

Review Article

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Microbial Biotransformation of Neonicotinoid Insecticides in Soil – A Review

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ABSTRACT

Neonicotinoid insecticides are widely used nowadays to control many sucking insect-pests in several horticultural crops. They are neurotoxic and systemic in nature and their indiscriminate use may affect both target as well as beneficial insects. They are persistent insecticides and can enter food chain through soil application because of high water solubility. Microbes play an important role in removing toxic insecticides from soil environment and microbial degradation can be considered to be a cost effective mechanism to detoxify the insecticides. This article focuses on microbial biotransformation of neonicotinoid insecticides in soil environment. Many bacterial strains have been isolated from soil, which are capable of degrading neonicotinoids to non-toxic compounds by using these insecticides as additional carbon source. Microbes can fasten the transformation of insecticides in soil and thereby reducing the chance of their entry into food chain. Studies have indicated that enhanced biodegradation of neonicotinoids can be achieved with microbial consortium under favourable environmental conditions. However, substantial research on identification of neonicotinoids-degrading microbial strains and identification of the genes and enzymes responsible for their degradation need to be carried out to understand the transformation pathways and advance bioremediation efforts.

Keywords

Neonicotinoid insecticides, Biodegradation, Bacteria, Soil environment

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Introduction

During the last two decades neonicotinoid insecticides have become the most widely used, popular and fastest growing class of insecticides in modern agriculture including horticulture. They are broad spectrum systemic insecticides used to control many sucking and some chewing pests *viz.* aphids, thrips, jassids, mites, whiteflies, leaf miners,

leaf hoppers, vine weevil, etc. With a global market share of >25% and spread in 120 countries, neonicotinoids are proved to be the most important new class of synthetic insecticides. The name neonicotinoids are derived from nicotine and they are relatively new to market compared to other already established organochlorines, organophosphates, carbamates and synthetic pyrethroids insecticides. They act by binding

strongly to nicotinic acetylcholine receptors (nAChR) in the central nervous system of insects causing over stimulation of their nerve cells, paralysis and death. Being highly water soluble and systemic in nature, they can migrate into all parts of treated plants. Neonicotinoids can be divided into three major groups:

Chloropyridinyl compounds (imidacloprid, nitenpyram, acetamiprid and thiacloprid)

Chlorothiazolyl compounds (thiamethoxam, clothianidine, imidaclothiz)

Tetrahydrofuryl compounds (dinotefuran)

Imidacloprid is the first neonicotinoid insecticide marketed by Bayer in 1992 and is the most widely used insecticide worldwide. Because of their specific mode of action and low resistance development among insects, neonicotinoids are continually used in agricultural and horticultural crops (Table 1). Due to this versatility in physicochemical properties, many types of agricultural applications including foliar spray, seed treatment, soil drench and stem injection are possible with them. Seed treatment with neonicotinoids is a proven and effective plant protection technique resulting not only in the increase in efficiency in protection but also in the reduction of labour cost. About 60% of these insecticides are applied as seed treatment especially for transgenic crops expressing *Bacillus thuringiensis* (Bt) toxin genes, as the treatment protects the plant seedlings before production of sufficient Bt toxin to provide effective pest resistance (Jeschke *et al.*, 2011).

Imidacloprid, the first insecticide registered from this group, can be used as seed dressing, as soil treatment and foliar treatment in different crops like rice, cotton, cereals, maize, mango, sugar beet, vegetables, etc. to

control sucking insects, soil insects, termites and some biting insects (Elbert *et al.*, 1998). The IUPAC name for imidacloprid is [1-(6-chloro-3-pyridinyl methyl)-N-nitro-2-imidazolidinimine] and its chemical formula is $C_9H_{10}ClN_5O_2$. Acetamiprid is another insecticide from this group which was first registered during 1989 by Nippon Soda. Its chemical formula is $C_{10}H_{11}ClN_4$ and IUPAC name is N -[(6-chloro-3-pyridyl)methyl]-N'-cyano-N-methyl acetamide. This insecticide is used to control aphids, thrips, mirids, spider mites, whiteflies, European pine sawflies, leaf miners, leaf hoppers and vine weevil in leafy and fruiting vegetables, fruits like apple, citrus, pears, grapes, cotton, ornamental plants and flowers (Yao *et al.*, 2006). Another compound from chloropyridinyl group is thiacloprid whose IUPAC name is [(2Z)-3{(6-chloropyridin-3-yl) methyl}-1,3-thiazolidin-2-ylidene] cyanamide and chemical formula is $C_{10}H_9ClN_4S$. It is effective against aphids, codling moth, leaf hoppers, leaf miners, psylla and whiteflies in potatoes, rapeseed, pome fruit, vegetables and ornamentals (Schuld and Schmuck, 2000). The fourth chloropyridinyl compound is nitenpyram which is a C-nitro compound consisting of 2-nitroethene-1,1-diamine where one of the nitrogen bears ethyl and (6-chloro-3-pyridinyl) methyl moieties and the other nitrogen carries a methyl moiety. Its chemical formula is $C_{11}H_{15}ClN_4O_2$ and IUPAC name is (E)-N-(6-chloro-3-pyridyl methyl)-N-ethyl-N'-methyl-2-nitrovinylidenediamine. Nitenpyram is used mainly to kill fleas on dogs, puppies, cats and kittens (veterinary purpose) and less in agriculture (Plumb, 2015). Thiamethoxam is a second generation chlorothiazolylmethyl neonicotinoid insecticide discovered and registered by Syngenta Crop Protection in 1996. Its IUPAC name is 3-[(2-chloro-1, 3-thiazol-5-yl)methyl]-5-methyl-N-nitro-1,3,5-oxadiazinan-4-imine and chemical formula is $C_8H_{10}ClN_5O_3S$. Thiamethoxam can effectively be used to control hopper, seed

weevil, scale insect and mealy bug in mango, other sucking soil and leaf-feeding pests like aphids, jassids, thrips and whitefly in vegetables, ornamentals, coffee, cotton, tropical plantations, rice and potatoes (Elbert *et al.*, 2008). Like imidacloprid, it can also be used as foliar application, seed treatment and soil treatment. Clothianidin is another second generation neonicotinoid which is found effective against a wide variety of insects from Hemiptera, Thysanoptera, Diptera, Coleoptera and Lepidoptera families in various agricultural crops at small doses (Jeschke *et al.*, 2011). The relatively less used and recently developed third chemical from second generation neonicotinoid is imidaclothiz whose chemical formula is $C_7H_8ClN_5O_2S$ and IUPAC name is (EZ)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-N-nitroimidazolidin-2-ylideneamine. It is found effective against sucking and chewing insect pests like aphids, plant hoppers, whitefly, leaf hoppers, beetles, etc. on various crops like vegetables including crucifers, tomatoes, citrus fruit, rice and tea (Liu *et al.*, 2013). The last and third generation neonicotinoid commercialized by Mitsui Chemicals (Tokyo, Japan) in 1994 is dinotefuran. It is used for the control of aphids, whiteflies, thrips, leafhoppers, leafminers, sawflies, mole cricket, white grubs, lacebugs, billbugs, beetles, mealybugs, and cockroaches in/on leafy vegetables (except Brassica), in residential and commercial buildings, and for professional turf management (USEPA, 2004). It is also used in veterinary medicine. Its IUPAC name is 2-methyl-1-nitro-3-[(tetrahydro-3-furanyl) methyl] guanidine and chemical formula is $C_7H_{14}N_4O_3$.

Persistence and fate of neonicotinoids in soil

The persistence of neonicotinoid insecticides in soil depends mainly on environmental conditions and varies accordingly.

Temperature, pH, moisture content, organic matter, soil structure and soil texture are some of the environmental factors affecting the degradation of these insecticides. Besides these, the nature of the insecticide, initial concentration and type of formulation used can also affect their persistence in soil. Among the neonicotinoid insecticides, imidacloprid and clothianidin are very highly persistent in soil with half-life ranging from 28–1250 and 148–6931 days, respectively; thiamethoxam and acetamiprid are moderate to highly persistent with half-life ranges from 7–353 and 31–450 days, respectively; thiacloprid and dinotefuran are less persistent with half-life of <90 days in soil (Goulson, 2013). In a laboratory study the half-life values of imidacloprid in three different types of soil (alluvial, laterite and coastal alkaline) were found between 34-45, 28-44 and 36-48 days, respectively, and it was found persistent up to 120 days in all three soils (Sarkar *et al.*, 2001). A conversion of 75 per cent of the applied dose (90 g/ha) of imidacloprid to four different metabolites in sugar beet field soil was reported by Rouchaud *et al.*, (1994) where residual half-life was found to be 40 to 44 days without the application of any organic fertilizer. Imidacloprid and its metabolites become strongly bound to soil with the passage of time and thereby increasing the risk of their persistence (Cox *et al.*, 1997; 1998). However, indirect application of imidacloprid (sprayed to mango trees) can lead to lower persistence in soil with a half-life of 17.5 days (Bhattacharjee *et al.*, 2019). Soil organic matter content has an impact on the sorption of imidacloprid and its metabolites as evidenced by increasing sorption with increasing soil organic matter content which is significantly correlated (Liu *et al.*, 2006). Thiamethoxam has low soil sorption and high leaching capability which makes it a potential contaminant of surface and underground water (Gupta *et al.*, 2008). However, literature on bioavailability and

sorption studies for other neonicotinoid insecticides is very sporadic.

Environmental risks of neonicotinoids to non-target taxa

Due to their high persistence and potential harmful effects on beneficial and non-target taxa, the use of neonicotinoid insecticides is currently generating concerns for the environment. Neonicotinoids are water soluble and possibility of leaching to soil water or ground water is always there though fishes are found less susceptible as compared to aquatic insects with LC_{50} values between 16 and 177 ppm depending on type of insecticides (Goulson, 2013). Being systemic in nature they are easily absorbed by plant root/leaves and translocated to other plant tissues via phloem/xylem without discriminating between harmful insects and beneficial insects (Krupke *et al.*, 2012). Small amount of these insecticides can be found in pollen and nectar of seed-treated crops. They are also used to control many insect-pests in various fruit crops as foliar spray e.g. imidacloprid and thiamethoxam in mango, thiamethoxam in peach, thiacloprid in raspberries, etc. These fruit crops are pollinated either by cultured pollinators or by wild ones which can be affected by neonicotinoids. Pollinators can also be intoxicated when these insecticides are sprayed to vegetables and flowers in kitchen garden. Neonicotinoids can induce mortality to both honeybees and other pollinators like bumblebees, butterflies, dragonflies, wild bees, melipona bees, lacewings, lady bugs, bats, etc. (Krischik *et al.*, 2007). However, supportive research data on bad/lethal effect of neonicotinoids on pollinators is not available. Cresswell (2011) has reported that imidacloprid at field-realistic dose under laboratory and semi-field conditions have very little lethal effect on honeybees. The recent data suggest that field-realistic

exposure of bees to neonicotinoids present in nectar and pollen of seed-treated crops may not cause any substantial direct mortality (Marzaro *et al.*, 2011; Tapparo *et al.*, 2012). This type of research has not been conducted on other pollinating taxa like hoverflies and butterflies and mainly concentrates on the exposure of honeybees to seed-treated crops though there is a possibility of direct mortality if pollinators forage on crops which are treated with neonicotinoids mixed in irrigation water or as foliar spray. Important sublethal effects may occur to bees when exposed to sublethal doses of neonicotinoids which include reduced learning, less foraging ability and homing ability in both honeybees and bumblebees (Yang *et al.*, 2008; Han *et al.*, 2010; Mommaerts *et al.*, 2010; Henry *et al.*, 2012).

Many researches have been conducted to examine the toxicity of neonicotinoid insecticides to both target and non-target organisms *viz.* insects, birds, fishes, crustaceans, molluscs and mammals and insects are found as the most sensitive organisms, whether exposed via contact or ingestion. The most and least sensitive insects are brown plant hopper (*Nilaparvata lugens*) and Colorado potato beetle (*Leptinotarsa decemlineata*) whose weights and LD_{50} values are 1 and 130 mg and 0.82 and 0.67 mg/mg body weight, respectively, indicating that variation in LD_{50} values depends of the weight of the particular insect (Goulson, 2013). Though the experiments over short period assess only mortality of insects, there are proof for important sublethal effects e.g. reduced feeding, less movement and reproduction, damaged immune system can be happened with much lower doses also as suggested by Alexander *et al.*, (2007) in case of mayfly (*Epeorus longimanus*) where feeding was shortened for 4 days after exposure to water containing 0.1 ppb of imidacloprid for 24 h. Crustaceans, annelids

and vertebrates are less susceptible than insects, though studies on toxicity of neonicotinoids to these groups of taxa are few. Goulson (2013) has reported that LC₅₀ values for these insecticides ranged between 7.1 ppb (over 28 days) in the amphipod *Hyalella azteca* to 361 ppm (over 48 h exposure) in the brine shrimp *Artemia* sp. He has also reported that LD₅₀ value in rats varies from 140 mg/kg body weight for acetamiprid to 5000 mg/kg body weight for clothianidin. Birds, especially insectivorous birds, are directly or indirectly affected by these insecticides with LD₅₀ values ranging between 14 mg/kg body weight for imidacloprid in grey partridge and 1333 mg/kg body weight in mallard ducks for clothianidin. For aquatic animals, fishes are comparatively less susceptible than aquatic insects with LC₅₀ values varying from 16 to 177 ppm. When neonicotinoids are used as seed treatment, only 1.6 to 20 per cent of active ingredient is absorbed by the crop to protect it from target insect-pests, whilst the remainder pollutes the surrounding environment (Sur and Stork, 2003) damaging mainly soil microorganisms (Sabourmoghaddam *et al.*, 2011) along with populations of earthworms, amphibians and aquatic insects (Kreutzweiser *et al.*, 2008; van der Sluijs *et al.*, 2014).

Though reviews discussing the environmental fate of neonicotinoids in details are available (Bonmatin *et al.*, 2015), microbial biotransformation of neonicotinoids in soil is recently investigated topic of interest to reduce the persistence of these insecticides in soil. This chapter focuses on microbial biodegradation of neonicotinoid insecticides in soil either by single isolated bacterium or by a microbial consortium as microbial biodegradation may hold the key to successful bioremediation of the widespread neonicotinoids contamination of soil environment.

Microbial degradation of neonicotinoid insecticides

Microbial degradation of neonicotinoids mostly deals with the degradation by bacteria—either by pure culture or by consortia. Microbial degradation of imidacloprid mainly revolves around the bacterial degradation of imidacloprid as it is the widely used neonicotinoid insecticide compared to other members of this group. Few researchers have studied the same for thiamethoxam, acetamiprid and thiacloprid. However, almost nothing has been done for clothianidin, nitenpyram and dinotefuran till date. Biodegradation of neonicotinoids by bacterial strains can be either catabolic, where the insecticide acts as a sole source of carbon/nitrogen for bacterial growth and development, or cometabolic, where biodegradation depends on both insecticide and supplementary or additional source of carbon or nitrogen. The factors affecting the bacterial degradation of a pesticide or a group of pesticides are chemical structure of pesticide and the catabolic activity of the degradation bacteria under some particular environmental conditions (Hussain *et al.*, 2016).

Imidacloprid

Many bacterial strains were isolated and identified with imidacloprid degrading potential (Table 2). First report on isolation of imidacloprid degrading microorganism was probably published by Anhalt *et al.*, (2007) where the authors isolated *Leifsonia* sp. strain PC-21 from agriculture soil and found that it was able to degrade imidacloprid in tryptic soy broth 37-58 per cent at 27°C after 21 days of incubation. High performance liquid chromatography coupled with mass spectroscopy (LC-MS) analysis revealed the formation of 6 metabolites from degradation pathway among which two were identified as

imidacloprid-guanidine and imidacloprid-urea. The authors also reported that 6-chloronicotinic acid was not detected during the experiment. They have also mentioned that degradation of imidacloprid by strain PC-21 was a process of cometabolism which means imidacloprid can be metabolized without being used by the bacteria as a source of energy, carbon or nutrient. Pandey *et al.*, (2009) have reported that *Pseudomonas* sp. 1G has the ability to transform imidacloprid and thiamethoxam to nitrosoguanidine and urea via aldehyde oxidase enzyme activity using glucose as supplementary carbon source. Dai *et al.*, (2010) have observed that imidacloprid can be transformed to olefin metabolite via hydroxylation and dehydrogenation by the bacterial isolate *Stenotrophomonas maltophilia* CGMCC 1.178 with the help of glucose. An aerobic bacterium, isolated from agriculture field soil by enrichment culture and identified as *Burkholderia cepacia* strain CH9, was found capable of degrading imidacloprid (69% of 50 µg/g) within 20 days of inoculation to a mineral-salts medium (Gopal *et al.*, 2011). Shetti and Kaliwal (2012) have isolated *Brevundimonas* sp. MJ15 (SP-1) from cotton field soil with a history of imidacloprid exposure which can degrade imidacloprid through catabolic reaction in liquid minimal salt medium. Phugare *et al.*, (2013) have studied cometabolic degradation of imidacloprid by *Klebsiella pneumoniae* BCH-1 and concluded that 6-chloronicotinic acid (6-CNA) was the final product of imidacloprid biotransformation via nitrosoguanidine and guanidine intermediates identified by gas chromatography-mass spectroscopy (GC-MS).

Hu *et al.*, (2013) have isolated a gram negative rod shaped bacterium, *Ochrobacterium* sp. strain BCL-1, from tea rhizosphere soil which can catabolically degrade 67.67 per cent of 50 mg/L

imidacloprid within 48 hours of application. The authors have also noticed that the biodegradation rate of imidacloprid by strain BCL-1 is significantly higher in tea soil compared to cabbage, potato and tomato soil. Ma *et al.*, (2014) have noticed the formation of olefin and 5-hydroxy imidacloprid metabolites during cometabolic biotransformation of imidacloprid by a soil isolated bacteria *Pseudoxanthomonas indica* CGMCC 6648. Akoijam and Singh (2015) have noticed that dissipation of imidacloprid followed pseudo first-order kinetics after applying at 50, 100 and 150 mg/kg in sandy loam soil amended with *Bacillus aerophilus* with respective half-life values of 14.33, 15.05 and 18.81 days. Imidacloprid urea, olefin, 5-hydroxy imidacloprid, 6-CNA, nitrosimine and nitroguanidine were identified by HPLC as metabolites. A soil isolated bacterium *Bacillus weihenstephanensis* can catabolically degrade imidacloprid to 6-CNA in minimal salt medium and tryptic soy broth up to 46 and 78 per cent, respectively, in four weeks (Shetti *et al.*, 2014). Among 50 bacterial isolates, collected from soils of vegetable forming areas, *Rhizobium* sp. showed the maximum imidacloprid degradation potential (45.48%) and *Bacillus* sp. the minimum (25.36%) (Sabourmoghaddam *et al.*, 2015). *Mycobacterium* sp. strain MK6 was found capable of converting 99.7 per cent added imidacloprid (150 µg/mL) in less than 2 weeks ($t_{1/2} = 1.6$ days) to 6-CNA as its major metabolite and desnitro-olefin and desnitro-degradates as minor metabolites by using imidacloprid as sole nitrogen source (Kandil *et al.*, 2015). Sharma *et al.*, (2016) have reported that *Bacillus aerophilus* has maximum potential to degrade imidacloprid in clay loam soil under autoclaved condition with 93.45, 95.41 and 95.02 per cent degradation from 50, 100 and 150 mg/kg doses, respectively, compared to degradation under unautoclaved condition (80.93, 87.57

and 85.95% from respective doses) after 56 days. *Enterobacter* sp. strain ATA1, isolated from paddy field soil at Punjab (India) with a history of 9-10 years of imidacloprid contamination, was found able to degrade imidacloprid as a co-metabolite in the presence of glucose in minimal salt medium. The degradation ranged between 30-40 per cent after 72 h of incubation resulting imidacloprid urea and imidacloprid guanidine as metabolites (Sharma *et al.*, 2014). Ganvir and Sathe (2018) have observed that among 20 isolates from contaminated agricultural soil, *Bacillus* sp., *Azotobacter* sp., *Azospirillum* sp. and *Pseudomonas* sp. showed degradation potential of imidacloprid after 48-72 hours of incubation in minimal salt medium. Concentration of imidacloprid degraded by *Azospirillum* sp. was up to 500 mg/L, whereas for other three bacteria the concentration was up to 200 mg/L. Imidacloprid can be degraded by *Pseudomonas* sp. up to 97 per cent in mango orchard soil after 28 days of application at 8 mg/kg (Garg *et al.*, 2018). Proposed degradation pathways of imidacloprid by various microorganisms are presented in Figure 1.

However, several metabolites produced during microbial biotransformation of imidacloprid in soil are more toxic and persistent than imidacloprid itself. Three widely reported metabolites are olefin, 4-hydroxy imidacloprid and 5-hydroxy imidacloprid. Both 4-hydroxy and 5-hydroxy imidacloprid can easily be converted to olefin, which is 10 times more toxic to insects and mammals than imidacloprid (Nauen *et al.*, 1999; Suchail *et al.*, 2004).

Acetamiprid

The second neonicotinoid insecticide which was studied for microbial degradation is acetamiprid. Two microbes were identified

for acetamiprid biotransformation in soil – *Stenotrophomonas* sp. strain THZ-XP and *Pigmentiphaga* sp. strain AAP-1 (Tang *et al.*, 2012; Wang *et al.*, 2013b). The authors have reported that both the bacterial strains could transform acetamiprid into N-methyl-(6-chloro-3-pyridyl) methylamine (ACE). In fact *Pigmentiphaga* sp. strain AAP-1 could utilize acetamiprid as a sole carbon, nitrogen and energy source, but with low growth rates (Wang *et al.*, 2013b). Though ACE was identified as N-deacetylation metabolism product by FT-IR, GC-MS and NMR analysis, but a full mineralization/degradation pathway was yet to be finalized. Cometabolism of acetamiprid by *Rhodococcus* sp. strain BCH 2 was studied in the presence of ammonium chloride and glucose as nitrogen and carbon sources, respectively (Phugare and Jadhav, 2015). Both ACE-VI and 6-CNA were detected as acetamiprid biodegradation products by GC-MS analysis. Restriction of acetamiprid biodegradation by bacterial strain at high concentrations was also described by Wang *et al.*, (2013a) using *Ochrobactrum* sp. D-12 which is capable of degrading acetamiprid at concentrations from 0 to 3000 mg/L within 48 h of incubation. The authors used Haldane inhibition model to fit the degradation rate at different concentrations and calculated maximum specific acetamiprid degradation rate (q_{max}) as 0.6394 for 6 h, half-saturation constant (K_s) as 50.96 mg/L and the substrate inhibition constant (K_i) as 1879 mg/L. Cometabolism of acetamiprid by *Pseudoxanthomonas* sp. strain AAP-7 in the presence of glucose as alternate carbon source was also studied where (E)-3-(((6-chloropyridin-3-yl) methyl) methyl) amino) acrylonitrile and N-(((6-chloropyridin-3-yl) methyl)-N-methylprop-1-en-2-amine were identified as hydrolytically demethylation product and both converting to ACE, reported as a dead-end product (Wang *et al.*, 2013c). Biodegradation kinetics of acetamiprid for

Pseudoxanthomonas sp. strain AAP-7 using concentrations ranging from 100 to 600 mg/L was also reported by the authors where degradation decreased with the increase in concentration after 60 h of incubation. Fusant-AC, an intergeneric fusion from *Pigmentiphaga* sp. strain AAP-1 and *Pseudomonas* sp. CTN-4 was constructed using protoplast-fusion technique and studied for degradation of acetamiprid and chlorothalonil (Wang *et al.*, 2016). The fusant strain AC completely degraded 50–300 mg/L concentrations of acetamiprid within 5 h indicating a strong capability for acetamiprid degradation.

A substrate inhibition model was used to describe the degradation kinetics of acetamiprid by bacterium *Stenotrophomonas maltophilia* CGMCC 1.1788 where it was found transformed with a maximum specific degradation rate, half-saturation constant and inhibit constant of 1.775/36 h, 175.3 mg/L and 396.5 mg/L, respectively, explaining that the rate of degradation of acetamiprid was restrained at high concentration (Chen *et al.*, 2008). Dai *et al.*, (2010) have reported that yeast *Rhodotorula mucilaginosa* strain IM-2 was able to degrade acetamiprid in sucrose mineral salt medium with half-lives of 3.7 days, while it did not degrade imidacloprid and imidaclothiz. Identification of metabolites indicated that the yeast selectively converted acetamiprid by hydrolysis to form an intermediate metabolite IM 1-3 (Figure 2). The yeast strain displayed biodegradability of acetamiprid in clay soils. In a partial cometabolic pathway for acetamiprid biodegradation by *Pigmentiphaga* sp. strain D-2 proposed by Yang *et al.*, (2013), three metabolites namely N'-[(6-chloropyridin-3-yl) methyl]-N-methylacetamide, N'-cyano-N-methyl-N-(pyridin-3-ylmethyl) ethanimidamide and N-methyl (6-chloro-3-pyridyl) methylamine were identified by LC-MS analysis. The authors have also reported

that a dechlorinated metabolite was detected for the first time in bacterial degradation of acetamiprid by LC-MS analysis and release of chloride ions during biodegradation. Zhou *et al.*, (2014b) have mentioned that the nitrile hydratase enzyme of *Ensifer meliloti* CGMCC7333 is capable of degrading acetamiprid to an unstable metabolite N-amidoamide which further degrades to chlorinated pyridyl methylmethanamine compound (Figure 3). Some others possible transformation pathways of acetamiprid by different microorganisms are presented in Figure 1.

Thiacloprid

Hydroxylation of thiacloprid to 4-hydroxy thiacloprid by bacterium *Stenotrophomonas maltophilia* CGMCC1.1788 as a cometabolite with or without sucrose as a carbon and energy source has been reported in literature (Zhao *et al.*, 2009). Tenfold increase in the efficiency of the bacterium was observed due to the presence of sucrose. Though 4-hydroxy thiacloprid does not convert to thiacloprid olefin under acidic condition, under alkaline condition it is oxidized and decyanated to form 4-ketone thiacloprid imine. Dai *et al.*, (2010) have found that yeast *Rhodotorula mucilaginosa* strain IM-2 was able to degrade thiacloprid in sucrose mineral salt medium with half-lives of 14.8 days. Identification of metabolites indicated that the yeast selectively converted thiacloprid by hydrolysis of thiacloprid to form an amide derivative. The inoculated *R. mucilaginosa* IM-2 displayed biodegradability of thiacloprid in clay soils. The hydrolysis of the N-cyanoimino group to a N-carbamoylimino group containing metabolite (thiaclopride amide) is supposed to be the major degradation pathway of thiacloprid by a bacterium *Variovorax boronicumulans* strain J1 (Zhang *et al.*, 2012). Expression of nitrile hydratase enzyme from *V. boronicumulans* by the resting cells of

Escherichia coli can confirm the biodegradation of thiacloprid to thiacloprid amine by *V. boronicumulans*. Mediation of the major hydration pathway of thiacloprid biotransformation by nitrile hydratase enzyme activity was also proposed by the authors, similar to the biotransformation of acetamiprid by *Ensifer meliloti* CGMCC7333 as suggested by Zhou *et al.*, (2014b). Nitrogen fixing bacterium *E. meliloti* CGMCC7333 is also capable of transforming thiacloprid into N-carbamoylimine derivative presumably via the same nitrile hydratase enzyme activity (Ge *et al.*, 2014). The biodegradation rate of thiacloprid varied from 0.11 to 2.89 $\mu\text{g/mL/h}$ with *E. meliloti* CGMCC7333, which hydrolysed thiacloprid to thiacloprid amide most rapidly. Therefore, it can be suggested that acetamiprid and thiacloprid share a common biodegradation pathway involving nitrile hydratase enzyme, which can provide an excellent opportunity to study microbial biotransformation pathway of neonicotinoid insecticides through expression of this enzyme in non-host bacteria. Proposed microbial biodegradation pathways of thiacloprid by numerous microorganisms are presented in Figure 4.

Thiamethoxam

Pandey *et al.*, (2009) have mentioned that *Pseudomonas* sp. strain 1G is able to degrade thiamethoxam by producing the same 'magic-nitro' ($=\text{N}-\text{NO}_2$) group metabolites, the same way it transforms imidacloprid. The magic-nitro group of thiamethoxam was converted to nitrosoguanidine, desnitrosoguanidine and urea metabolites by pure bacterial culture of *Pseudomonas* sp. strain 1G under microaerophilic growth conditions when supplemented with 10 mM glucose. This study indicated that magic-nitro group of both imidacloprid and thiamethoxam might be transformed by bacterial enzymes in a non-specific fashion. Another study by Zhou *et*

al., (2013) on biotransformation of thiamethoxam by the nitrogen-fixing and plant growth promoting rhizobacterium *Ensifer adhaerens* strain TMX-23 has also suggested that the transformation of N-nitroimino group ($=\text{N}-\text{NO}_2$) to N-nitrosoimine or nitrosoguanidine ($=\text{N}-\text{NO}$) and urea ($=\text{O}$) metabolites was the major metabolic pathway of thiamethoxam biodegradation. Biodegradation of thiamethoxam (50 $\mu\text{g/mL}$) in agricultural soil by *Bacillus aeromonas* strain IMBL 4.1 and *Pseudomonas putida* strain IMBL 5.2 was reported to be 45.28 and 38.23 per cent, respectively, in 15 days (Rana *et al.*, 2015). Biodegradation of thiamethoxam in clay loam soil by *Bacillus aerophilus* strain IMBL4.1 has also been reported very recently with half-life values ranging from 11.15 to 12.54 days for 25, 50 and 100 mg/kg doses (Rana and Gupta, 2019). Microbial biotransformation pathways of thiacloprid by different microbes are presented in Figure 4.

Biodegradation of neonicotinoids by microbial consortium

Now-a-days researchers are exploring the idea of using microbial consortia and unculturable microbes for biodegradation of neonicotinoid insecticides in soil. Results showed that microbial consortia along with unidentified microbes might play a significant role in rapid *in situ* biodegradation of insecticides in soil. Microbial degradation of four neonicotinoid insecticides imidacloprid, acetamiprid, thiacloprid and imidaclothiz in soil was studied by Liu *et al.*, (2011). Much faster degradation for acetamiprid and thiacloprid (94.0 and 98.8%, respectively) was observed within 15 days compared to imidacloprid (22.5%) and imidaclothiz (25.1%) in unsterilized soils after 25 days. In sterile soils, the degradation rates were much slower for these insecticides (21.4, 27.6, 9.0 and 0% for acetamiprid, thiacloprid, imidaclothiz and

imidacloprid, respectively). The degradation products identified were olefin, nitrosoguanidine metabolites for imidacloprid and imidaclothiz and an amide metabolite for thiacloprid). A consortium of four bacteria *Bacillus subtilis* GB03, *Bacillus subtilis* FZB24, *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* SE34 was reported capable of degrading 11-22 per cent of thiamethoxam in liquid culture medium (Myresiotis *et al.*, 2012). Sharma *et al.*, (2014) have reported that biodegradation of imidacloprid by a consortium of *Bacillus aerophilus* and *Bacillus alkalinitrilicus* led to the formation of 6-CNA and imidacloprid nitrosoguanidine as metabolites where 50, 100 and 150 mg/kg doses of imidacloprid can be degraded in clay loam soil under autoclaved condition with half-life ranging from 14-16 days after 56 days of treatment.

Degradation of imidacloprid in soil was 69 per cent by a consortium of three bacteria isolated from agricultural field soil of Uttarakhand, India after 20 days as compared to only 15 per cent degradation in control soil (Negi *et al.*, 2014). However, imidacloprid degradation in soil slurry was 3.6 times higher in consortium than in control (76 and 21%, respectively). Shaikh *et al.*, (2014) have reported that a consortium of four *Bacillus sp.* showed maximum degradation of imidacloprid between 48-72 hours after incubation and 6-CNA was the degradation product identified by HPLC. Though most of the imidacloprid biodegradation pathways conclude that 6-CNA is the final metabolite, a 6-CNA mineralizing chemolithoautotrophic bacterium *Bradyrhizobiaceae* strain SG-6C has also been mentioned (Pearce *et al.*, 2011; Shettigar *et al.*, 2012) which indicates that a pathway of complete mineralization of imidacloprid is possible. Soil microbial degradation of imidacloprid and thiamethoxam in unsterilized soil resulted three degradation products (olefin, olefin

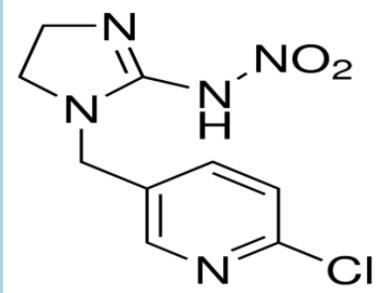
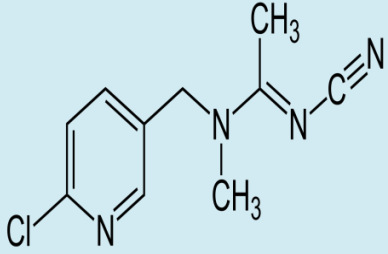
desnitro and urea) for imidacloprid and two degradation products (clothianidin and clothianidin TZMU) for thiamethoxam compared to minimal detection of these metabolites in sterilized soil (Vineyard and Stewart, 2017). *Comomonadaceae* sp., the uncultivable beta proteobacteria, was found capable of biodegradation of thiamethoxam in soil (Zhou *et al.*, 2014a).

These studies indicate that microbial consortia can be used successfully to detoxify neonicotinoid insecticides in contaminated soil. However, the complexity of culturing/harnessing such type of microbial consortia makes them difficult to apply for bioremediation of neonicotinoid insecticides in soil environment without detailed knowledge of bacteria, other microbes and enzymatic processes involved.

Optimum conditions for biodegradation of neonicotinoids

Biodegradation of pesticides by bacterial isolates can be affected by environmental factors like biotic and abiotic parameters. These parameters include soil texture, soil pH, temperature, aeration, status of soil nutrients, chemical structure of pesticides and their bioavailability along with inoculum size of microbial community and their catabolic activity. For successful bioremediation of a pesticide in a particular soil environment with accelerated microbial activity, the optimization of environmental conditions is highly necessary. Desired results may not be sometimes obtained for bioremediation of pesticides in soil due to poor application and improper handling of biotic and abiotic factors required for the growth and activity of degrading microbes. The optimum conditions for biodegradation of neonicotinoid insecticides by different microorganisms are provided in Table 2.

Table.1 Dosages of neonicotinoid pesticides in different crops used against various insect-pests and their waiting periods in the soil. (Source: [http://agritech.tnau.ac.in/crop protection/pdf/5 major use insecticides.pdf](http://agritech.tnau.ac.in/crop%20protection/pdf/5%20major%20use%20insecticides.pdf))

Name of pesticide	Crop (Dosage g a.i./ha)	Waiting Period (days)	Insect-pests	Chemical structure
Imidacloprid	Chilly (25-50)	40	Aphid	
	Tomato (30-35)	3	Whitefly	
	Okra (20)	3	Jassid, Thrips	
	Mango (0.4-0.8 g/tree)	45	Brown plant hopper	
	Citrus (10)	15	Whitebacked	
	Grapes (0.06-0.08)	32	plant- hopper	
	Sunflower (20)	30	Green leaf	
	Groundnut (20-25)	40	hopper	
	Sugarcane (70)	45	Termite	
	Cotton (20-25)	40	Hopper	
	Paddy (20-25)	40	Leaf miner Psylla Flea beetle	
Acetamiprid	Okra (15)	3	Aphids	
	Cabbage (15)	7	Jassids	
	Chilli (10-20)	3	White flies	
	Cotton (10-20)	15	Thrips	
	Rice (10-20)	7	Brown plant hopper	
Thiamethoxam	Tomato (50)	5	Stem borer	
	Brinjal (50)	3	Gall midge	
	Potato foliar application (25)	75	Leaf folder BPH	
	Okra (25)	5	WBPH	
	Cotton (25)	21	GLH	
	Mustard (12.5-25.0)	21	Thrips	

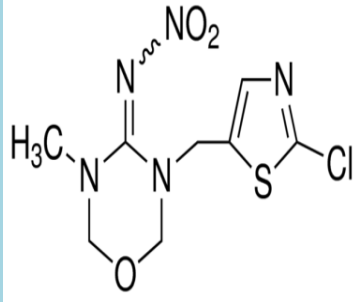
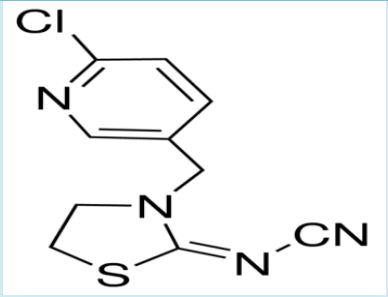
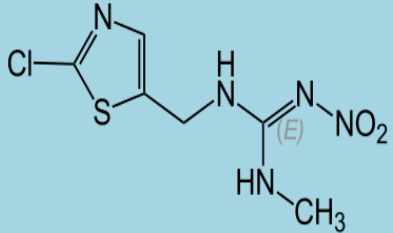
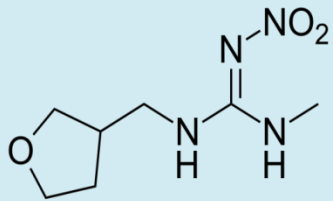
	Rice (25) Wheat (12.5) Mango (25) Citrus (25) Tea (25) Soil drench (50)	14 21 30 20 7 77	Jassid Aphid White flies Hoppers Mosquito bug Psylla	
Thiacloprid	Brinjal (180) Chilli (54-72) Cotton (24-30) Soybean (180) Paddy (120) Tea (90)	5 5 52 17 30 7	Aphid Thrips Jassid Whitefly Stem borer Mosquito bug Shoot & fruit borer Girdle beetle	
Clothianidin	Rice (10-12) Cotton (20-25) Cotton in soil drench (100-125) Sugarcane in soil drench (125)	12 20 20 310	Brown plant hopper Jassids White fly Aphids Thrips White fly Termite	
Dinotefuran	Rice (30-40)	10	Brown plant hopper	

Table.2 Microbial strains capable of degrading neonicotinoid insecticides with optimum biodegradation conditions

Microorganism	Source	Mode of degradation	Optimal biodegradation conditions	Reference
Imidacloprid				
<i>Bacillus alkalinitrilicus</i> and <i>Bacillus aerophilus</i>	Sugarcane field soils	Cometabolic, mixed culture	Soil slurry	Akoijam and Singh (2015) Sharma <i>et al.</i> (2014)
<i>Bacillus sp.</i>	Rhizospheric soil	Catabolic (C, N)	30 – 35 °C, pH 7	Shaikh <i>et al.</i> (2014)
<i>Brevundimonas sp.</i>	Cotton field soils	Catabolic (C, N)	37 °C, 120 ppm	Shetti and Kaliwal (2012)
<i>Bacillus weihenstephanensis</i>	Soil	Catabolic (C,N)	Liquid minimal Medium, 22 °C, pH 7.0	Shetti <i>et al.</i> (2014)
<i>Burkholderia cepacia</i>	Agriculture field soil	Catabolic	Liquid tryptic soy broth, spiked imidacloprid (50 µg/mL)	Gopal <i>et al.</i> (2011)
<i>Klebsiella pneumoniae</i> BCH-1	Pesticide-contaminated agricultural soil	Cometabolic	pH 7, 30 °C, static	Phugare <i>et al.</i> (2013)
<i>Leifsonia sp.</i> PC-21	Agricultural soil	Cometabolic (glucose, succinate)	–	Anhalt <i>et al.</i> (2007)
<i>Mycobacterium sp.</i> strain MK6	Agricultural soil	Catabolic (N)	Liquid minimal medium	Kandil <i>et al.</i> (2015)
<i>Ochrobactrum sp.</i>	Tea rhizosphere soil	Catabolic (C)	30 °C, pH 8	Hu <i>et al.</i> (2013)
<i>Pseudomonas sp.</i> 1G	Neonicotinoid-exposed golf course soil	Cometabolic (glucose)	28 °C, microaerophilic	Pandey <i>et al.</i> (2009)
<i>Pseudoxanthomonas indica</i>	Rhizospheric soils	Cometabolic (glucose)	Liquid minimal medium	Ma <i>et al.</i> (2014)
<i>Rhizobium sp.</i>	Vegetable farming areas	Catabolic (C)	Liquid minimal medium	Sabourmoghaddam <i>et al.</i> (2014)
Acetamiprid				
<i>Ensifer meliloti</i> CGMCC7333	Rhizosphere soils	Catabolic (N) N-Aminoamide IM-1-2	30 °C, resting cells	Zhou <i>et al.</i> (2014b)
<i>Fusarium sp.</i> CS-3	Acetamiprid-contaminated soil	Acetamiprid as sole carbon source	20-42 °C, pH 5.0-8.0	Shi <i>et al.</i> (2018)
<i>Ochrobactrum sp.</i> D-12	Polluted agricultural soil	Catabolic	25 – 35 °C, pH 6 – 8	Wang <i>et al.</i> (2013a)
<i>Pigmentiphaga sp.</i>	Pesticide-	Catabolic	30 °C, resting cells,	Wang <i>et al.</i> (2013b)

AAP-1	contaminated factory soil		pH 7	
<i>Pigmentiphaga</i> sp. D-2	Wastewater from acetamiprid-manufacturing factory	Catabolic (C)	30 – 45 °C, pH 5 – 10	Yang <i>et al.</i> (2013)
<i>Pseudomonas</i> sp. FH2	Sludge from pesticide factory	Cometabolic	30 °C, pH 7	Yao and Min (2006)
<i>Pseudoxanthomonas</i> sp. AAP-7	Pesticide-contaminated factory soil	Cometabolic	30 °C, resting cells, pH 7	Wang <i>et al.</i> (2013c)
<i>Rhodococcus</i> sp. BCH-2	Pesticide-contaminated soil	Cometabolic (6-CNA)	35 °C, pH 7, static	Phugare and Jadhav (2015)
<i>Rhodotorula mucilaginosa</i> IM-2	Clay soil	Catabolic (C)	Mineral salt medium	Dai <i>et al.</i> (2010)
<i>Stenotrophomonas</i> sp. THZ-XP	Sludge from an acetamiprid-producing factory	Cometabolic ACE-3	30 °C, pH 7	Tang <i>et al.</i> (2012)
<i>Stenotrophomonas maltophilia</i> CGMCC 1.178	Purchased	Cometabolic	30 °C, pH 7.2	Chen <i>et al.</i> (2008)
Thiacloprid				
<i>Ensifer meliloti</i> CGMCC7333	Rhizosphere soils	Catabolic (N)	30 °C	Ge <i>et al.</i> (2014)
<i>Rhodotorula mucilaginosa</i> IM-2	Clay soil	Catabolic (C)	Mineral salt medium	Dai <i>et al.</i> (2010)
<i>Stenotrophomonas maltophilia</i> CGMCC 1.178	Purchased	Cometabolic	30 °C, pH 7.2 (resting cells)	Zhao <i>et al.</i> (2009)
<i>Variovorax boronicumulans</i> J1	Agricultural soil	Cometabolic (resting cells)	30 °C, pH 7.2	Zhang <i>et al.</i> (2012)
Thiamethoxam				
<i>Bacillus aeromonas</i> IMBL 4.1 and <i>Pseudomonas putida</i> IMBL 5.2	Agricultural soil	Cometabolic	37 °C, pH 6.0-6.5, mineral salt medium	Rana <i>et al.</i> (2015)
<i>Bacillus aerophilus</i> IMBL4.1	Clay loam soil	Cometabolic	–	Rana and Gupta (2019)
<i>Ensifer adhaerens</i> TMX-23	Rhizosphere soil around soybean plant	Catabolic (C, N)	30 °C	Zhou <i>et al.</i> (2013)
<i>Pseudomonas</i> sp.1G	Neonicotinoid-exposed golf course soil	Cometabolic	28 °C, microaerophilic	Pandey <i>et al.</i> (2009)

Fig.1 Proposed degradation pathways of imidacloprid and acetamiprid by different microbes. (Source: Hussain *et al.*, 2016, FEMS Microbiology Letters)

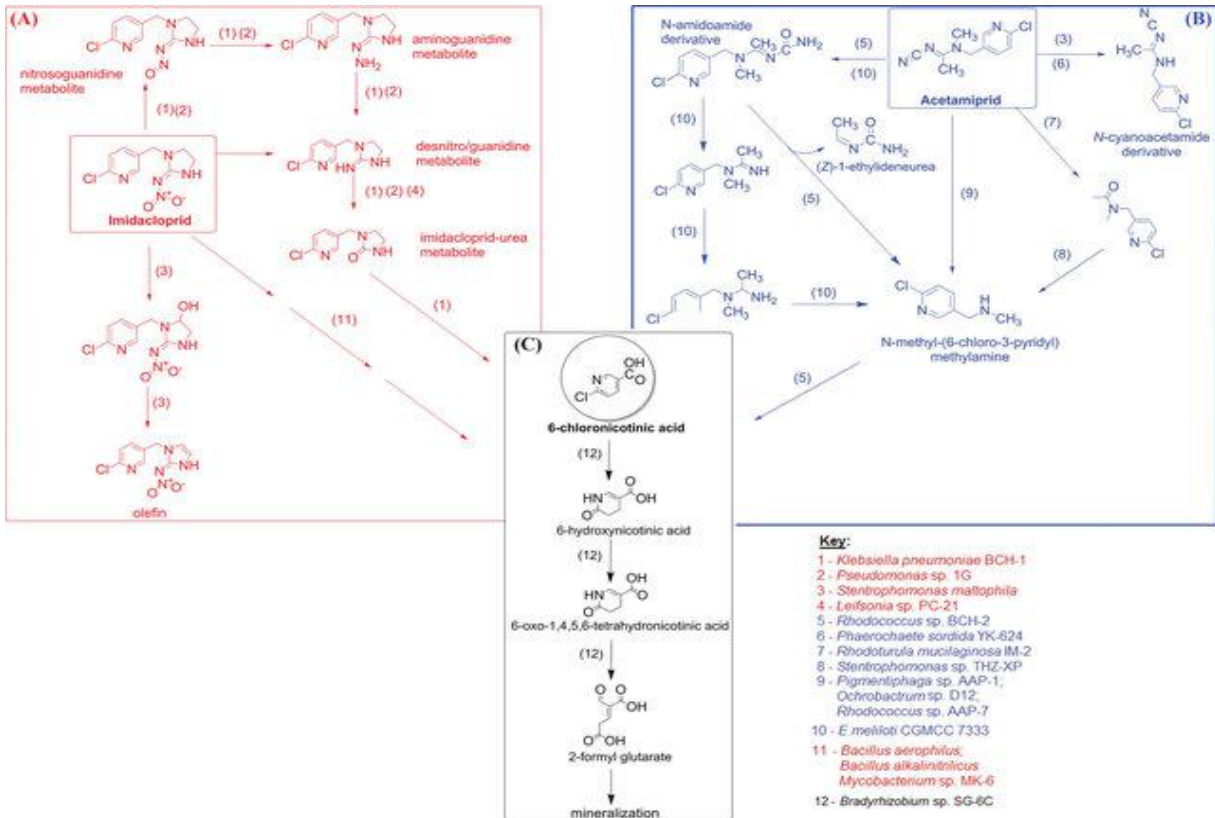


Fig.2 Proposed degradation pathways of acetamiprid and thiacloprid by yeast strain in sucrose mineral salt medium. (Source: Dai *et al.*, 2010, J Agric Food Chem)

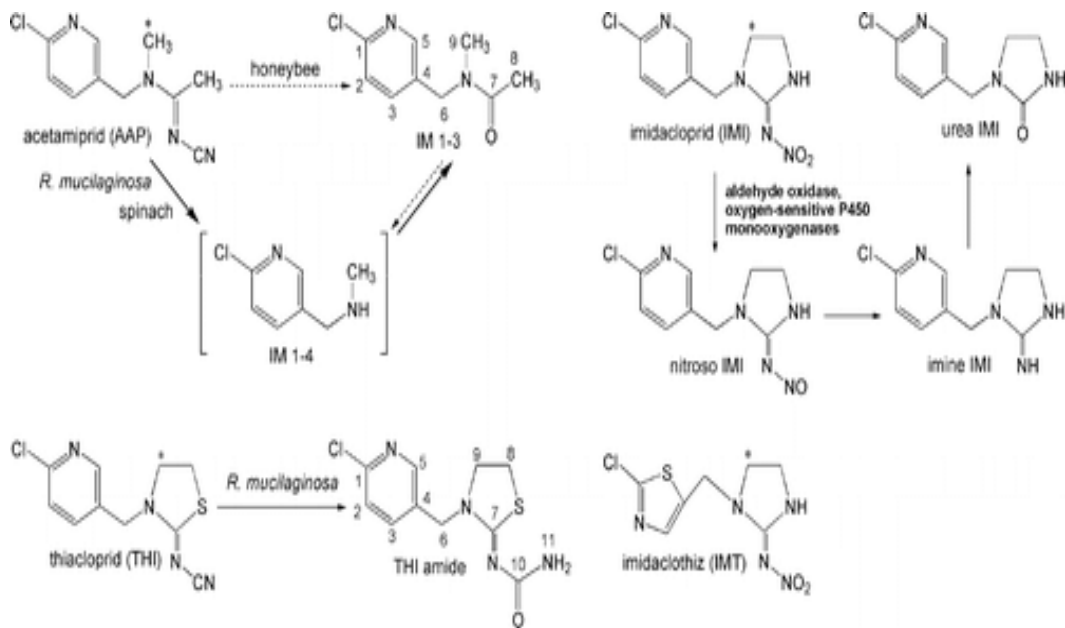


Fig.3 Possible degradation products of acetamiprid by nitrogen-fixing bacteria *Ensifer meliloti* CGMCC7333 (Source: Zhou *et al.*, 2014b, J Agric Food Chem)

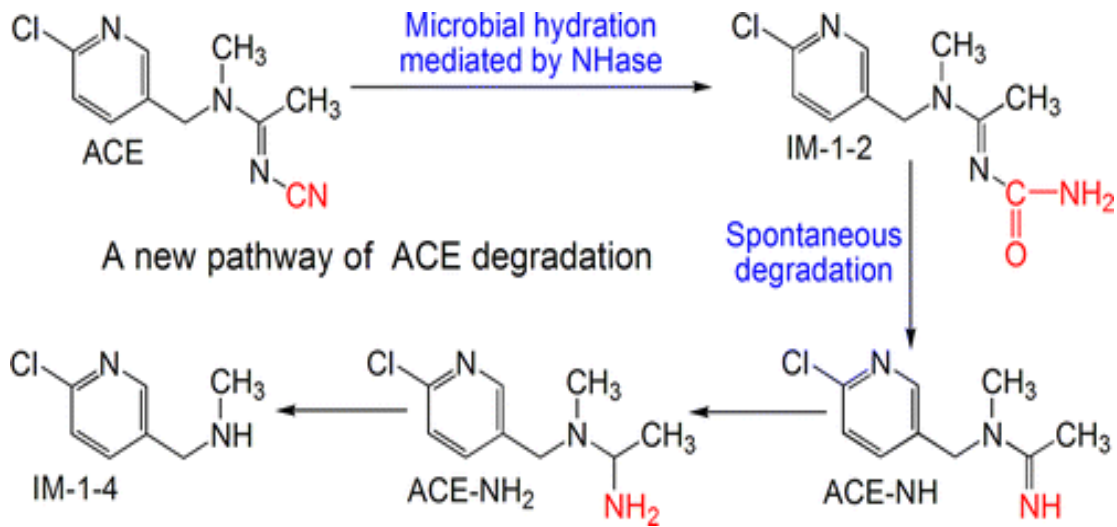
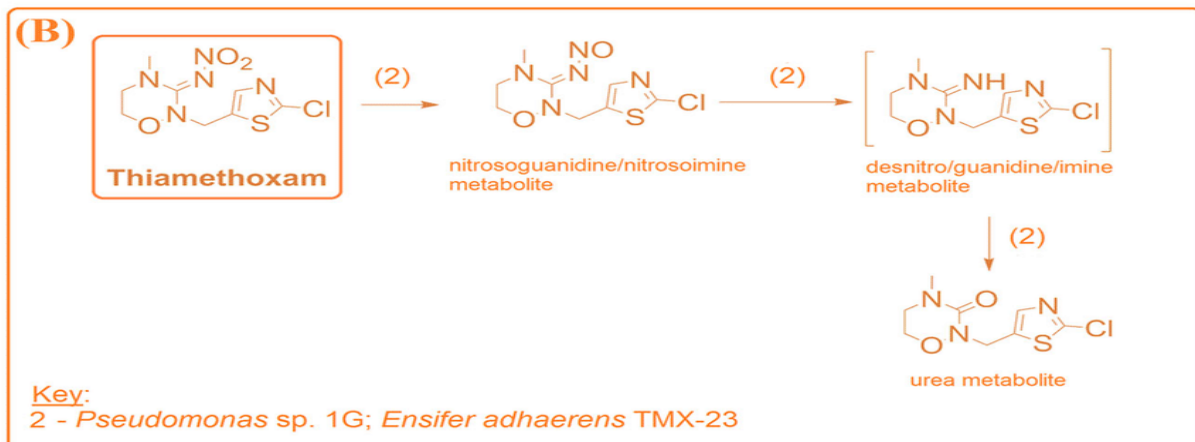
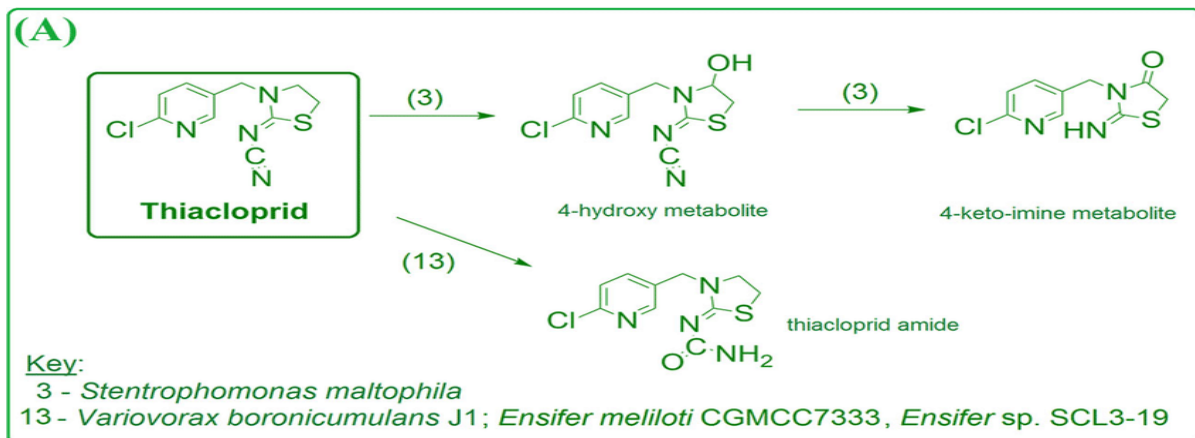


Fig.4 Microbial degradation pathways of thiacloprid (A) and thiamethoxam (B) in soil (Source: Hussain *et al.*, 2016, FEMS Microbiology Letters)



Acetamiprid-degrading bacteria *Pseudomonas* sp. strain FH2 could grow optimally at pH 7.0 and 30°C temperature in mineral medium with 800 mg/L concentration and about 53.3 per cent acetamiprid was degraded after incubation for 14 d, while nearly 96.7 per cent was degraded when incubated in acetamiprid-yeast mineral medium at 30°C for 14 d (Yao and Min, 2006). This paper describes phylogenetic and degradation characterization of a pure bacterium being able to mineralize acetamiprid for the first time. The effect of different temperatures (20, 25, 30, 35 and 40 °C) and pH (5, 6, 7, 8 and 9) was tested on imidacloprid biodegradation rate and it was noticed that the optimum conditions for biodegradation were a pH of 8 and 30°C temperature (Hu *et al.*, 2013). Sharma *et al.*, (2014) have reported that *Enterobacter* sp. strain ATA1 was a competent bacterium for imidacloprid degradation at pH between 6.0 and 7.0 and 37°C temperature. optimum imidacloprid degradation efficiency of four isolates was achieved at 25°C temperature and at neutral pH 7.0 after carrying out the study at three temperatures (65, 25 and 5 °C) and three pH (4, 7 and 10) (Shaikh *et al.*, 2014). Maximum rate of biodegradation of imidacloprid and acetamiprid by *Klebsiella pneumonia* and *Rhodococcus* sp., respectively, occurred at neutral pH (7.0) and temperature ranging between 30 to 35°C after considering the effect of various physico-chemical parameters like temperature, pH, initial concentration of insecticides and application of additional nutrient sources (Phugare *et al.*, 2013; Phugare and Jadhav, 2015). However, imidacloprid degradation was slightly better under alkaline conditions than acidic conditions. Acetamiprid degradation could be maximized at a temperature of 35°C under mesophilic conditions suitable for degrading bacteria. They concluded further that lower degradation rate under acidic and alkaline conditions than under neutral conditions

might be due to the suppressed bacterial growth under these conditions. Microbial growth and enzymatic activity might be the reason for the effect of temperature on biodegradation rate. Substrate toxicity could slow down the biotransformation rate due to increased initial concentration of insecticides as reported by them. They observed that imidacloprid degradation was 78.3 per cent with an initial concentration of 50 mg/L which significantly reduced to 9 per cent with an initial concentration of 250 mg/L. Similarly, 85 per cent biodegradation rate was achieved with 50 mg/L of acetamiprid, but only 14 per cent biodegradation rate was recorded with 250 mg/L of acetamiprid. The application of additional nutrient sources also significantly affected the rate of biodegradation of acetamiprid. Yeast extract as a carbon and nitrogen source accelerated the degradation rate, while citric acid retarded it. Likewise, acetamiprid degradation rate was repressed with the application of sodium nitrate as a nitrogen source. Yang *et al.*, (2013) have isolated *Pigmentiphaga* sp. D-2 capable of degrading acetamiprid in a liquid medium from 0.22 mM to a non-detectable level within 72 h at a wide temperature range of 30 to 45°C under pH range of 5 to 10. Bacterial species *Bacillus aeromonas* strain IMBL 4.1 and *Pseudomonas putida* strain IMBL 5.2 could grow optimally at 37 °C under shake culture conditions in MSMT medium at pH of 6.0–6.5 where improved thiamethoxam degradation by these bacterial species was noticed (Rana *et al.*, 2015). These species caused maximum thiamethoxam degradation only in the presence of thiamethoxam as sole source of carbon and energy. In our laboratory it was observed that *Pseudomonas mosselii* strain NG1, an imidacloprid degrading bacteria isolated from mango orchard soil, could show optimum degradation potential at 35°C temperature and neutral pH 7.0 (Bhattacharjee *et al.*, 2018). These studies clearly suggest that for

successful bioremediation of neonicotinoid insecticides establishment of optimum biotic and abiotic environmental conditions is extremely desirable.

Looking ahead

The metabolic fate of imidacloprid is still not clear from the researches mentioned above which leaves a gap to understand biotransformation of neonicotinoid insecticides. Better biodegradation of imidacloprid can be achieved when its metabolic fate by all the reported and similar bacteria will be crystal clear. Till date not a single study is available on complete mineralization of imidacloprid by a single bacteria isolate. Therefore, isolating or devising such a bacterium or a bacterial consortium may be crucial for successful biodegradation of not only imidacloprid but other neonicotinoid insecticides also in the soil environment. A clear observation on neonicotinoid biodegradation pathway in soil isolated bacteria / other microbes is somehow missing today though metabolism of these insecticides in several other biological systems has been extensively studied. The identified common mechanism for the biotransformation of chloropyridinylmethyl neonicotinoid insecticides in some biological systems is spontaneous conversion of an unnamed intermediate to 6-CNA via N-methylene hydroxylation (Casida, 2011). Though complete mineralization of imidacloprid by a single bacterium is yet to be studied, 6-CNA has been reported as deadend product of both imidacloprid and acetamiprid metabolism for many bacteria (Figure 1). It can be suggested from these reports that complete mineralization pathway for chloropyridinylmethyl neonicotinoids by microorganisms might proceed via 6-CNA. N-Methylene hydroxylation (yet to be reported in bacteria) or the sequential catabolism of the cyclic N-nitroimine moiety

in imidacloprid, the N-cyanoimine moiety in thiacloprid, the acyclic N-cyanoimine moiety in acetamiprid or the 2-nitromethylene moiety in nitenpyram might be the possible reason for the formation of 6-CNA as a metabolic intermediate due to bacterial degradation of chloropyridinylmethyl neonicotinoid insecticides. Some researchers have identified a chemolithoautotrophic bacterium from Bradyrhizobiaceae family, strain SG-6C, capable of mineralizing 6-CNA (Pearce *et al.*, 2011; Shettigar *et al.*, 2012). This strain (SG-6C) possessed a novel gene encoding a 6-CNA dechlorinating hydrolase enzyme through an integrative and conjugative element, a 139-kb mobile element capable of conjugative transfer and integration into the genome at a particular 48-bp recognition sequence. This hydrolase enzyme feeds 6-CNA into a pre-existing/possible nicotinic acid mineralization pathway in strain SG-6C.

This study is the only research available till date regarding the evolution of neonicotinoid catabolism in bacterial gene despite the fact that the introduction of neonicotinoid insecticides can provide an alluring model system to examine the catabolic pathway evolution for xenobiotic pesticides in bacteria. The expansion of this research area might not only enlighten the microbial biotransformation of neonicotinoid insecticides but also significantly enhance the progress towards the research on genetic engineering of a neonicotinoid-mineralizing microorganism especially bacterium.

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