

Impact of Deltamethrin on Environment, use as an Insecticide and its Bacterial degradation – A preliminary study

Shrivastava Bhanu¹, Shrivastava Archana², Kumar Ajay¹, Bhatt J.L¹, Bajpai S.P¹, Parihar Surendra Singh¹, Bhatnagar Vandana³

1- Department of Life Sciences, Institute of Applied Sciences and computer Application (IASCA) ITM Universe Gwalior M.P

2- Department of Engineering chemistry, Gwalior Institute of Information Technology (GIIT) Gwalior M.P

3- Faculty of Life Sciences in Microbial Biotech Research & Training M.P
abs_micro@yahoo.com

ABSTRACT

Deltamethrin is a synthetic pyrethroid pesticide that kills insects through dermal contact and digestion. It is applied for a range of commercial crops and recreational uses, and by extension controls a variety of pests. The cytological effects of deltamethrin, a synthetic pyrethroid used as an insecticide, have been studied using root meristems of *Allium cepa*. Deltamethrin treatment resulted in a dose-related reduction in mitotic index. Both chromosomal and mitotic abnormalities were encountered at all concentrations ranging from 0.05 to 2 ppm. Most of the chromosomal and mitotic abnormalities were apparently due to disturbance in the mitotic spindle. Induction of chromosome and chromatid breaks with higher concentrations (0.5 to 2.0 ppm) suggested clastogenic action of this compound but Deltamethrin is not mobile in the environment because of its strong adsorption on particles, its insolubility in water, and very low rates of application; however, it still presents risks to the ecosystem in which it is applied. The level of deltamethrin biodegradation in mixed cultures of benthic and planktonic bacteria after 5, 10, and 15 days of incubation was higher than that in homogenous cultures. It was demonstrated that microorganisms from the *Sphingomonas paucimobilis* species and the *Moraxella* genus, among planktonic bacteria, as well as *Burkholderia cepacia* and *Bacillus mycoides* species, among benthic bacteria, were the most effective in reducing the concentration of this insecticide

Keyword: Pyrethroid, pesticide deltamethrin,

1. Introduction

Deltamethrin is a synthetic pyrethroid pesticide that kills insects through dermal contact and digestion. It is applied for a range of commercial crops and recreational uses, and by extension controls a variety of pests. It was first synthesized in 1974, and since has been used primarily on cotton, coffee, maize, cereals, fruits, and stored products; however, deltamethrin is also applied in animal health and public health capacities. Deltamethrin is considered the most powerful and therefore the most toxic of the pyrethroids, up to three orders of magnitude more so than some Deltamethrin(DLT)- a type-II pyrethroid insecticide has also been reported to increase intracellular free Ca^{2+} (Muralidhara et al., 1995). And it is a Ca^{2+} channel agonist and the primary target site for pyrethroid action, in both insect and mammals, is the axonal sodium channel through a dissociable interaction with macromolecular component (Narahashi, 1992). DLT has been reported safe to adult mammals and had been studied using biochemical and electrophysiological tools. In studies done on workers in agricultural settings, deltamethrin can produce a variety of acute health conditions, but these

can be prevented with necessary precautions. The chemical also carries several ecological risks, particularly by causing algal blooms and reducing bee populations and their associated pollination service.

Chemical Structure

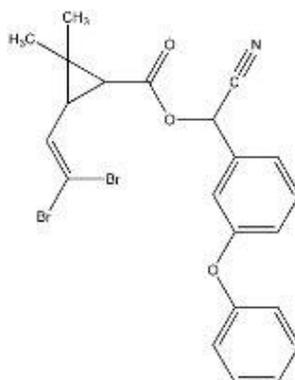


Figure 1: Deltamethrin, Use: pesticide, Source: synthetic chemistry, Absorption: rapidly following oral ingestion, slowly when absorbed through skin, Sensitive individuals: agricultural workers and those who use the pesticide.

The level of deltamethrin biodegradation in mixed cultures of benthic and planktonic bacteria after 5, 10, and 15 days of incubation was higher than that in homogenous cultures. It was demonstrated that microorganisms from the *Sphingomonas paucimobilis* species and the *Moraxella* genus, among planktonic bacteria, as well as *Burkholderia cepacia* and *Bacillus mycoides* species, among benthic bacteria, were the most effective in reducing the concentration of this insecticide

1.1 Chemical Description

Deltamethrin belongs to the chemical class of pyrethroids, naturally occurring insecticidal compounds that are synthesized from chrysanthemum flowers. Its most common appearance is either as a colorless or slightly beige powder, both of which are odorless. As a lipophilic compound, deltamethrin is not soluble in water and therefore is highly stable in the physical environment.

1.2 Deltamethrin, Impact on plants

The cytological effects of deltamethrin, a synthetic pyrethroid used as an insecticide, have been studied using root meristems of *Allium cepa*. Deltamethrin treatment resulted in a dose-related reduction in mitotic index. Both chromosomal and mitotic abnormalities were encountered at all concentrations ranging from 0.05 to 2 ppm. Most of the chromosomal and mitotic abnormalities were apparently due to disturbance in the mitotic spindle. Induction of chromosome and chromatid breaks with higher concentrations (0.5 to 2.0 ppm) suggested clastogenic action of this compound.

2. Deltamethrin, Impact on Animals

Many environmental factors claim to have practically no influence on the adult nervous system, sometimes present frank cytological and organization changes in developing nervous

tissues. Such effects are even reflected in behavioral systems. In earlier studies with deltamethrin, provided evidences to suggest that so prominently proclaimed 'safe to man' pyrethroid insecticides have various degrees of toxicological impacts in developing rat brain at doses much below the safe limit to exposure. If this is acceptable then we must find out suitable neurotropic agent which can counteract or help revival of such pathological changes. It is now known that DLT promotes Ca^{2+} uptake in neurons. In conditions of nerve injury Ca^{2+} influx is being promoted as a factor responsible for synthesis of macromolecules including apoptosis. In another independent study this laboratory recorded the Ca^{2+} channel antagonist, flunarizine to be capable of not only promoting neuronal survival following axonal injury, but also general recovery from the damage. (Patro et al 1999; Bajpai, 2002). The above two major observations helped in developing the hypothesis that Ca^{2+} channel antagonist like flunarizine may act as positive antidotes to deltamethrin associated developmental neurotoxicity. Cerebellum was chosen as the tissue of priority because developing cerebellum is most vulnerable to any type of experimental changes and most of the environmental factors. It also facilitates studying impact of any factor on different types of neuronal development like those of macroneurons, interneurons and microneurons. Of the various phases of cerebellar development the development during 9-13 days was considered for this study because, i > this is similar to first year of postnatal life of man, ii > this is active phase of granule cell migration and histogenesis and synaptogenesis which are vital processes for future nervous functions, iii > maternal rejection rate is higher in rats if the pups are handled during first week of postnatal life which could cause in either death of the pups or changes related to nutritional deficits. Extensive period of development beginning from the embryonic organogenesis to the postnatal infantile period, the high susceptibility of the undifferentiated neural cells and the lack of self renewing ability of neurons make the mammalian brain highly vulnerable to the environmental factors (Koestner and Norton 1991; Inouye 1995). During early postnatal development of the brain, the improper acquisition of position of the migrating neurons, through active migration, ultimately affects their morphology, synaptic connectivity and position. Any disturbances to the migrating neurons may cause congenital brain anomalies (Barth 1987; Hatten 1990).

During the normal development of the cerebellar cortex, the granule cell proliferation and migration to the inner granular layer and deeper part of molecular layer are completed by the end of the postnatal day 20 and the external layer almost disappears in the 21 days postpartum animals (Close et al 1977; Lewis et al 1975).

2.1 Effects on Aquatic Organisms

As is common with many pyrethroids, deltamethrin has a high toxicity to fish under laboratory conditions. However, in field conditions under normal conditions of use, fish are not harmed. Deltamethrin had an impact on aquatic herbivorous insects. This impact led to an increase of algae. Although the fish (fathead minnows) accumulated the deltamethrin, no mortality could be observed (Haug, G. et al 1990).

2.2 Impact on Aarthopoda

The study was undertaken to evaluate the impact of deltamethrin-impregnated mosquito nets on malaria incidence, mosquito density, any adverse side effect among users. A field trial was carried out over a period of three years in two adjacent military stations at Allahabad (UP), keeping one as a trial and other as a control station. During first year, baseline data were collected and during next two years residual spray was replaced with use of deltamethrin

impregnated mosquito nets in trial station. The use of deltamethrin-impregnated mosquito nets/insecticide treated bed nets resulted in a significant decline in malaria incidence and Annual Parasite Index (API). The average mosquito density of Anopheline mosquitoes decreased by 67.8% and Culex by 49.7%. The insecticide was found safe for use amongst troops. Use of deltamethrin-impregnated mosquito nets has beneficial impact on integrated control of malaria.

2.3 Impact on Environment

Deltamethrin is not mobile in the environment because of its strong adsorption on particles, its insolubility in water, and very low rates of application; however, it still presents risks to the ecosystem in which it is applied. Under laboratory conditions, deltamethrin has been found to be highly toxic to a range of aquatic organisms such as amphibians, crustaceans, mollusks, and various forms of plankton. Although these laboratory investigations demonstrate that the chemical is harmful to fish, field studies have not confirmed this finding.

Additionally, because deltamethrin reduces local insect populations, its use can indirectly cause the proliferation of algal blooms. With fewer insect-consumers to control algae population growth, these blooms can in turn harm fish and other aquatic life through clogging gills and decreasing the water's level of oxygen. Other ecological risks of deltamethrin use are seen in decreased pollination, as the chemical is toxic to bees.

3. Methods and Materials

Bacterial strains isolated from individual samples and cultured on peptone iron agar plates (IPA) were tested for purity and then used for preparation of preliminary cultures. Subsequently, bacterial inoculum for the analyses of deltamethrin degradation was obtained from these preliminary cultures. 804 *Agnieszka Kalwasińska et al.* Five cm³ of sterile mineral medium, containing (g dm⁻³ of distilled water) KH₂PO₄ – 1, KNO₃ – 0.5, MgSO₄ · 7H₂O – 0.4, CaCl₂ – 0.2, NaCl – 0.1, FeCl₃ – 0.1, glucose – 1, was poured into test tubes, inoculated with pure bacterial cultures, and incubated at 20°C for 7 days on in a rotary shaker (WL – 2000, JW Electronic). Bacterial *inoculum* was retrieved from the culture with 2 inoculation loops. The medium contained deltamethrin with the final concentration of 1 µg dm⁻³. For each bacterial strain, we also carried out control analyses, which involved culturing bacteria on a mineral medium without the pesticide.

3.1 The Measurement of the Culture Optical Density

In order to determine the adaptability of the microorganisms to growth in the presence of a xenobiotic, we measured the optical density of preliminary cultures in a medium with and without deltamethrin after 7 days of incubation in accordance with the above method.

3.2 Analysis of the Reduction of Deltamethrin Concentration

Degradation of deltamethrin was monitored using the gas chromatography technique (GC) –. Two cm³ culture samples after 5, 10, and 15 days of incubation were collected to determine the amount of the pesticide. In order to extract deltamethrin from bacterial culture samples, 2 cm³ samples were treated three times with 2 cm³ dozes of hexane (POCH). Prior to the extraction, 50 mm³ dozes of an internal standard – 10 mm³ solution of decachlorobiphenyl in acetone (POCH) – were added to each sample. Extracts were combined and dry-evaporated with a slow flow of ultra-pure nitrogen at ambient temperature. After the evaporation, the

remains were dissolved in 50 mm³ of methanol (POCH). The extracts were stored in dark-glass vials at -40C in preparation for chromatographic analyses. Analyses were conducted in a capillary column Equity-5 (30m 0.25 mm i.d., 0.25 mm d.f.). Five mm³ samples were used in the chromatographic analysis. Nitrogen (N₂) with the flow rate of 60 cm³ min⁻¹ was used as carrier gas. The temperature program was set to: 150°C (1 min), 35 min⁻¹ to 200°C, 15°C min⁻¹ to 280°C. Temperatures of a detector and an injector equaled 320°C and 260°C, respectively. The amount of the pesticide in a sample was determined based on comparison of retention times of peaks in the sample relative to the internal standard with the relative retention times of standard reference chemicals. The calibration curve was prepared using decachlorobiphenyl as the internal standard. All plots were linear with a correlation coefficient of 0.99 or higher (least squared method). The level of deltamethrin biodegradation (%) was calculated from the

equation:

$$B = a - b / 100 . a$$

where:

B – biodegradation (%),

a – concentration of deltamethrin in a culture after *t*₀,

b – concentration of deltamethrin in a culture after *t*₅, *t*₁₀, *t*₁₅

4. Results and Discussion

Deltamethrin is a pyrethroid insecticide that kills insects on contact and through digestion. It is used to control apple and pear suckers, plum fruit moth, caterpillars on brassicas, pea moth, aphids (apples, plums, hops), winter moth (apples and plums), codling and tortrix moths (apples). Control of aphids, mealy bugs, scale insects, and whitefly on glasshouse cucumbers, tomatoes, peppers, potted plants, and ornamentals. It also controls numerous insect pests of field crops. Formulations include emulsifiable concentrates, wettable powders, ULV and flowable formulations and granules. There are no known incompatibilities with other common insecticides and fungicides (Agrochemicals Handbook. 1983). Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which rapidly paralyze the insect nervous system giving a quick knockdown effect . Deltamethrin has a rapidly disabling effect on feeding insects and for this reason there is hope that it may be useful to control the vectors of "non-persistent" viruses (viruses that can be passed on by the vector within a few minutes of starting to feed on the plant) . Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Death of insects seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours (Leahey, J. P.1985). Deltamethrin poisoning occurs through cuticular penetration or oral uptake. The susceptibility of insects is dependent on a variety of factors and can vary, as with many insecticides, according to the environmental conditions.

Flies are most susceptible to pyrethroid poisoning shortly before dawn. The LD₅₀ drops by the factor of 2 as compared to full daylight activity . In the natural environment relatively easily as a result of photodegradation and biodegradation; the latter is thought to be the primary process responsible *Agnieszka Kalwasińska et al.* for breakdown of this compound in alkaline or neutral pH waters with limited amount of light. The principal mode of pyrethroid decomposition includes hydrolysis of ester bonds and oxidation of acid and alcohol functional groups (Demuot 2006) This study demonstrated that as early as 7 days after the

xenobiotic was added, both pure and mixed cultures of analyzed groups of lacustrine bacteria were developing relatively well in the presence of deltamethrin with the concentration of $1 \mu\text{g dm}^{-3}$. The $1 \mu\text{g dm}^{-3}$ concentration of deltamethrin was found to have a stimulating effect on the majority of bacterial strains. Zhang et al. (1984) observed an increase in bacterial and actinomycetes populations in soil containing deltamethrin during a 180-day experiment on degradation of this compound in organic soil. However, certain organic micropollutants may have a toxic effect on populations of microorganisms, inhibiting their metabolism; as a result, degradation of these compounds is possible only to a small degree (Warrens et al. 2003). According to available literature data regarding decomposition of deltamethrin in soil, this insecticide undergoes microbiological degradation in 1-2 weeks (Kidd, James 1991). In order to confirm the priority of biodegradation over abiotic modes of transformation in decomposing pyrethroids, Champman et al. (1981) investigated stability of permethrin, cypermethrin, deltamethrin, fenpropathrin, and fenvelaret in sterile and non-sterile soil samples. The initial concentration of pesticides equaled 1 ppm. The remaining percentages of pesticides after 8-week incubation were as follows: fenpropathrin – 2%, permethrin – 6%, cypermethrin – 4%, fenvelaret – 12%, and deltamethrin – 52%. In sterile soil samples, the content of pesticides exceeded 90%, which suggests that biodegradation plays a key role in decomposing these compounds in soil. In biodegradation tests with deltamethrin as the only source of carbon and energy conducted on bacterial strains isolated from soils, the initial concentration of the insecticide was reduced by 35.7–44.4% within a week and by 59.7–72.5%, within two weeks.

In samples without microorganism, the initial concentration of this compound was reduced by 3–10% (Khan et al. 1988). Research on degradation of the following pyrethroids: biosmerin, permethrin, cypermethrin, deltamethrin, fevelaret, chlorpyrifos, and pesticide Nurelle D 550 EC (mixture of 50 g cypemethrin and 500 g of chlopyrifos in 1 dm^3) in a model of an aquatic ecosystem show that, among the analyze compounds, deltamethrin is characterized by the highest rate of degradation with decomposition time up to 21 days (Lutnikka et al. 1999). Microorganisms inhabiting various ecological niches of a water body are characterized by different metabolic activity levels and, in consequence, a different response to xenobiotics introduced to the environment. The results indicate that planktonic bacteria are characterized by higher average capacity *Biodegradation of Deltamethrin...* 811 to decompose deltamethrin than benthic bacteria. Among 25 analyzed strains of planktonic bacteria, all but one strain were capable of decomposing deltamethrin. In the case of bacteria isolated from bottom sediments, 13 strains degraded the insecticide, while 12 showed no such ability. The half-life of deltamethrin in cultures of planktonic bacteria equaled on average ca. 10 days. Benthic bacteria, on average, needed 15 days to reduce the concentration of fungicide by 23%.

According to numerous studies related to the metabolic activity of heterotrophic bacteria that occur in the water column and bottom sediments, bacterioplankton is characterized by a higher metabolic and biochemical activity than benthic bacteria (Fisher et al. 2002, Haglund et al. 2003). In spite of relatively high abundance of the latter microorganisms (Niewola 1968, Kalwasins, Donderski 2005) and the fact that cells of bacteria from deep bottom sediments are capable of growing on solid mediums in laboratory conditions (viable bacteria) (Miskin et al. 1988, Parkes et al. 1994), their activity is often low, which indicates that these bacteria are characterized by a low cell-specific activity. It is probable that precisely this low cell-specific activity of bacteria inhabiting bottom sediments is responsible for their limited ability to decompose deltamethrin. Comparing pure strains and mixed cultures of bacteria, it was concluded that during the entire period of the experiment, heterogeneous cultures were, on

average, more effective in decomposing deltamethrin than pure strains. More effective biodegradation of deltamethrin in mixed cultures could be attributed to the prevalence of stimulating interaction over antagonistic interactions between organisms belonging to different genera and species. Chodynieski (1968), when analyzing various combinations of bacteria from the *Pseudomonas*, *Aeromonas*, *Vibrio*, and *Flavobacterium* genera, found that 12% of bacterial interactions were of antagonistic character, and 8% of stimulating. This latter type of interactions and, most probably, also bacterial synergism that occurred in heterogenous mixtures were responsible for the fact that these cultures decomposed deltamethrin more effectively than single strains of bacteria. Screening microorganisms with particularly high capacities for decomposing deltamethrin is very important in bioremediation of this type of pollutants. In spite of this, reports regarding isolation and identification of pure bacterial cultures capable of degrading deltamethrin are scarce and primarily pertain to soil environments. According to Maloney et al. (1988), pure cultures of the *Bacillus cereus* and *Pseudomonas fluorescens* species and bacteria from the *Achromobacter* genus are capable of decomposing deltamethrin with the concentration of 50 $\mu\text{g dm}^{-3}$ in the presence of Tween 80.

The half-time of deltamethrin in oxygen cultures of these microorganisms ranged from 21 to 28 days. Grant et al. (2002) 812 *Agnieszka Kalwasińska et al.* discovered that *Pseudomonas* sp. and *Serratia* sp. isolated from soil are useful in breaking down synthetic pyrethroids. Lee et al. (2004) isolated 56 bacterial strains capable of decomposing synthetic pyrethroids from bottom sediments polluted with pesticides. According to Tallur et al. (2007), deltamethrin is degradable by bacteria from the *Micrococcus* sp. This study demonstrated that among the planktonic bacteria, species *Sphingomonas paucimobilis* and microorganisms from the *Moraxella* genus were characterized by the highest ability to decompose the analyzed insecticide, while *Burkholderia cepacia* and *Bacillus mycoides* were the most effective among benthic bacteria. The bacterial species *Sphingomonas paucimobilis* and *Burkholderia cepacia*, which are characterized by an ability to decompose a wide spectrum of organic pollutants, including pesticides, are useful in bioremediation. *Sphingomonas paucimobilis* is, for example, capable of decomposing hexachlorocyclohexane and biosorption of cadmium (Pal et al. 2005, Tangaromsuk et al. 2002) and shows ability to biodegrade phenyl-methyl-ethers (Nishikawa et al. 1998). The *Burkholderia cepacia* species demonstrates exceptional capability to decompose many structurally complex organic compounds. The abilities of this microorganism to decompose 2,4,5-trichloroacetic acid (Daubras et al. 1996), benzo(a)pyrene, dibenz(a,h)anthracene, coronene (Juhász et al. 1997), p-nitrophenol (Bhushan et al. 2000), and other polyaromatic hydrocarbons (Kim et al. 2003) have been confirmed.

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