



Bioremediation of Deltamethrin Pesticide through Plant Growth Promoting Rhizobacteria (PGPRs)

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Abstract

Deltamethrin is a synthetic product of Pyrethroid compounds. Several countries have banned the use of this pesticide in their agricultural fields including USA, Germany, Indonesia, Sri Lanka, China and Japan. However, it is still being used in Pakistan, India and other third world countries as a broad spectrum pesticide. The exposure to Deltamethrin pesticide is not only caused through pesticide spraying, but the consumption of pesticide residues through fruits and vegetables have also been reported through oral route. The present study was designed to determine the possibility of the biodegradation/bioremediation of residual pesticides in view of emerging severe side effects of Deltamethrin due to slow but continuous exposure or consumption through fruits and vegetables. In this study, eight strains of *Bacillus spp* have been used that have already been used as biofertilizers. Deltamethrin pesticide was inoculated in broth cultures of these strains at concentrations 0.05 and 1% (w/v) and incubated at 37°C for one month. After one week, 20 ml of each experiment was collected and centrifuged to get cell free supernatant; absorption spectra of these cell free supernatant samples were taken at UV-Visible spectrophotometer in the UV range of (200-400) nm. This degradation of the Deltamethrin by the inoculated strains could be visualized through the spectroscopy readings. It was very encouraging to note that such large concentrations of the pesticides have been successfully degraded by the strains which also acts as plant growth promoting rhizobacteria (PGPR) when inoculated as biofertilizer in several vegetables and cotton crop, as well. In view of the degrading capability, these strains could also be used for the remediation of pesticide contaminated soil and other bioaugmentation purposes as well.

Key Words Deltamethrin, PGPR(s), bioremediation, Bioaugmentation

Introduction

Pakistan is an agricultural country whose 65-70% of GDP is supported through agricultural earnings. The demand for the agricultural crops is increasing day by day due to the ever increasing population. Therefore, there is a huge need for the improved quality and quantity of the agricultural produce. Farmers are using huge quantities of the broad spectrum pesticides to control the problem of pests in cash and other edible crops. These extra quantities of the pesticides deposited in the fruits/vegetables as residual pesticide and are ultimately consumed by the consumers

which results in serious diseases related to lungs, reproductive system and even cancer etc.

Deltamethrin is classified as Pyrethroid (WHO environmental health criteria 97, 1990). Pyrethroids are synthetic chemicals modeled after the Pyrethrin components of Pyrethrum (Davis, 2009) having one pure compound in its structure (Toxicological Profile for Pyrethrin and Pyrethroids, 2009). It is a broad spectrum insecticide (Tomlin, 1985) included in the class of extremely hazardous pesticides by WHO. Its toxicity has prompted several countries like USA, Germany, Indonesia, Sri Lanka and Tanzania to ban this product and also several other countries such as Colombia, Korea, China and Japan to restrict its use. However, it is being regularly used in many developing countries including Pakistan, India etc. Deltamethrin pesticide of Pyrethroid group was selected for the biodegradation or bioremediation studies due to the hazardous nature and very frequent use of this pesticide in Pakistan.

Materials and Methods

Microorganisms used

The isolated *Bacillus* species, earlier used as PGPR(s) in form of biofertilizer for several vegetable and cotton crop, were used for bioremediation study. Eight bacterial strains were inoculated in broth containing Deltamethrin pesticide at 0.05% and 1% concentrations. Biodegradation was detected through VIS-UV spectrophotometer at 200-400nm.

Degradation of pesticide Deltamethrin

Bacterial strains were propagated in selected Pikovskaya medium (PVK) and were incubated at 37°C. After one week 20 ml of each experiment was collected and was centrifuged at 2000 rpm for 20 minutes. Absorption spectra (OD) of these cell free supernatants were taken at UV-Visible Spectrophotometer in the UV range (200-400 nm).

Results and Discussion

The present study was initiated in view of the importance of the presence of residual pesticide above the acceptable limits and the hazardous effects of the Deltamethrin pesticide being used on different consumable crops in Pakistan. Later, these crops such as okra, pumpkin, cotton seed, etc are consumed by the population resulting in severe diseases in their consumers, with the passage of time. Efforts were made to characterize the strains that can be used for bioremediation/biodegradation of Deltamethrin pesticide so that the population could be saved from the hazards of the residual pesticide present in edibles.

The optical densities of samples of cell free supernatant collected after one week showed the degradation of pesticide by the inoculated strains. Results are shown in Table 1 and 2. All these strains have successfully degraded large concentrations of pesticides. The ability of these bacteria could be used for making the edibles, pesticide free in form of some formulation. These formulations not only can rescue the health of

the population but also prevent the rejection of the export quality fruits due to presence of residual pesticides.

Table 1: Spectrophotometer Reading of Eight Bacterial Strains at 0.05% Concentrations

Wave Length (nm)	Total Number of Cultures & Optical Densities of the samples							
	1	2	3	4	5	6	7	8
300	3	3	3	4	4	3	3	3
310	2	3	3	4	4	3	3	4

Table 2: Spectrophotometer Reading of Eight Bacterial Strains at 1%Concentration

Wave Length	Total Number of Cultures & Optical Densities of the samples							
	1	2	3	4	5	6	7	8
300	3	3	3	2	2	4	3	2
310	3	4	3	2	1	3	3	1

The misuse of pesticides results in environmentalcontamination and health hazards and is one of the major reasons for pesticide poisoning in developing countries (Konradsen*etal*2003). It was reported that poor education level, use of improper instruments for spraying of pesticides and lack of information and training on personal protection, and pesticide safety all results in intoxication (Hurtig*etal*(2003), Atreya*etal*(2008), Damalas*etal*(2006a), (2006b), Ajayi and Akinnifesi(2008), Chalermphol and Shivakoti(2009), Plianbangchan*etal*(2009), Sosan and Akingbohunge(2009)). Exposure to pesticides is one of the important occupational risks among farmers in developing countries (Wesseling*etal*(2001), Konradsen*etal*(2003), Coronado *etal*(2004)). Pesticides play very important role in meeting the food and cotton demands of the world of today by protecting the crops against controlling the vector borne diseases. The pesticide enters in the body through three major routes: skin, lungs and gut. Skin is the major route of exposure of acute pesticide intoxication incidences among farmers. Similarly, respiratory tract also play very important role in absorption of pesticides whether they are in form of vapors, particles or drops. Pesticides are very beneficial for protecting the crops against pest problems, but are very harmful for the human health, as well. Pesticide intoxication may further be complicated due to the impurities, which the pesticide manufacturers are using in their formulations as carrier materials for increasing the efficacy of the pesticides. For example, Deltamethrin is a synthetic product of Pyrethroid which has less health hazards than its synthetic formulation. There are several reported disadvantages of residual Deltamethrinpesticides present in the fruits and vegetables and in the environment being consumed and inhaled by the population.

According to the results mentioned in table 1, these strains can successfully degradeDeltamethrinpesticide not only at low concentrations like 0.05% but also at high concentrations upto 1%, which is very encouraging. Regular and frequent use of pesticides may result in the development of resistance in the pests with the passage of time then there will be dire need of the nonpathogenic strains which could degrade these residual pesticides in fruits and vegetable to make them harmless for the human health.

Organophosphorus and Organochloride pesticides are being used frequently in Pakistan and worldwide due to their good results despite the fact that their accumulation in skin or consumption through fruits and vegetables could lead to different diseases and even death (Quintero *et al* 2008). While several countries have started the pesticide monitoring programs for accumulation of Organophosphorus pesticides in fruits and vegetables (Amoah *et al* 2006, Zhou *et al* 2008). The accumulation of several chlorinated pesticides at different levels in fruits and vegetables are reported which are exceeding the maximum residual limits for consumption by the consumers in Pakistan (Musud *et al* (1992) and Tahir *et al* (2001).

There are several reported disadvantages of Deltamethrin pesticides for example it was mentioned in a report by US Department of Health and Human Resources (entitled, "Toxicological profile of Pyrethrin and Pyrethroids", (2003) that Pyrethrin and Pyrethroid compounds could affect nerves and brain functions. It results in tingling, numbness and burning effects on exposed skins, it enters in body through breathing in the pesticide contaminated air during spraying in the field and in the houses as well. Several other problems in childrens and adults were also reported due to severe exposure to high doses of these compounds such as muscle twitching, tremors, convulsions, loss of consciousness that may last for several hours. It was further reported by cancer assessment review committee for Pyrethrin that these compounds are likely to be human carcinogen by the oral route.

The technical grade Pyrethrins and Pyrethroids are less harmful than the formulated products because in these products manufactures are using several carriers to increase the effect of the pesticide. Due to this reason several severe side effects were reported related to Deltamethrin product which is a synthetic formulation of Pyrethrin compound. Wagner (2000) has reported that even due to the allergic reactions triggered by these compounds may lead to death in children in less than 3 hours. Wax Hoffman (1994) has also reported the death of a child due to the onset of severe allergic reaction caused by the dog shampoo containing only 0.2% pyrethroid as an ingredient. In view of this severe side effect, it may be speculated that the farmers inhaling these pesticides, though not consuming, are at highest risk because the allergic reactions could be triggered in the body anytime by the exposure to this pesticide that may lead to death. This is more harmful than the consumption of slow doses of Deltamethrin pesticide by the consumer in form of residues present in consumable goods such as fruits and vegetables. It was reported by Copplestone (1985) that annually 10,000 farmers and field workers and about 60-70% of affectees got acute unintentional occupational poisoning due to exposure to different pesticides.

It was further reported by Kolaczinski (2004) and Lengetal (2003) that over the period of last decade in Germany in view of chronic diseases due to long term exposure to low doses of synthetic Pyrethroid insecticide, Deltamethrin, the point has reached when the pest control operators have refused to use Pyrethroid compounds and its synthetic product Deltamethrin in their fields and houses. Prohletal (1997) have reported several sources of exposure that can cause chronic diseases due to long term exposure to low doses of the pesticides such as carpets, wall papers in houses, and volatile insecticides, usually Pyrethroid compounds, being used on electric evaporator as mosquito repellents etc. Chronic diseases such as Gulf War Syndrome and Multiple Chemical Syndrome are also reported due to the long term exposure to the low doses

of neurotoxin compounds such as Deltamethrin (Twombly(1994), Anonymous, (1995a),(1996);Fiedler *etal*,(1996). Muller Mohnssen(1984)has reported that the synthetic Pyrethroid compounds including Deltamethrin, Cypermethrin and Fenvalerate are having irreversible and cumulative effect on nerve tissues. Muller Mohnssen(1991) has further reported that widespread use of Pyrethroid compounds may also lead to severe disorders to human nervous system due to accumulation of small doses of the pesticides in the body tissues. Based on these reported diseases due to exposure to Pyrethroid synthetic compounds it was suggested by Muller Mohnssen(1991) and Tippe(1993) that all these compounds should be withdrawn from the market until the adverse effects of these products are not ruled out. In Germany following this media coverage of chronic diseases due to long term exposure of synthetic Pyrethroid compounds 500 individuals reported their symptoms of diseases due to domestic exposure (MullerMohnssen and Hahn (1995).Symptoms include nausea, dizziness, respiratory pain to delayed loss of weight, hair and skin rashes, and loss of muscular response, memory and immune response. Even it was reported that some of the victims become permanently handicapped due to long term exposure to these compounds (Anonymous (1992), Haas (1992).There are several conditions which are caused byPyretheroid compounds such as chronic fatigue syndrome, sick building syndrome and gulf war syndrome to multiple clinical synderom (MCS) (Altenkirch(1997), Rekitke(1997),Pehrke(1998),Merz(1999). Other diseases which were reported due to use of Deltamethrin pesticide in low doses in furniture or else are Cerebro-organic disfunctions, locomotory disorder remiscent of multiple sclerosis or Parkinson's disease, polyneuropathy and immuno suppression. Patients with chronic exposure to organic solvents, heavy metals organophosphates and Pyrethroids describes symptoms of fatigue, depression and memory loss and reduction in attention time or span (Hartman (1988), Singer (1990), Lohmannetal(1996), Prohletal(1997)). Narahashi(1992)did not agree that irreversible chronic poisoning could result due to the Pyrethroid poisoning, though the acute Pyrethroid poisoning symptoms are established facts as has been reported by Moshammer(1996), Nasterlalack and Dietz (1996).

Pakistan is basically an agriculture country. It contributes 21% of the total GDP and 44% of the employment of Pakistan is generated through this sector, while two third of the Pakistan's population lives in rural areas (WHOEnvironmental Health Criteria 97, 1990). In Pakistan, Organophosphorus pesticides and insecticides are being used for controlling the pest problems in vegetable crops. In spite of their toxicological profiles, farmers are using these pesticides in large amounts to make their crops pest free and good crop yield (Baig 2009).It has been reported that due to spray of these pesticides in huge amounts, pesticides start accumulating in the fruits as residues and are ultimately consumed by the consumers and result in health hazards and even death. These are registered for use in various crops including cotton, cereals, soybeans and vegetables for pests such as mites, ants, weevils and beetles (Pesticide Product 2007).

Paresthesia was the most commonly reported symptom from dermal exposure in occupational studies involving pyrethroids. Usually skin sensations were characterized a tingling, burning and numbness of skin after dermal exposures which is reversible or lasting upto 24-48 hrs (Ray (1994), Soderlund(2002).Greiger(1993) has reported that workers in pesticide industries related to manufacturing, formulation and packing are at higher risk than the consumers.

Cotton is a cash crop of Pakistan and is more vulnerable to pest problems at different stages of its growth. Organophosphates, Carbamate and Pyrethroid pesticides are used against infectious pests and insects. Hassan (1998) has reported that there is no regulatory program for the use of pesticides by the farmers for different crops. The doses are not precisely calculated, manufacturer's safety instructions are not followed properly, appropriate instruments are not provided for spraying of the pesticides to the farmers, and hence they are at the highest risk of pesticides intoxication. Their families and animals are next in the ladder of effectees due to these pesticides. One farmer dies every minute due to the pesticide poisoning (Ref). Ahmed (1998) reported that in Pakistan generally organophosphates and pyrethroid pesticides are used and not only the farmers but general consumers are also very much affected by the consumption of these pesticide residues present in fruits and vegetables.

The inoculation of these tested Deltamethrin degrading bacterial strains in the fruits and vegetables before marketing them will make them pesticide free in a more natural and harmless way besides protecting the consumers against the severe health hazards such as even cancer due to continuous exposure with slow doses in fields or consumption of these pesticides.

Anwer *et al.* (2004, 2005) has reported the presence of several residual pesticides in drinking water, fruits and vegetable in Pakistan. It was suggested that epidemiological studies related to pesticides are required on the farmers in different areas of Pakistan so that the risk assessment policies could be developed for the safety of these agricultural workers.

In a study conducted by Sajjad *et al.* (2009) it has been reported that most of the farmers in Pakistan are using Organophosphorus pesticides including Trizophos, Profenos and Chlorpyrifos and are being extensively used in Layyah, Muzafargar, Multan and Khanewal districts on the vegetable crops such as Okra, Eggplant, Pumpkin etc. It was further reported by the author that out of 108 samples 38 were found to be contaminated with OP pesticide residues. Profenose is the most frequently used pesticide in Pakistan, and its residues detected and reported in different crops are: 25% in okra 17% in eggplant and 5% in pumpkin, which are directly proportional to the frequency of its application. Chloropyrifos is second most used pesticide in Pakistani agricultural lands, according to Sajjad *et al.* (2009). It was reported cumulatively that in Okra Profenofos 25%, Triazophos 14%, and Chlorpyrifos 19%, Eggplant Profenosfos 17%, Trizophos 11% and Chlorpyrifos 22% and in Pumpkin Profenosfos 5%, Trizophos 2.5% and Chlorpyrifos 2.5% found respectively.

Based on all these reported disadvantages due to the use of pyrethroid synthetic compounds like Deltamethrin and detection and consumption of residual pesticides in fruits and vegetable in Pakistan, it is imperative to take effective measures to reduce the risks of acute and chronic health problems to the consumers being affected directly or indirectly by the consumption of residual pesticides. Bioremediation may prove to be an effective measure for alleviation of the hazards of pesticides. Formulations containing indigenously isolated bacterial cultures can degrade the residual pesticide in edible consumables such as fruits and vegetables. Moreover, bioaugmentation of soil with the selected degrading bacterial strains will

also help in reducing the pesticide contaminants from soil and environment subsequently reducing the waste water pollution.

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