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Biodegradation of imidacloprid in an open compost pile

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Abstract

The aim of the study was to determine in which way and at what speed a composting process affects the degradation of imidacloprid N-{1-[(6-chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl} nitramide, one of the most effective and most widely used insecticide in the world, but also at the same time one of the most accused insecticide for bee colony collapse disorder. The process of imidacloprid degradation through composting was monitored at four experimental open piles, with an additional fifth pile composed of random green parts of plants and soil and used as a simulation of natural and spontaneous degradation of organic matter in a field. Two of the compost piles were kept under aerobic conditions and forced aerated through perforated pipes with a ventilator, while two were kept under anoxic conditions (the compost was wrapped in impermeable foil). *Pseudomonas aeruginosa* FN culture was added in two composting piles, one under aerobic conditions and one under anoxic conditions. It has been established that the half-life of imidacloprid occurred after 29 days in the pile inoculated with bacterial cells P. *aeruginosa* FN under anoxic condition, which is considerably faster than in spontaneous degradation in the soil, which was simulated in the fifth pile (60 days). The physical and chemical properties of compost piles did not significantly affect the dynamics of degradation rate, which was faster in the mesophilic and thermophilic phase and slower in the maturation phase. The half-life of imidacloprid was shorter in piles with anoxic condition. The added culture, *P. aeruginosa* FN, has accelerated the imidacloprid degradation rate in both piles with anoxic and aerobic conditions, faster than in the piles without the *P. aeruginosa* FN culture. That is lesser influence than was expected, probably because of the abundance of microorganisms from animal manure, which created the competitive relations with *P. aeruginosa* FN cultures.

Key words: Imidacloprid, composting process, dynamics of degradation, half-life time, aerobic, anoxic, *Pseudomonas aeruginosa* FN.

Introduction

Imidacloprid belongs to the group of pesticides called neonicotinoids and it has been highly efficient in pests control in agriculture. However, there are doubts about the negative effects of imidacloprid and some other active ingredients belonging to the group of neonicotinoids on pollinators [11]. Many crops are routinely treated with neonicotinoid insecticides as a seed dressing or as a foliar treatment [12]. Soil or seed applied plant protection products (PPPs) aim at bringing the amount of active substance involved to the only parts of the plant that have to be protected [2]. These compounds are systemic, migrating in the sap to all parts of the plant and providing protection against insect herbivores [18]. Despite a reduced exposure of non-target organisms by this way, an exposure of honey bees through residues in pollen and/or nectar may not be excluded for substances that migrate towards the upper plant parts [11]. In modern cereal farming systems, honey bees are readily exposed to pesticides because they rely heavily on common blooming crops, like oilseed rape (*Brassica napus*), maize (*Zea mays*) or sunflower (*Helianthus annuus*), which are now routinely treated against insect pests [13]. The most widely used of these compounds is imidacloprid, which is routinely used on most major crops including cereals, oilseed rape, corn, cotton, sunflower and sugar beets. Potentially, bees could come in touch with neonicotinoids through plant (nectar, pollen, guttation water, propolis and plant surface such as leaves, petioles, axils, etc.) and non-plant (droplets of spray and solid particles in air, water and/or soil compartments) routes of exposure [11]. Applying to the seeds, imidacloprid protects the roots and seedling of field crops after germination. The whole plant is protected during growth because, as a systemic pesticide, imidacloprid moves into all parts of the plant in various stages of growth. However, because of this mobility, bees become exposed to imidacloprid through pollen and nectar when they feed. Even at sub-lethal doses imidacloprid may cause the inhibition of acetylcholine receptors in the bees, and further have an impact on their orientation and colony collapse disorder [17]. Imidacloprid, which is used as a seed treatment in maize and oilseeds as well as other field crop production, can accumulate in soils with low humus content through the years [9]. In the flowering stage of subsequent crops grown in contaminated soils, it can provide a serious threat to the bees and other beneficial insects [4].

Composting of organic materials is the decomposition by which microorganisms rapidly consumes organic compounds. Main products of biological metabolism in presence of oxygen are CO_2, H_2O, microbial biomass, heat and compost [10]. The composting of organic materials in agriculture is the topic of many studies, which examine the influence of different materials [8] on composting process and quality of compost [16]. The subject of the study is the influence of different materials [7] on composting process and

quality of compost [16]. In a line with strong development of pesticide application, these studies tend to explain pesticides degradation mechanisms in soil [6] and the composting process. Studies have shown that microorganisms can use pesticides and imidacloprid as carbon source very successfully [6]. Published studies on *Pseudomonas* sp. 1G isolated from soil indicate that micro-organisms have a significant impact on nitro group degradation within neonicotinoids [14].

Composting agricultural crop residues that had been treated with imidacloprid were subjected to ecological and cost-effective way to reduce the accumulation of imidacloprid in the soil. At the same time, by adding compost during fertilization of crops, one increases microbial activity, which further contributes to degradation of imidacloprid and other pesticides accumulated in agricultural soils.

Materials and Methods

Raw material for composting: The research was carried out at the municipality bio waste disposal – ZG Holding, Zrinjevac Office, PJ Markuševac. All fresh and dry components were taken from biowaste disposal. The material for composting consisted of 1.53 m^3 horse manure, 0.32 m^3 vegetable waste, 0.84 m^3 compacted wet leaves, 4.8 m^3 dry leaves and grass and 0.2 m^3 soil. The total amount of composite material was 7.69 m^3. Raw materials for compost piles were selected from the plant material and manure manner as to ensure favourable C/N ratio (25-30:1) and acceptable humidity compost material of 60% RH [5]. This ratio is determined by the calculator for measuring compost (Compost Mixture Calculation Spreadsheets, Cornell Waste Management Institute). Laboratory measurements determined a lower C/N ratio of 23.74:1 that is close to the optimum C/N ratio (25:1) in the use of horse manure [1].

Composting conditions: Composting was set up in five wooden containers, 1m × 0.96 m ×1 m, four of them contained mixed raw compost materials, and one contained randomly selected plant material and soil (Fig. 1). Nutrient broth (5 L) was inoculated with bacterial cells *P. aeruginosa* FN and the overnight culture was grown on a rotary shaker at 37°C and 160 rpm. Inoculum was diluted 1:1 w/w in tap water and applied to the composting material, for two composting piles. The first composting pile was aerated without the addition of *P. aeruginosa* FN (AO). The second composting pile was aerated with the addition of *P. aeruginosa* FN (APS). The third composting pile was kept in anoxic conditions without bacterium (ANO) while in the fourth composting pile, *P. aeruginosa* FN (ANPS) was added under anoxic condition. The aeration was carried out by an air compressor (model Einhel BT-AC 270/50, 1.8 kW, Landau/Isar, Germany) connected to PVC tubes (6 cm Ø and 3 m long), perforated in two diametrically opposite lines, and placed at the bottom of the pile. Additionally, through the tubes water was added to maintain optimal moisture 60% during the composting process. Anoxic conditions were kept by means of a waterproof and photo-impermeable foil into which compost material was placed. The fifth compost pile (BL) was prepared for the simulation of spontaneous degradation of imidacloprid on agricultural land. It was composed of 0.3 m^3 dry leaves and grass, 0.3 m^3 compacted wet leaves, 0.1 m^3 vegetable waste and 0.2 m^3 soil with initial C/N ratio 1:32.7. Neither manure as a composting process activator [3] nor bacterium were added in the fifth compost pile. Formation of H_2O is one of the products of composting process. To collect drainage water under each compost pile glass containers were placed to determine the loss of imidacloprid. Dimensions of composting containers enabled the smooth running of the thermophilic phase in composting process [5].

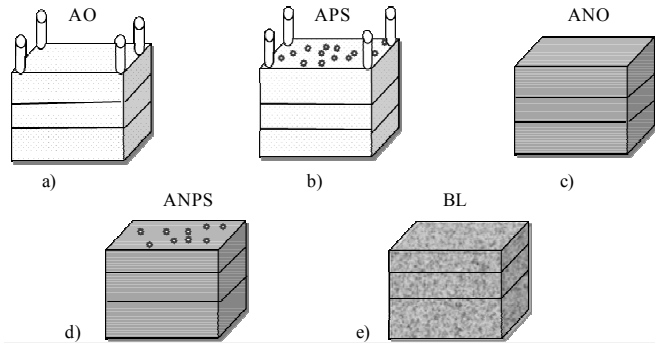

Figure 1. Design of composting piles: a) AO – aerobic conditions without bacterium, b) APS – aerobic conditions with bacterium, c) ANO – anoxic conditions without bacterium, d) ANPS – anoxic conditions with bacterium, e) BL – spontaneous degradation without bacterium and forced aerating.

Imidacloprid: A plant protection product with 20% of active substance imidacloprid, the Boxer© 200 SL (ChromosAgro, Croatia) was used. Four compost piles were prepared by dividing well mixed composting material and spread in a thin layer on the plastic sheet. The insecticide diluted in 250 ml of water was applied as 0.1 concentration. According to the manufacturer's instructions, the applied amount of 250 ml of diluted insecticides is enough to cover 40 m^2 of plant surface. The application of insecticide on the composting material carried out with an applicator nozzle, which allowed an even application. The treated material was several times mixed, to obtain good distribution of imidacloprid in the compost.

Monitoring and sampling: Monitoring of the composting process and sampling was planned to last 60 days. Temperature was monitored daily (at 10, 60 and 80 cm depth from the top) using a thermometer with probe (model TM 200, Kimo© Instruments, Montpon, France). Removable device, mode SPH, Agra, Čakovec, Croatia, was used to measure pH and the presence of oxygen was measured with oxygen meter model Greisinger GOX 100, Regenstauf, Germany, respectively. During the sampling period, laboratory tests were also carried out as a correction of the field measured results. During the composting process, samples were taken on a weekly basis. Samples (200 g, fresh weight) were taken after mixing the composting materials. Standard probe for pedological research of 1.20 m length with a transverse groove of 60 cm to collect samples was used. A composite sample was used to determine the amount of imidacloprid, and the other one to determine the changes of the pH and C/N ratio in composting material. All collected samples were stored at -18°C until laboratory processing. All analyses were carried out for two replicate samples.

The analytical procedures: Imidacloprid standard (purity 99.4%) molecular weight 255.7 g/ mol, supplied from the manufacturer SAF in Germany, was used as an external standard. For extraction process, a standard method [15] was used. Composting material (25 g) after drying at room temperature was ground in a mortar and sieved through 200 μm sieve. As extraction solvent, a mixture of

acetonitrile (HPLC grade) and water in a ratio 80:20 v/v was used. Extraction was performed on a shaker for two hours. After 10 min when the precipitate had settled, the extract was separated by decantation and filtered on a system for rapid filtration through blue ribbon filter paper. The extraction was repeated once more. The entire sample was filtered through the same system and the residues were rinsed with a solvent. The combined filtrate was evaporated for the most part on a rotary evaporator. Then the sample was filtered and injected in a HPLC-MS/MS system. For detection and quantification of imidacloprid in samples, HPLC-MS/MS method was developed on Agilent 1200 HPLC, which was connected to the mass spectrometer with triple quadrupole Agilent 6410 (Agilent Technologies, Santa Clara, CA). All samples were analysed in two replicates to ensure the accuracy and repeatability.

Statistical analysis: First-order kinetics analysis was used to estimate the degradation rate of imidacloprid. The statistical analysis of the results was performed using MicroMath Scientist Model File (Scientist© for Windows 3.0, MicroMath, Saint Louis, USA).

Results and Discussion

The initial temperature in the compost material ranged between 37°C (ANO) and 39°C (ANPS), depending on the composting pile (Fig. 2). Mesophilic phase (<45°C) lasted for four days which was followed by thermophilic phase. The highest temperature (62°C) in a thermophilic phase was recorded in the compost pile with anoxic conditions (ANO) at 60 cm depth. Maximum oxygen consumption was observed in the pile with anoxic condition and *P. aeruginosa* FN (ANPS) after three days when the oxygen content was only 3%. After the initial turbulent microbial activity and peak thermophilic phase, the measured oxygen content ranged between 15% (ANPS) and 20% (APS).

The initial C/N ratio in compost material was 23.74 (Fig. 3) and fell on 19.81 (ANPS) where lowest was recorded 16.8 (APS), respectively. Humidity of compost pile was kept by periodic addition of water through perforated pipes for aerating or by moistening.

The pH reaction in the starting material was 7.86 (Fig. 4). The increase of pH was recorded after 1 week in all piles up to a maximum of 9.1 (ANPS). Between the compost piles, there were no significant differences in pH value. The initial pH value increased in the beginning but thereafter slightly decreased between 8 and 9.

The fifth pile (BL) of randomly selected plant material and soil was also monitored. In this pile changes of temperature, pH and C/N ratio were recorded, which indicated the activity of microorganisms from soil and moist vegetables. The results obtained show difference from those in composting piles. The temperature increased only slightly from the start, and the pH range of 8.2 was reached after 2 weeks. After 60 days C/N ratio was 19.1. These results indicate that the process of degradation of organic matter was also present in this pile, although significantly slower than in the compost piles.

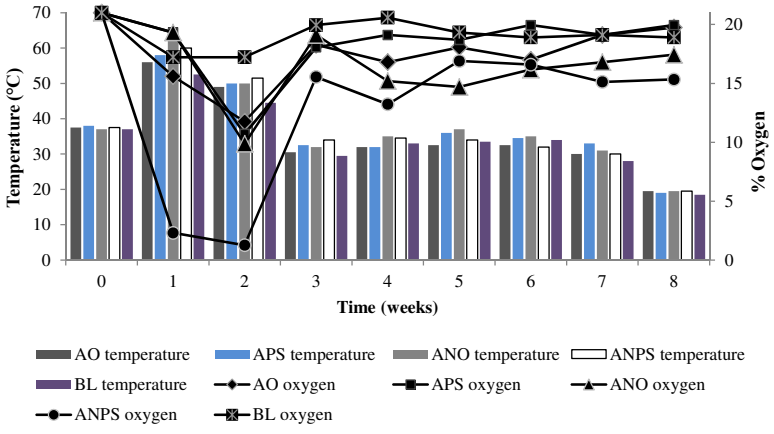

Figure 2. Relation between changes of temperature and oxygen consumption in composting piles during the monitoring period.
AO – aerobic conditions without bacterium, APS – aerobic conditions with bacterium, ANO – anoxic conditions without bacterium, ANPS – anoxic conditions with bacterium, BL – spontaneous degradation without bacterium and forced aerating.

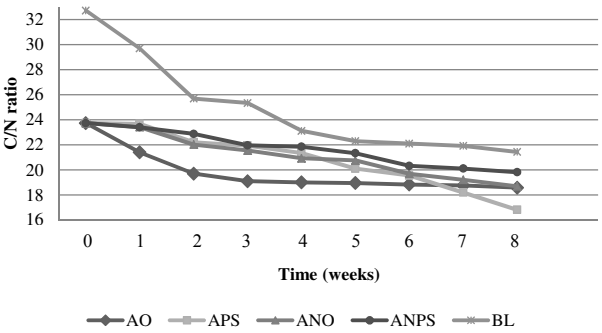

Figure 3. Changes of C/N ratio in the composting piles during eight weeks.

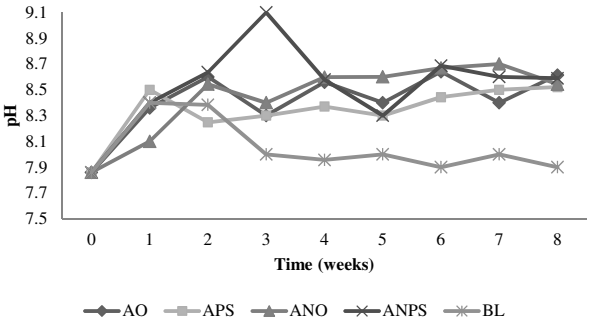

Figure 4. Changes in pH reaction in composting piles during eight weeks.

***Degradation rate of imidacloprid*:** The initial concentration of imidacloprid (Fig. 5) in primary composting material was 1.104 mg kg^{-1}. In the aerated compost pile (AO), at the end of eighth week records showed 0.404 mg kg^{-1}, in the aerated pile with the addition of *P. aeruginosa* FN (APS) 0.390 mg kg^{-1}, in the anoxic pile 0.286 mg kg^{-1} and in the anoxic pile with the addition *P. aeruginosa* FN it was 0.288 mg kg^{-1}. In pile with random stacks of various plants and soil, the final concentration of imidacloprid was 0.695 mg kg^{-1}, which was significantly higher than in the other compost piles, shown in Table 1. When comparing degradation rate within piles in aerobic condition and in anoxic condition, it is obvious that added bacterium slightly influenced on faster reduction of imidacloprid as is shown in Table 1. The half-life of imidacloprid in three compost piles was four weeks while in the compost pile with aerobic conditions without bacteria (AO) it was five weeks. In the pile with random

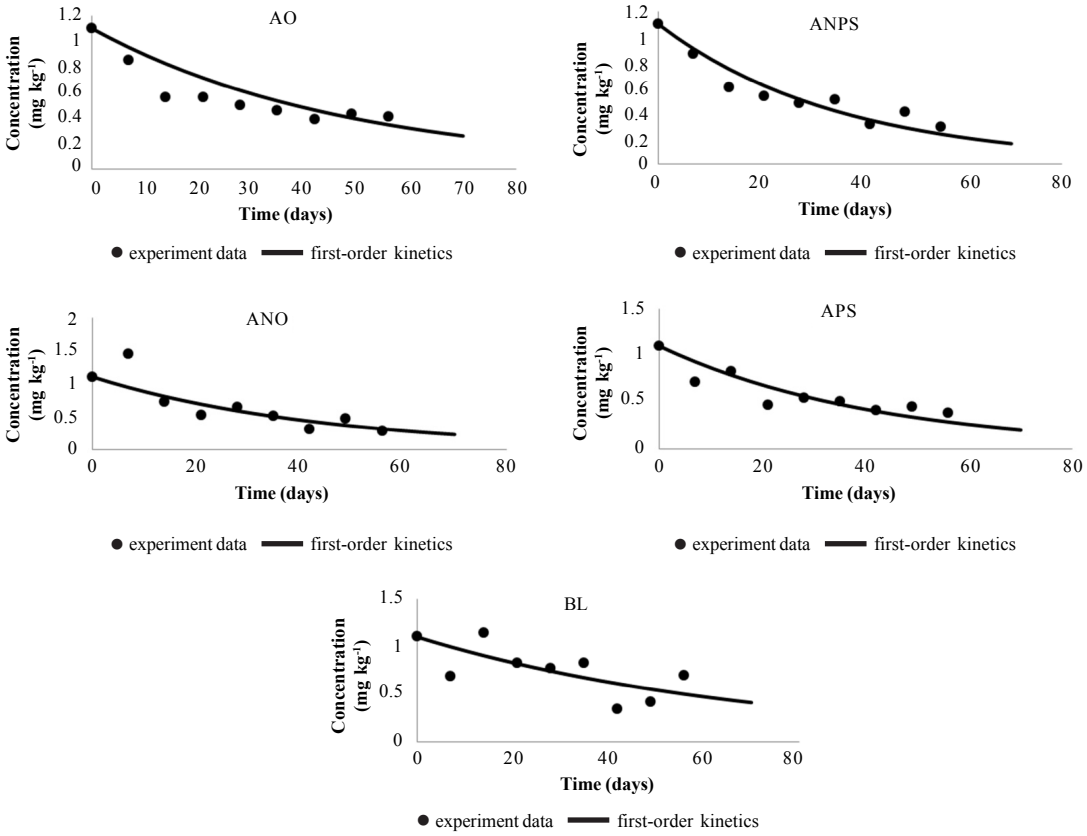

Figure 5. Estimation of experimental data with first-order kinetics model.

Table 1. The degradation rate of imidacloprid and half-life in days.

	kd (mg kg^{-1}/d)	SD	$t_{1/2}$ (days)
AO	0.0208	±0.0045	36
APS	0.0236	±0.0029	32
ANO	0.0224	±0.0046	34
ANPS	0.0278	±0.0025	29
BL	0.0141	±0.0033	60

stacks (BL), concentration of imidacloprid did not reach half-life within 60 days. Recently studies have reported degradation of about 70% of an initial concentration of 50 ppm imidacloprid by *Pseudomonas* cultures within 14 days [14].

The largest deviation of experimental data from the assumed mathematical model emerged in pile with a randomly selected material (BL) because of undishomogeneous of the material collected by the probe.

Relation of physical and chemical conditions of the compost piles and alterations in the degradation coefficient: In the observed test containers, there was no significant difference in the coefficient of degradation in its bearing on the physical and chemical characteristics composting process. The degradation rate of imidacloprid in all containers was carried out quite linearly regardless of the change in the dynamics of physical and chemical condition within the compost piles.

Conclusions

The degradation of imidacloprid in the composting process was significantly faster in compost piles than in the pile with spontaneous degradation (BL). A slightly faster half-life of imidacloprid occurred in compost piles with anoxic conditions. The addition of *P. aeruginosa* FN culture affected not significantly but slightly the degradation, probably because of competitive relations with microorganisms from animal manure and limited amounts of carbon as a nutrient. The research hypothesis that composting crop residues is an ecological and cost-effective way to reduce accumulated imidacloprid in the agricultural soils was confirmed. Results of this study showed significantly faster degradation of imidacloprid in compost piles than in pile that simulated spontaneous degradation in agricultural soil. Composting process can be used as treatment method for crop residues especially in a system of intensive agricultural production where imidacloprid is used to protect plants against harmful insects. At the same time, the compost as a final product of composting process can be returned back in agriculture soil surface as a fertilizer.

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References

[1]Airaksinen, S., Heinonen-Tanski, H. and Heiskanen, M. L. 2001. Quality of different bedding materials and their influence on the compostability of horse manure. J. Equine Vet. Sci. **21**:125-130.

[2]Alix, A., Chauzat, M. P., Duchard, S., Lewis, G., Maus, C., Miles, M. J., Pilling, E., Thompson, H. M. and Wallner, K. 2009. Guidance for the assessment of risks to bees from the use of plant protection products applied as seed coating and soil applications – conclusions of the ICPBR dedicated working group. Hazards of pesticides to bees – 10th

International Symposium of the ICP-Bee Protection Group (Eds. Oomen, P. A. and Thompson, H. M.). Julius-Kühn-Archiv **423**:15-27.

[3]Alkoaik, F. and Ghaly, A. E. 2006. Influence of dairy manure addition on the biological and thermal kinetics of composting of greenhouse tomato plant residues. Waste Manage. **26**:902–913

[4]Bonmatin, J. M., Moineau, I., Colin, M. E., Fleché, C. and Bengsch, E. R. 2000. Insecticide imidacloprid: availability in soils and plants, toxicity and risk for honeybees. Pesticides in Food and Drink, EPRW, 134 p.

[5]Epstein, E. 1997. The Science of Composting. Technomic Publishing Company, Lancaster, Pennsylvania, USA, 35 p.

[6]Fernández-Gómez, M. J., Nogales, R., Insam, H., Romero E. and Goberna, M. 2011. Role of vermicompost chemical composition, microbial functional diversity, and fungal community structure in their microbial respiratory response to three pesticides. Bioresour. Technol. **102**:9638–9645

[7]Fogarty, A. M. and Tuovinen, O. H. 2001. Microbiological degradation of pesticides in yard waste composting. Microbiol. Rev. **55**(2):225-233.

[8]Goyal, S., Dhull, S. K. and Kapoor, K. K. 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. Bioresour. Technol. **96**:1584–1591.

[9]Greatti, M., Sabatini, A. G., Barbattini, R., Rossi, S. and Stravisi, A. 2003. Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. Bulletin of Insectology **56**(1):69-72.

[10]Haug, R. T. 1993. The Practical Handbook of Composting Engineering. Lewis Publisher, Boca Raton, 287 p.

[11]Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., Aptel, J., Tchamitchian, S. and Decourtye, A. 2012. A common pesticide decreases for aging success and survival in honey bees. Science **336**:348-350.

[12]Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G. and Given, K. 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS ONE **7**:e29268.

[13]Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdrop, D. and Pettis, J. S. 2010. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS ONE **5**(3):e9754.

[14]Pandey, G., Dorrian, S. J., Russell, R. J. and Oakeshott J. G. 2009. Biotransformation of the neonicotinoid insecticides imidacloprid and thiamethoxam by *Pseudomonas* sp. 1G. Biochem. Biophys. Res. Commun. **380**:710–714.

[15]Placke, F. J. and Weber, E. 1993. Method of determining imidacloprid residues in plant materials. Pflanzenschutz Nachrichten Bayer **46**(2):109-182.

[16]Sanchez-Monedero, M. A., Roig, A., Paredes, C. and Bernal, M. P. 2001. Nitrogen transformation during organic waste composting by the Rudgers system and its effects on pH, EC and maturity of the composting mixtures. Bioresour. Technol. **78**:301-308.

[17]Stokstad, E. 2012. Field research on bees raises concern about low-dose pesticides. Science **335**:1555. .

[18]Whitehorn, P. R., O'Connor, S. O., Wickers F. L. and Goulson, D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Scienceexpress, http://www.sciencemag.org/content/early/recent /29 March 2012/ Page 1/ 10.1126/science.1215025.