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Non-target effect of continuous application of chlorpyrifos on soil microbes, nematodes and its persistence under sub-humid tropical rice-rice cropping system

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ABSTRACT

Application of pesticide in agricultural fields is "unnecessary evil" for non-target microflora and fauna. Hence, to identify the safer pesticide molecules against non-target microbes, a long-term pesticide experiment was initiated at National Rice Research Institute, Cuttack, India. In the present study, the effect of continuous application of chlorpyrifos (0.5 kg ha^{-1}) in rice fields on non-target groups of soil microbes and nematodes was studied for seven seasons (four wet and three dry seasons) during 2009–2013. Treatments were arranged in a randomized complete block design with four replications of chlorpyrifos-treated (0.5 kg a.i. ha^{-1}) (CT) and untreated control (UT) plots. During seven seasons of experimentation, regular application of chlorpyrifos had no significant effect on population of heterotrophic aerobic, anaerobic, oligotrophic and copiotrophic bacteria in CT compared to UT, whereas, population of asymbiotic aerobic nitrogen fixer, nitrifiers, denitrifiers, gram positive and spore-forming bacteria were significantly reduced by nearly 0.25–2 fold in CT than UT. However, comparatively less deviation in population of actinomycetes, fungi, phosphate solubilizing and sulfur oxidizing bacteria were observed in CT than UT. Significant interactions were found between effects of chlorpyrifos with time in population dynamics of microbes. In plant parasitic nematode species, *Meloidogyne graminicola* (RRKN) and *Hirschmanniella* spp. (RRN), were significantly lower ($p < 0.01$) in CT compared to UT after first year onwards. The overall observation of five years data indicated that the RRKN population showed a decreasing trend (R^2=0.644) whereas RRN showed increasing trend (R^2=0.932) in CT. The drastic chlorpyrifos dissipation was noticed after 15 days of application from the initial residue of 0.25 mg kg^{-1} soil, which indicated that chlorpyrifos residue in rice field soil was not persistent and its half-life was found to be 4.02 days. Overall, the present findings revealed that non-target effect of repetitive application of chloropyrifos (0.5 kg ha^{-1}) on soil microbes and nematodes was found less under rice-rice cropping system.

1. Introduction

Rice is one of the major agricultural crops and consume maximum amount of pesticides. Application of pesticides inevitable for rice cultivation; however accumulation of these in soil may poses several risks to non-target groups (Li et al., 2015; Zhang et al., 2010). Though one time application of pesticide per year may not have much impact on agricultural environment, however repetitive application of these poses threats to environmental health and sustainability of agricultural soils (Imfeld and Vuilleumier, 2012). An ideal pesticide should have the ability to destroy target pest without harming the non-target organisms and should be able to degrade non-toxic substances. On the contrary, the previous reports revealed about presence of pesticide residues in soil and their toxicity to the soil microflora and microfauna (Babendreier et al., 2015; Hua et al., 2009; Singh et al., 2015; Sahoo et al., 2016). Several researchers have documented the effect of frequently used organophosphate pesticides on specific groups of soil microorganisms such as nitrogen fixers, nitrifiers, heterotrophic bacteria and fungi (Mitra and Raghu, 1998; Singh et al., 1999; Das and Mukherjee, 2000). One of the most repeatedly used organophosphate pesticides on agricultural crops is chlorpyrifos.

Chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is a moderately toxic broad spectrum organophosphate (OP) insecticide and acaricide. In the year 2007, globally, maximum amount of chlorpyrifos (3.64–4.99 million kg) was produced (Grube et al., 2011). In India, it was reported that chlorpyrifos was the second most

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used agricultural insecticide (9540 t) in 2013–14 (Ministry of Chemicals and Fertilizers, GOI, 2014). Moreover, it is widely used for pest control on cereals including rice, cotton, fruit, vegetable crops, lawns and ornamental plants (Fang et al., 2008). Although, a diverse range of bacterial, fungal and algal species such as *Arthrobacter* sp., *Bacillus pumilus, Pseudomonas* sp., *Trichoderma viride, Aspergillus niger, Spirulina platensis, Synechocystis* sp., etc., have been shown their ability to degrade chlorpyrifos in soil (Yadav et al., 2016). But excessive and wide-scale application of chlorpyrifos may lead to the microbial imbalance (Adak et al., 2016; Fang et al., 2008; Gupta et al., 2013; Kumar et al., 2012; Sasikala, et al., 2012), environmental pollution due to contamination of soil (Johnsen et al., 2001), surface and ground water and health hazards (Banks et al., 2005).

The persistence of chlorpyrifos and its half-life in rice field soil has been given much importance as it causes environmental pollution and also affects the microbial diversity. Chlorpyrifos has high soil-absorption coefficient and low water solubility (2 mg L^{-1}) (Racke et al., 1990), this pesticide is degraded in environment by many ways like photolysis, chemical hydrolysis and microbial attack but still resistant to biodegradation and remains in environment for 5–17 years (Baskaran et al., 1999; Zhang et al., 2012).

The short-term effect of chlorpyrifos on soil microbes were reported in different crops like groundnut (Pandey and Singh, 2004), cotton (Ajaz et al., 2005) and soybean (Sarnaik et al., 2006) and rice conditions (Adak et al., 2016). However, little information is available on long-term effect of non-target group of soil microflora and fauna after continuous application of pesticides (Smith et al., 2000). Interestingly, so far no reports are available on impact of continuous application of chlorpyrifos on soil microflora, nematode and its persistence under rice ecosystem. Hence, the present investigation was initiated to generate long-term data to observe the effect of repetitive application of pesticides on non-target microflora, nematodes and their residue in paddy soil. In the present paper, attempts were made to analyze the effect of repetitive application of chlorpyrifos during five years (seven seasons) on 1) specific groups of soil microflora 2) nematodes and 3) persistence of chlorpyrifos in soil under subtropical rice-rice cropping system.

2. Materials and methods

2.1. Study site

The experimental site is situated at ICAR-National Rice Research Institute (NRRI), Cuttack, India (latitude 20° 25′ N, longitude 85° 55′ E and mean sea level 24 m). Mean annual maximum and minimum temperatures are 39.2 and 22.5 °C, respectively. Annual precipitation is 1500 mm of which 75–80% is received during June to September. The soil at the experimental site is sandy clay loam texture (31% clay, 17% silt and 52% sand) which falls under Aeric Endoaquept soil type (Soil Survey Staff, 2010). Other soil physical and chemical properties viz. bulk density (1.40 Mg m^{-3}), cation-exchange capacity (15.2 cmol (p+) kg^{-1}), pH (6.6), organic carbon (0.66%), available-N (172 kg ha^{-1}), available-K (163 kg ha^{-1}) and available-P (31 kg ha^{-1}) were analyzed and recorded in the experimental field at the beginning of the study.

2.2. Long-term pesticide experimental plot

A long-term pesticide experiment was initiated at NRRI, Cuttack during wet season of the year 2009–10. Two crops per year of *Oryza sativa* L. were raised as one in wet (July-November; variety-Tapaswini) and another one in dry (January-May; variety-Naveen) seasons. Treatments were arranged in a randomized complete block design with four replications (plot size: 20×20 m^2) of control (no application of pesticides) and different pesticides two insecticides (chlorpyrifos: 0.5 kg a.i. ha^{-1}; cartap hydrochloride: 1 kg a.i. ha^{-1} and one each of fungicide (carbendazim: 0.1%) and herbicide (pretilachlor: 0.75 kg a.i. ha^{-1}). The chlorpyrifos (1.5% DP) was purchased from Shree Ramcided Chemical Pvt. Ltd. Chennai (India) with the trade name Robon. The herbicide was applied after 2 days of transplanting (DAT) and other pesticides were applied as per treatments after 30 days of planting. The chemical fertilizer schedules were 60-40-40 and 80–40–40 N-P_2O_5-K_2O kg ha^{-1} for wet and dry seasons, respectively, and applied according to the treatments.

2.3. Soil sampling and analyses

Soil samples were collected in polythene bags from the surface layer (0–15 cm) after 3 days of application of chlorpyrifos. A composite representative sample were collected from each plot and stored at 4 °C in refrigerator till analysis. The collected part of soil sample was air-dried and ground to pass through a 2 mm sieve and analyzed for soil organic carbon (SOC), available-N (Subbiah and Asija, 1956), P (Bray and Kurtz, 1945), K (Piper, 1966) and pH.

2.4. Population dynamics of different groups of soil microbes

Soils (10 g in 90 mL, 0.85% sterile saline distilled water) suspensions were diluted to 10^{-2} to 10^{-6} level, 100 µl suspension was spread on petriplates (with suitable medium) and maintained three replications for each groups. The plates were incubated at 30 ± 0.1 °C generally for 3 to 7 d in a BOD incubator and the numbers of colonies were counted. The exact media composition for each groups are referred as per Pelczar (1957) and Holt (1993).

For enumeration of heterotrophic aerobes (HA), plates containing nutrient agar (NA) (HiMedia) were incubated at 30 ± 0.1 °C for 72 h in a BOD incubator; whereas for heterotrophic anaerobes (HAN), the same plates were incubated in an anaerobic jar containing Oxygen absorbing pack along with anaerobic indicator tablet (HiMedia) at room temperature. The visible bacterial colonies appeared on plates were counted and expressed as colony forming units per gram of soil (CFU g^{-1} soil). The Rose Bengal (Himedia) and actinomycetes isolation agar medium was used for enumeration of fungi and actinobacteria, respectively (Kutzner, 1986).

The nitrifying (NH_4^+ oxidizing) bacterial population was determined in *Nitrosomonas* medium (Winogrodsky's medium). The plates were incubated for 25–30 days at 30 ± 0.1 °C and flooded with sulfanillic acid reagent and the pink colonies were counted. The denitrifying (NO_3 reducing) bacterial population was determined on Winogrodsky's medium replacing ammonium sulphate with potassium nitrate or sodium nitrate. The plates were inoculated with soil suspensions, incubated for 72 h at 30 ± 0.1 °C, flooded with sulfanillic acid reagent and the pink colonies were counted.

The phosphate solubilizing bacteria was determined on calcium phosphate (PSB) agar medium. Plating was done after sterilization without allowing the medium to solidify. The soil suspensions were plated and incubated for 72 h or more at 30 ± 0.1 °C and the colonies having a clear zone around them were counted. To determine the population of sulfur oxidizing bacteria, soil suspensions were plated in *Thiobacillus* medium, incubated in a BOD at 30 ± 0.1 °C for 7 d or more. The organisms producing black (or brownish black) colonies due to sulfur deposition were counted.

To determine the population of oligotrophic bacteria, soil suspensions were plated on tryptone soya yeast extract medium, the plates were incubated in a BOD at 30 ± 0.1 °C for 72 h. The organisms producing brownish color were counted. To determine the population of copiotrophic bacteria, soil suspensions were plated on copiotrophic bacteria isolation medium, the plates were incubated in a BOD at 30 ± 0.1 °C for 72 h. The organisms producing brownish color were counted. To determine the population of asymbiotic nitrogen fixing bacteria, soil suspensions were plated on Jensen's medium. The plates were incubated in a BOD at 30 ± 0.1 °C for 72 h. The organisms producing transparent color were counted.