and is at the focus of environmental scientists, its adverse effects in soil environment is not yet understood (Shahidi Bonjar, 2004; Shahidi Bonjar, 2005). To combat the problem of MTBE soil contamination, several workers reported laboratory methods of remediation and especially bioremediation (Salanitro, *et al.,* 1994; Hardison 1997; Hanson, *et al.,* 1999; Fortin, 2001; Steffan, *et al.,* 2001). However, these methods are not practically established for wide spread use in the natural soil environment.

Soil is the main constituent of our environment, keeping it healthy should be brought to the focus of all people. At the present study, to investigate biological effects of V-Guard and E-Guard against beneficial microorganisms of soil, fifteen soil-inhabitant *Streptomyces* isolates were tested. Two ubiquitous soil pathogens of plant roots, *Erwinia carotovora* a bacterium, and *Fusarium solani* a fungus, were also included in the test to evaluate if there is any differential activity in these additives. The growth-inhibitory activity of these additives was measured by *In Vitro* assay using Agar-well diffusion method. In other words, the aim of the study was to elucidate effects of these additives on the growth of soil-beneficial *Streptomyces* in comparison with their effect on two of soil plant-pathogenic microorganisms.

Beside vast amount of spills in cities gas stations during car refills which contaminate soil and underground water, a major way in spread of gasoline in agricultural soils is by spills or leakage of gasoline during in and out refills in the vicinity of reservoirs or gasoline pumps constructed in farms. From there, by many ways as irrigation, runoffs after precipitations, field animals, soil levelers and contaminated mud on field machinery-tires, gasoline leaks and spills throughout the farms. As a result, concentration of hazardous additives gradually increases in soil and consequently causes suppression of the beneficial *Streptomyces*. This leads to eruption of harmful microorganisms causing detrimental changes in soil health and fertility. It is important to know that members of the genus *Streptomyces* produce about 80% of antibiotics and several classes of biologically active secondary metabolites (Keiser, *et al.,* 2000).

MATERIALS & METHODS

Pure cultures of *E. carotovora*, *F. solani* and fifteen *Streptomyces* isolates, obtained from the Research Laboratory of Department of Plant Pathology, College of Agricultural Engineering, Bahonar University of Kerman, Iran. The gasoline additives, V-Guard and E-Guard were purchased from public gas stations in Kerman, Iran. The bacterium, *E. carotovora*, was cultured on Mueller-Hinton-Agar medium (MHA). For bioassays, suspension of approximately 1.5×10^8 cells/mL in sterile normal saline were prepared as described by Baron et al. (1990), and about 1.5 mL of it was uniformly seeded on MHA in 9×1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers and then aliquots of V-Guard and E-Guard administered to fullness in the corresponding wells. *F. solani*, the plant root pathogenic fungus, was cultured on Potato Dextrose Agar medium (PDA). For bioassays, suspension of fungal spores was uniformly seeded on PDA medium using sterile cotton swabs and assayed as mentioned. *Streptomyces* isolates were cultured on Casein Glycerin Agar medium (CGA). For bioassays, suspension of spores was uniformly seeded on CGA using sterile cotton swabs and assayed as above. Culture plates, were incubated at 29 °C for 24 h for *E. carotovora* and 3-5 days for *F. solani* and *Streptomyces* isolates. All samples were tested in triplicate. Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm. Solvent controls of di-methyl sulfoxide (DMSO): methanol (1:1, v/v) were included, although no antimicrobial activity noted in the solvent employed for the test. In order to determine Minimum Inhibition Concentration (MIC), dilution series of 1:10, 1:100, 1:200, 1:400, 1:800 and 1:1600 of V-Guard and E-Guard were prepared in dimethyl sulfoxide (DMSO): methanol (1:1, v/v) solvent and tested as mentioned.

RESULTS & DISCUSSIONS

During incubation at 29 °C, no growth inhibition was observed neither against *Erwinia carotovora* after 24 h, nor against *Fusarium solani* after 3-5 days; but strong inhibitory effects of V-Guard and E-Guard was noticed against all *Streptomyces* isolates after 3-5 days. Inhibitory effect against *Streptomyces* isolates was quite noticeable indicating complete lack of mycelial growth.

Clear inhibition zones were representative of probable bactericidal activity of V-Guard and E-Guard against *Streptomyces* isolates. Growth suppression of the *Streptomyces* and Minimum Inhibitory Concentration (MIC) values against these microorganisms are presented in Table 1. As indicated in the table, MIC of V-Guard and E-Guard is approximately 1/800 of the original additives concentrations against *Streptomyces* isolates.

Table 1. Inhibitory effects of V-Guard and E-Guard against fifteen *Streptomyces* spp. isolates, *Erwinia carotovora* and *Fusarium solani*, tested in Agar-well diffusion method and indicated by diameter of growth-inhibition zones in mm

	V-Guard Dilutions					E-Guard Dilutions				
Microorganisms	1:100	1:200	1:400	1:800	1:1600	1:100	1:200	1:400	1:800	1:1600
Streptomyces sp. isolate No. 14	24	22	17	11	0	27	25	19	11	0
Streptomyces sp. isolate No. 19	25	19	13	11	0	27	21	14	10	0
Streptomyces sp. isolate No. 29	45	37	26	20	0	47	36	27	19	0
Streptomyces sp. isolate No. 32	27	24	17	13	0	29	26	19	14	0
Streptomyces sp. isolate No. 41	24	22	17	11	0	26	25	18	12	0
Streptomyces sp. isolate No. 52	38	32	19	14	0	40	33	20	12	0
Streptomyces sp. isolate No. 62	45	37	26	20	0	47	38	27	20	0
Streptomyces sp. isolate No. 63	27	24	17	13	0	29	25	19	14	0
Streptomyces sp. isolate No. 70	21	17	13	11	0	23	18	15	12	0
Streptomyces sp. isolate No. 93	21	17	13	11	0	23	19	16	11	0
Streptomyces sp. isolate No. 100	20	16	12	9	0	22	17	12	10	0
Streptomyces sp. isolate No. 112	22	18	14	11	0	24	20	19	12	0
Streptomyces sp. isolate No. 113	23	19	15	12	0	25	19	15	13	0
Streptomyces sp. isolate No. 117	16	12	9	8	0	19	13	11	10	0
Streptomyces sp. isolate No. 120	19	15	12	10	0	22	14	11	9	0
Average of inhibition zones (mm)	26	22	16	12	0	30	23	17	13	0
Erwinia carotovora (Jones)	0	0	0	0	0	0	0	0	0	0
Fusarium solani (Mart.) Sacc.	0	0	0	0	0	0	0	0	0	0

CONCLUSION

In the soil environment, *Streptomyces* are of the major contributors to the biological buffering of soils and have roles in decomposition of organic matter conductive to crop production. Additionally, *Streptomyces* have been much studied as potential producers of antibiotics and exert antagonistic activity against wide range of harmful bacteria and fungi of the soil (Sykes and Skinner 1973, Okami and Hotta 1988, Keiser, *et al.,* 2000). Fading their role in the soil may alter the balance of soil micro flora and dominate microorganisms having adverse biological or ecological effects in soil environment. *E. carotovora* and *F. solani* are major plant-root pathogens causing diseases in underground parts of plants, but under normal conditions, they are partially suppressed by antagonistic activity of soil *Streptomyces* (Demain 1998, Getha and Vikineswary 2002). Evidently, suppression of beneficial microorganisms as *Streptomyces* would lead to eruption of harmful microorganisms causing detrimental changes in soil health and fertility. Future studies are required for better elucidation of adverse effects and spectrum of bioactivity, inhibitory and cytotoxic effects of these additives against wider range of soil-inhabitant organisms. Results of the present study highlights that fuel spills could have a potential ecotoxicological impact in the soil environment.

ACKNOWLEDGMENT

Support of Research Affairs Office of Bahonar University of Kerman is appreciated. Thanks to helpful work of Mrs. Najmeh Hoseinpour and Maryam Shekari. The spiritual result of this research is dedicated to Mr. A. Afzalipour and F. Saba, the founders of Bahonar University of Kerman. Author would like to emphasize that all of the expressed materials, results and discussion of this article should be considered as pure research findings only. It is published for the enhancement of science and is not intended to imply any criticism upon any private or governmental organization.

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