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Original article

Effect of synthetic pyrethroid insecticides on N_2-fixation and its mineralization in tea soil

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A B S T R A C T

Microorganisms degrade a great variety of chemical substances including pesticide residues in soil. By virtue of low persistence and broad-spectrum efficacy, pyrethroid insecticides are sometimes used in tea garden to combat insect pests but the residual effects of these chemicals on microbial activities in soil have rarely been studied. The present experiment has been conducted under laboratory conditions to investigate the effect of four synthetic pyrethroid insecticides, viz. cypermethrin, deltamethrin, fenvalerate and permethrin at their field application rates (225, 200, 225, 210 g a.i. ha^{-1}, respectively), on growth and activities of microorganisms in relation to non-symbiotic N_2-fixation and N mineralization in a tea garden soil of the Himalayan *terai* (at the lower base) region of West Bengal, India. Application of insecticides, in general, decreased growth and activities of ammonifying and nitrifying bacteria but increased proliferations of non-symbiotic N_2-fixing bacteria, resulting in greater fixation of atmospheric N_2, more so with permethrin (7.8%) followed by cypermethrin (5.2%). Most of the insecticides had a deleterious effect on the accumulation of oxidizable organic C and total N, more pronounced with fenvalerate and permethrin. The availability of exchangeable NH_4^+ was also significantly reduced, more prominently with fenvalerate (15.6%) followed by deltamethrin (11.2%), while permethrin followed by cypermethrin accentuated greater accumulation of soluble NO_3^- in soil. Therefore, the effects of synthetic pyrethroid insecticides on microbial activities in relation to non-symbiotic N_2-fixation and its mineralization in tea soil of West Bengal can not be generalized, rather in most cases the effects were inclined more towards detrimental than a few stimulative one.

1. Introduction

In the complex and dynamic soil matrix, autochthonous microorganisms govern the biological equilibrium of the ecosystem [48] through their vital roles in the myriad of soil processes like nutrient cycling, soil aggregation and degradation of agrochemicals [23]. Perturbations in soil caused by the presence of xenobiotic substances like pesticides due to agricultural practice in crop fields influence the composition of soil microbial community [9,45] through the modulation of their metabolism [38]. After reaching to the soil ecological niche, pesticides are degraded by both biotic and abiotic pathways [10], of which microbial biodegradation is considered to be the primary and most important mechanism of pesticide breakdown [12] and detoxification [11,31] because microorganisms are scavengers in soil and degrade a great variety of chemical substances including the incorporated pesticides to derive carbon, energy and other nutrients for their growth and metabolism [30]. This biodegradation of the applied chemicals obviously cause an increase in the activities of the insecticide-utilizers [14], excepting for those species performing co-metabolism in which, microorganisms break down the compounds without extracting neither nutrients nor even energy from it [4], while other players are awaiting in rest for their turn to come. As a result, insecticides and their derivatives in soil may stimulate [17] or decrease [25,42] or keep unchanged [7] the microbiological activities leading to subsequent unpredictable amplification [18] or reduction of plant nutrients availability or maintain their accessibility at par untreated control soil [35].

By virtue of medium to low persistence [17], high effectiveness [5] and broad-spectrum efficacy [32], synthetic pyrethroid insecticides are being widely used in India, somewhere in tea gardens of Himalayan *terai* (at the lower base) regions to combat insect

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pests. Multidimensional effects including shifts in microbial counts [44] and differential biochemical processes [15] have been reported due to application of synthetic pyrethroid insecticides to different field crops, but their comparative residual effects on microbial growth and activities in the tea garden soils of the Himalayan *terai* regions of West Bengal have rarely been studied.

The objectives of the present study were to investigate the effects of four commonly used synthetic pyrethroid insecticides viz., cypermethrin, deltamethrin, fenvalerate and permethrin at their recommended field application rates on microbiological activities in relation to aerobic non-symbiotic N_2-fixation and mineralization of N in a tea garden soil of the Himalayan *terai* region of West Bengal, India.

Characteristically, cypermethrin [(Cyano-(3-phenoxyphenyl) methyl)3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate], deltamethrin [{(*S*)-Cyano-(3-phenoxyphenyl)-methyl}(*1R,3R*)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate] and permethrin [3-Phenoxybenzyl (*1RS*)-*cis*, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] are broad spectrum non-systemic insecticides having neurotoxin activity through dermal contact and digestion to a variety of sucking, chewing and boring type of insects (fruit moths, caterpillars, aphids, mealy bugs and whiteflies) in a number of agricultural crops including cotton, coffee, tea, maize, wheat, alfalfa, cereals, vegetables and many fruit crops, while fenvalerate [(*RS*)-α-Cyano-3-phenoxybenzyl(*RS*)-2-(4-chlorophenyl)-3-methylbutyrate] is a mixture of four optical isomers having different insecticidal activities (the 2-*S*-α configuration, known as esfenvalerate, is the most insecticidal active isomer) through contact and ingestion to chewing, sucking and boring type of insects belonging to the orders Lepidoptera, Coleoptera, Diptera, Orthotera and Hemiptera in cotton, fruits, cereals, vegetables and some plantation crops.

2. Materials and methods

2.1. Experimental methods

An experiment has been conducted under laboratory conditions with Himalayan *terai* soil collected from the tea garden of Anandapur Tea Estate, Jalpaiguri district, West Bengal, India (26º85′86″ N and 88º73′31″ E) by taking several thin slices from the surface soil layer (0–15 cm) by means of a spade as outlined [21]. The composite soil samples were air dried at 30–35 °C in shade and passed through a 2 mm (4–8 mesh cm^{-1}) sieve. The processed soils were stored in a screw-cap jar and used for the experiment. The soil belongs to fluventic eutrochrepts [43],[1] having the general characteristics as presented in Table 1. Four synthetic pyrethroid insecticides, viz. cypermethrin [10% emulsifiable concentrate (EC) - United Phosphorus Ltd], deltamethrin [11% EC - Bayer Crop Science Ltd], fenvalerate [20% EC – Rallis India Ltd] and permethrin [25% EC - Devidayal Agro Ltd] (Fig. 1), at their recommended field application rates (225, 200, 225, 210 g a.i. ha^{-1}, respectively), were mixed thoroughly with 2 kg of air-dried and sieved soil (≤2 mm) and were placed in earthenware pots having a soil depth of 15 cm. Soil moisture was adjusted to 60% of water holding capacity of the soil and maintained throughout the experimental periods. The pots were kept covered with black polyethylene sheets to avoid photodegradation of insecticides and evaporation loss of water from soil surface and incubated in the dark at 30 ± 1 °C for 60 days. There were three replications for each treatment.

[1] USDA:United States Department of Agriculture.

2.2. Soil sampling and analysis

Soil samples were collected after 0 (1 h), 15, 30, 45 and 60 days following insecticide applications by taking 3–5 soil cores as described[18]. Soil moisture content was measured from the subsamples at each sampling day. The subsamples were immediately analyzed to determine microbial populations, their biochemical activities and chemical transformations.

2.3. Enumeration microorganisms

The subsamples were immediately analyzed to enumerate the colony forming units (cfu) of ammonifying bacteria in Remy's nutrient agar [2] and aerobic non-symbiotic N_2-fixing bacteria in sucrose-calcium carbonate agar [22] media following serial dilution technique and pour plate method [34], and incubating the agar plates at 30 ± 1 °C for 7 days in a BOD (biological oxygen demand) incubator. The cfu of nitrifying bacteria were counted in modified [20] Alexander and Clark medium [1] following serial dilution technique and most probable number (mpn) method [46], and incubating at 30 ± 1 °C for 3 days in a BOD incubator followed by microscopic examination.

2.4. Analysis of non-symbiotic N_2-fixing capacity

The non-symbiotic N_2-fixing capacities of the soil samples were analyzed [19] by incubating 1 g soil of each sample in 50 ml Jensen's broth [22] containing 2% sucrose in conical (Erlenmeyer) flasks (150 ml capacity) at 30 ± 1 °C for 15 days followed by estimation of total N [6] in the broth.

2.5. Chemical analysis

Soil samples were analyzed to estimate oxidizable organic C by Walkley and Black method [28] and total N through a wet oxidation procedure (Kjeldahl method) [6]. Available mineral N of the soil samples were measured through extraction with *2N* KCl followed by estimation of mineral N (exchangeable NH_4^+ and soluble NO_3^-) using distillation technique [27].

2.6. Data analysis

The results were evaluated by analysis of variance (ANOVA) and the statistical significance ($p < 0.05$) of difference between means within factors (insecticides and sampling days) was evaluated using Fisher's protected LSD method [33]. The results were also evaluated by analysis of linear relationship between the variables and the statistical significance ($p < 0.01$) of correlation co-efficient (r value) was evaluated using statistical package for social sciences, version 16.0 (SPSS 16.0).

3. Results and discussion

3.1. Effect on ammonifying and nitrifying bacteria

Incorporation of synthetic pyrethroid insecticides in general, did not have a significant effect ($p < 0.05$) on the proliferation of ammonifying and nitrifying bacteria in soil (Table 2). Compared to untreated control, application of cypermethrin and permethrin increased the colony forming units (cfu) of ammonifying and nitrifying bacteria to the extent of 5.6 and 5.8%, respectively, while the other insecticides had a deleterious effect on the microorganisms in soil. This manifested that cypermethrin and permethrin including their degraded metabolites were preferably utilized by ammonifying and nitrifying bacteria, respectively to derive carbon,