Applied Soil Ecology 98 (2016) 150–158

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Aerobic biodegradation kinetics and pathway of the novel *cis*-nitromethylene neonicotinoid insecticide Paichongding in yellow loam and Huangshi soils

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ARTICLE INFO

Article history:
Received 26 March 2015
Received in revised form 8 October 2015
Accepted 12 October 2015
Available online 27 October 2015

Keywords:
Paichongding
Degradation pathway
Degradation rate
DT50
Sphingobacterium

ABSTRACT

Biodegradation of Paichongding (IPP), a recently developed *cis*-nitromethylene neonicotinoid insecticide, was investigated in two different soils under aerobic condition. IPP degradation rate was strongly affected by soil physic-chemical characteristics and the inoculation of IPP-degrading bacteria. Inoculation of IPP-degrading bacteria can increase degradation rate and decrease DT50 (half-life value). The removal ratio of RR-IPP, SS-IPP, SR-IPP and RS-IPP at 60 days after treatment (DAT) reached 30.17%, 28.06%, 51.48% and 45.76% in Yellow clayed soil (S1), 20.04%, 19.78%, 36.22% and 40.59% in Huangshi soil (S2), respectively. DT50 of IPP in S1 and S2 decreased after inoculation of *Sphingobacterium* sp. M3-1. Furthermore, based on the identified eight metabolites (M1–M8) by LC–MS/MS and their behavior, a biodegradation pathway of IPP in soils was proposed. New metabolites, M4, M6 and M7 were observed and determined in soils. Biodegradation of IPP involved continuous biocatalytic reactions such as nitro reduction and elimination, hydrolysis, C-N cleavage, de-methyl, and ether cleavage reactions. Finally, IPP was bio-transformed into M7 and M8.

1. Introduction

Neonicotinoid insecticides, such as imidacloprid, are one of the fastest growing insecticides because they are selective agonists of the insect nicotinic acetylcholine receptors (nAChRs) and extensively used in areas of crop protection and animal health to control a variety of insect pest species (Jeschke and Nauen, 2008; Matsuda et al., 2005; Ohno et al., 2010; Tomizawa and Casida, 2003, 2005). A new nicotinic family with several chiral members, *cis*-nitromethylene neonicotinoids, has been grow rapidly in recent years. Paichongding (IPP, 1-((6-chloropydidin-3-yl)methyl)-7-methyl-8-nitro-5propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-α-]-pyridine), is a novel *cis*-nitromethylene neonicotinoid recently developed in China (Fu et al., 2013a,b; Li et al., 2013; Wang et al., 2013; Zhao et al., 2010). IPP is different from the traditional neonicotinoids as it is the antagonist of the postsynaptic nicotinic acetylcholine receptors (nAChRs) of insects. IPP has excellent toxicity toward insects with a broad spectrum of sucking and biting insects, including Homoptera and Lepidoptera (Shao et al., 2008; Tian et al., 2007; Zhang et al., 2013). In addition, IPP activity is about 40–50 times higher than imidacloprid against imidacloprid-resistant insects (Shao et al., 2010, 2011).

Since 2009, IPP suspension (10% w/v) (the trade name is Paichongding) has been approved to be used extensively for insect control in China and the total amount of IPP would soon reach about 1000 t or 3.3 million hectares (Fu et al., 2013a,b; Li et al., 2013; Shao et al., 2011). IPP has two chiral carbon centers, which leads to four stereoisomers as *5R,7R*-Paichongding (RR-IPP), *5S,7S*-Paichongding (SS-IPP), *5S,7R*-Paingchongding (SR-IPP) and *5R,7S*-Paingchongding (RS-IPP) (Fig. 1). The application of chiral insecticide IPP may lead to the release of inactive isomers into the environment. Different chiral insecticides used in crops have been frequently detected in plants, soils and groundwater, resulting in harmful effects to human health, ecosystems and the sustainable development of crop productivity. Furthermore, the presence of chiral insecticides will also result in insecticide-resistance. The behavior of IPP and its biodegradation pathway in the environment are important requirements for its safe use on food crops (Fu et al., 2013a,b; Wang et al., 2013). Several reports have mainly focused on its photodegradation in aqueous solution, translocation in Youdonger (*Brassica campestris* subsp. *chinensis*), stereoselective fate in flooded soils and its metabolism, extractable and bound

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http://dx.doi.org/10.1016/j.apsoil.2015.10.009
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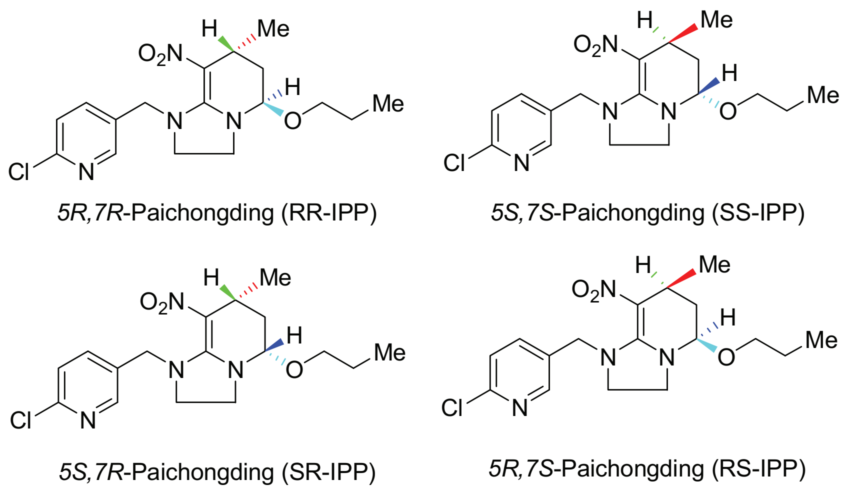

Fig. 1. Stereostructures of four isomers of Paichongding (IPP).

residue, transformation in soils (Cai et al., 2015a,b; Fu et al., 2013a, b; Li et al., 2013; Wang et al., 2013; Zhang et al., 2013; Zhao et al., 2010). Microbial degradation of IPP and its degradation pathway in aerobic soils system have not been reported until now.

In the present study, *Sphingobacterium* sp. P1-3, which was isolated from soil and could utilize IPP as sole carbon and energy source with greater IPP-degrading activity, was used to study microbial degradation of IPP, bioremediation of contaminated soils and the proposed biodegradation pathway of IPP in soils.

2. Materials and methods

2.1. Chemicals

Paichongding (IPP, chemical purity 96.3%; formula weight, FW 366, Fig. 1) was obtained from Jiangsu Kesheng Company Ltd., HPLC grade methanol and acetonitrile were purchased from Burdick & Jackson (MI, USA). All other reagents and common chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

2.2. Test soils

Two different types of soil from different agricultural fields were used in this study, which were yellow loam soil (S1, GB/T-A2111411) and Huangshi soil (S2, GB/T-G2511211), respectively. The soils was classified according to Chinese Soil Taxonomy (Gong, 2003) and Classification and codes for Chinese Soil (GB/T 17296-2009). The soil samples were taken from the surface zone (0 to 15 cm depth) in rice fields in Fujian and Jiangsu provinces, China. All the soil samples were air-dried, mixed and passed through a 1-mm sieve. Their basic physicochemical characteristics were determined by previously reported methods (Gee and Bauder, 1986) and listed in Table 1.

2.3. Microorganisms and culture media

Sphingobacterium sp. P1-3 (GenBank accession numbers: KP657689, CGMCC No. 10454) was isolated from soils in the south of Changzhou, Jiangsu province, China. It had good IPP-degrading activity and could use IPP as a sole carbon and energy source. *Sphingobacterium* sp. P1-3 was cultivated aerobically at 30 °C on Luria–Bertani (LB) broth. Stock cultures were stored in 20% glycerol at −80 °C.

2.4. Biodegradation experiments in soils

To study the behavior and degradation of IPP in aerobic soils, IPP was added into the test soils to give a final concentration of 20 mg kg^{-1} soil and thoroughly mixed. The experiment had a completely randomized block design with three replications that had the following treatments: each soil was separated into three sets: one set was sterilized to remove the microbial activity, the second was pre-incubated at 25 ± 1 °C to allow the microorganisms to acclimatize in natural (unsterilized) soils, and the third was inoculated *Sphingobacterium* sp. P1-3. The soil moisture content was adjusted to about 60% of the maximum water-holding capacity by adding Milli-Q (MQ) water.

During the incubation, the soil moisture content was maintained constant by weighing the flasks and correcting for any weight loss by adding MQ water. The incubation temperature was 25 ± 1 °C. The flasks with soil samples were sealed and connected with a series of air-tight test tubes, a slow and continuous air flow was maintained. At different time intervals (0, 5, 10, 20, 30, 45, 60, 75 and 100 days after treatment), 10 g of soil (dry weight equivalent) was sampled from the flask and was used for determining the concentration of IPP and its metabolites.

Table 1
Physico-chemical characteristics of the experimental soils.

	Yellow loam soil (S1)	Huangshi soil (S2)
Location	Longquan, Fujian Province	Changzhou, Jiangsu Province
pH (H_2O)	6.63	5.95
OM[a] (%)	2.67	1.52
CEC[b] (cmol kg^{-1})	14.09	7.11
Clay (%)	38.7	33.5
Silt (%)	50.4	49.8
Sand (%)	10.9	16.7
Total N (%)	0.24	0.08
P (mg kg^{-1})	21.25	7.65
K (g kg^{-1})	13.47	10.7
Texture (%)		
<0.01 mm	67.4	60.7
0.01–0.09 mm	28.3	32.6
>0.09 mm	4.3	6.7

[a] Organic matter.
[b] Cation exchange capacity.