



Isolation and Identification of Pesticide Resistant Bacteria from Agricultural Soil

Swati R. Bipte, Padmini S. Parmar and M. Musaddiq

P. G. Department of Microbiology, Shri Shivaji College, Akola-444 001, Maharashtra, India

Nat. Env. & Poll. Tech.

Website: www.neptjournal.com

Received: 25/4/2011

Accepted: 17/6/2011

Key Words:

Pesticide resistant bacteria
Fipronil
Imidacloprid
Agricultural soil

ABSTRACT

The present study reports the identification of pesticide resistant isolates i.e., *E. coli*, *Azotobacter* sp., *Rhizobium* sp., *Pseudomonas aeruginosa* and *Bacillus subtilis*, which are capable of utilizing the pesticides Fipronil and Imidacloprid as a sources of carbon. Utilization of these harmful chemical compounds by soil microorganisms is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. Results from the present study showed that the isolates such as *P. aeruginosa*, *E. coli*, *B. subtilis*, *Azotobacter* sp. and *Rhizobium* species are able of growing in a medium in presence of added pesticides and may, therefore, be used for bioremediation of pesticide contaminated soil.

The main occupation in India is farming with an objective of increasing the yields in order to fulfil the demands. The crops are attacked by various types of phytopathogens like bacteria and fungi, which affect the product yield. In order to overcome these problems, various commercial pesticides are used. Since pesticides are highly toxic by design, they have potential to adversely impact ecosystems (Rani & Lakshmi Vijaya 2008). There are many tangible benefits of pesticides to humanity. However, from the aspect of the environmental pollution extensive use of pesticides and other agro-chemicals not only increase the plant growth but may also induce mutagenic and carcinogenic effects on non-targeted microorganisms. As pesticides cause extensive damage to non-targeted microorganisms, studies regarding their degradation have received considerable attention from soil microbiologists (Singh et al. 2003). The pesticides are not degraded or detoxified rapidly enough in the soil. The risk of their migration may pose health risk to humans and other living beings. Pesticides acutely affect man, animals, plants, soil as well as the aquatic biota in variety of ways.

Various types of microorganisms are present in soil like bacteria, fungi, viruses and algae. The occurrence of greater number of microorganisms in vicinity of plant roots than in soil free from root penetration has become generally established. Energy available for bacteria in soil is largely in the form of organic carbon (Clark 1940). Biodegradation is a natural process, and is primarily aerobic and rapid because the surface soil has large number of microorganisms. The

rate of pesticide degradation by bacteria is primarily a strategy for their own survival (Visoottive & Kruawan 2004). Most of the microorganisms work in natural environment but some modification can be brought about to encourage the organisms to degrade the pesticides at a faster rate in limited time frame (Singh 2008).

Soil samples were collected from rhizosphere area of pesticide contaminated soil. Particularly the soil samples were collected from the jowar, soyabean, cotton and wheat fields. Commercial graded pesticide Fipronil 5% and Imidacloprid 17.8% was used in the experiment. They are most commonly used in Vidharbha for control of aphids, jassids, thrips, hopper and termites. Nutrient agar media enriched with Fipronil and Imidacloprid was used for isolation and characterization of Fipronil and Imidacloprid degrading bacteria. The carbon source in nutrient agar media was replaced with pesticide i.e., with Fipronil and Imidacloprid. Soil bacteria capable of degrading Fipronil and Imidacloprid was isolated from agricultural soil. The pesticide contaminated soil samples suspended in 2 flasks containing 50 mL of nutrient broth for 7 days at 30°C. At periodic intervals, a loopfull of bacterial growth from the flasks was streaked on to nutrient agar supplemented with Fipronil and Imidacloprid and plates were incubated at 37°C for 24 hours. Colonies were obtained after 24 hours of incubation. Bacterial isolates grown on nutrient agar media were subjected to morphological, cultural and biochemical studies. From these studies 5 isolates were obtained from pesticide contaminated soil (Rani & Lakshmi Vijaya 2008). The Nutrient broth was prepared by using pes-

Table 1: Observation colorimetric analysis at 530 nm.

Name of Isolates	Fipronil	Imidacloprid
<i>E. coli</i>	0.676	0.595
<i>Rhizobium</i> sp.	0.668	0.523
<i>Azotobacter</i> sp	0.642	0.561
<i>Bacillus subtilis</i>	0.561	0.308
<i>Pseudomonas aeruginosa</i>	0.688	0.507

ticide as a sole carbon source. The Nutrient broth was inoculated by the above bacterial isolates and kept for incubation period of 24 hours at 37°C. The turbidity was observed after 24 hours of incubation. The colorimetric analysis of test tubes was made with the help of a spectrophotometer at 530 nm (Table 1).

Pesticides are used to control unwanted and dangerous species of insects. These are the economic poisons employed to regulate the impact of various pests on our land, life and economy. Fipronil and Imidacloprid are most commonly used commercial insecticides, it is therefore logical that the bacteria from Fipronil and Imidacloprid contaminated fields could be able to degrade these pesticides. (Montonaga et al. 1995). Out of five isolates, one was found to be a Gram positive bacilli and other four isolates were found to be Gram negative rods and cocci. All the isolates were screened for their ability to resist Fipronil and Imidacloprid. The resistant

isolates were identified on the basis of morphological-cultural and biochemical characterization. The isolates were identified as *E. coli*, *Rhizobium* sp., *Azotobacter* sp., *Pseudomonas aeruginosa* and *Bacillus subtilis*, which are capable of degrading pesticides Fipronil and Imidacloprid (Bouquard & Quazzani 1977).

REFERENCES

- Bouquard, C. and Ouazzani, J. 1997. Dechlorination of Atrazine by *Rhizobium* sp. isolate. Applied Environmental Microbiology, 63 (3): 862-866.
- Clark, Francis E. 1940. Types of bacterial associates with plant roots trans- action. Kansas Academy of Science. 43: 75-84.
- Montonaga, K., Takagi, K. and Matamoto, S. 1995. Biodegradation of Chlorothalonil in soil after suppression of degradation. Journal Biology and Fertility of Soil, 23(3): 340-345.
- Rani Surekha and Lakshmi Vijaya 2008. Isolation and characterization of Chlorpyrifos degrading bacteria from agricultural soil and it's growth kinetics. African Journal of Microbiology, 2: 26-31.
- Singh, Dileep K. 2008. Biodegradation and bioremediation of pesticides in soil. Concept, method and recent development. Indian Journal Microbiol., 48: 35-40.
- Singh, Brahesg, Walker Allan, Alun J. and Morgan, W. 2003. Biodegradation of Chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soil. Applied and Environmental Microbiology, 69(9): 5198-5206.
- Visoottiei Seth P. and Kruawan, K. 2004. Isolation of bacterial culture capable of degrading triphenyltin pesticides. Applied Organic Chemistry, 9: 1-9.