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Evaluation of Insecticides on Soil Micro-Biota under Laboratory Condition

Sakil Dhamala[1] • Dwipendra Thakuria[1] • Sandip Patra[2] • Pankaj Baiswar[2] • Prabha Moirangthem[2] • Kennedy Ningthoujam[1]

[1]CPGSAS, Central Agricultural University, Umiam, Meghalaya, India.
[2]ICAR Research Complex for NEH Region, Umiam, Meghalaya, India.

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ABSTRACT

A pot experiment was conducted to study the effects of insecticides on soil micro-biota under laboratory condition. Soil was collected from control plot (0-15 cm depth) where no insecticides were applied and filled in plastic pots. Three insecticides *viz.* indoxacarb 14.5 SC, chlorfenapyr 10SC and chlorpyrifos 20EC with two doses were applied twice at 15 days of interval on the pot soil surface. Sampling of soil from pot was done by using PVC core (1 inch diameter) after 72 hours of each spray. Inoculation of serially diluted soil solution was done on culture media for microbial count. The number of colonies of bacteria and fungi on the plates were counted directly or with the help of a colony counter. The results revealed that the higher mean log CFU/g population of soil bacteria was recorded in the normal dose indoxacarb (5.77-5.99 cfu/g of soil) and chlorfenapyr (5.79-5.83cfu/g of soil) whereas soil fungi was higher in normal dose of indoxacarb (5.49-6.10 cfu/g of soil) and chlorpyrifos (5.54-5.99 cfu/g of soil).

1. Introduction

Soil microorganisms such as bacteria, fungi, protozoa, actinomycetes and other soil dwelling agents play an important role in agriculture. Soil microorganisms seem to be very appropriate and sensitive early-warning indicators or predictive tools in soil health monitoring (Pampulha and Oliveira, 2006). Recently, bacteria have been used in soil for the mineralization of organic pollutants for bioremediation of polluted soils (Burd et al. 2000; Middledrop et al. 1990; Zhuang et al. 2007; Zaidi et al. 2008). Plants show a different range of interactions like competitive, neutral, exploitative, commensal and mutualistic with the microorganisms dwelling in soil (Toor and Adnan, 2020). In modern agriculture, different classes of chemical pesticides are used to protect the crops from various pests like insects, diseases causing organisms, weeds etc., thereby pesticides residues are accumulating in soil system and disturbing the normal activities of microorganisms. Soil micro flora, mainly bacteria, fungi, algae and protozoa make an important role in making the soil fertile through degradation of many plants and animal residues in the soil (Chowdhury et al. 2008).

Pesticides that disrupt the activities of the soil microorganisms as well as affect the nutritional quality of soils that leads to serious ecological consequences (Handa et al. 1999). Large quantities of pesticides reaching to the soil have a direct effect on soil microbiota, which is a biological indicator of soil fertility influencing plant growth and development (Santos and Flores, 1995; Fabra et al. 1997; Hussain et al. 2009). As pesticides application in crop field is very quick, easy and one of the most effective and reliable method of pest control in short period of time, hence these are widely used in agriculture. Vegetables alone consume 14% of the total pesticides used in India, in which the share of different types of pesticides in Indian agriculture market shows that organophosphorus (50%) ranked first, followed by pyrethroids (19%), organochlorines (18%), carbamates (4%) and bio-pesticides (1%) (Dhaliwal and Singh, 2000). Pesticides may badly affect the multiplication of beneficial soil microorganisms and their associated biotransformation in the soil (Hussain et al. 2009). Indiscriminate use of these pesticides on crops has detrimental effects to different non-targeted organisms. It has been assessed that only about 0.1%

*Corresponding author: sakil.dhamala@gmail.com

of the pesticides reach the target organisms and the remaining bulk contaminates the surrounding environment (Carriger et al. 2006). Therefore, these necessities to study the effects of very commonly used insecticides on soil micro-biota to identify the safe use of insecticides in agriculture. Hence, the present experiment was conducted to evaluate the effect of three common insecticides with two different dose levels on soil micro-biota under laboratory condition.

2. Materials And Methods

Pot study

A pot experiment was conducted in 2017 to study the effects of insecticides on soil micro-biota under laboratory condition in ICAR Research Complex for NEH Region, Umiam, Meghalaya, India. Soil was collected from control plot (0-15 cm depth) totally free from insecticide use. Collected soil was filled in plastic pots. Each plastic pot received 500 grams of soil. Soil moisture content in pots was maintained at field capacity. Pots were arranged in rows with 7 treatments and 5 replications. Seven treatments *viz.* indoxacarb 14.5 SC @ 75 g a.i./ha (normal dose), indoxacarb 14.5 SC @150 g a.i./ha (double dose), chlorfenapyr 10 SC @100 g a.i./ha (normal dose), chlorfenapyr 10SC @ 200 g a.i./ha (double dose), chlorpyriphos 20EC @ 200g a.i./ha (normal dose), chlorpyriphos 20EC @ 400 g a.i./ha (double dose) and control (water spray) were applied using hand atomizer twice at 15 days of interval on the pot soil surface.

Soil sampling from pot

Sampling of soil from pot was done by using PVC core (1 inch diameter) after 72 hours of each spray. The effect of test insecticides on soil micro-biota (bacteria and fungi) in pots were determined in terms of population counts in colony forming unit in microbiology laboratory, College of Post Graduate Studies for Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya.

Preparation of microbial culture media

Preparation of nutrient agar (HiMedia) and rose Bengal agar (HiMedia) required for 500 ml of the medium was weighed and dissolved in 500 ml of distilled water in a 1 liter conical flask by shaking and sterilized at 121°C (15 psi pressure) for 15 minutes in an autoclave along with ten numbers of centrifuge tubes and 21 petri dishes. The sterilized media were removed from the autoclave and make them cool for some time, without allowing the medium to solidify. The sterilized warm molten nutrient medium was poured aseptically into the sterilized petri dishes so that the molten medium covers the bottom of the petri dishes completely. The plates were covered with their lids and allowed to cool, so as to solidify the medium in them.

Serial dilution for microbial counting

One gram of the soil sample was weighed and homogenized in 10 ml sterilized distilled water and shaken in vortex under aseptic condition and gives 10 times dilution (dilution = 10^{-1}). Serial dilution were prepared from an initial 10^{-1} diluted solution by taking 1 ml of dilution and adding 9 ml of sterilized water to make 10^{-2} dilution, similarly dilution of 10^{-3}, 10^{-4} and 10^{-5} were prepared from the dilution of 10^{-2}, 10^{-3} and 10^{-4} respectively.

Inoculation of serial diluted soil solution containing microbes on nutrient media

The petri plates containing nutrient agar media and rose Bengal agar media were inoculated with diluted soil solutions separately by dropping 1 µl of diluted solutions respectively. Then, the drops of suspension on the agar plates were spread aseptically by a sterilized L shaped spreader. Petri dishes were wrapped with parafilm and incubated in inverted position, top down, at 30°C for 24 hours in an incubator.

Microbial count

The number of colonies of bacteria and fungi on the plates were counted directly or with the help of a colony counter. From this, the number of bacteria and fungi per gram or ml of the original sample was calculated.

3. Results And Discussion

Effect of different insecticide treatments on soil bacteria and fungi

The effect of different insecticide treatments on soil bacterial population are presented in the Table 1. The results revealed that out of six insecticide treatments, the mean log CFU/g population of soil bacteria after first spray were highest in normal dose of indoxacarb (5.99) and it was followed by control (5.82), normal dose of chlorfenapyr (5.79), double dose of chlorfenapyr (5.75), normal dose of chlorpyrifos (5.72), double dose of indoxacarb (5.57) and double dose of chlorpyrifos (5.52). After second spray, pattern of bacterial population was different as compared to first spray. The highest mean log CFU/g population of soil bacteria was recorded in the control (6.14) followed by chlorfenapyr 100 g a.i./ha (5.83), indoxacarb 75 g a.i./ha (5.77), chlorpyrifos 200 g a.i./ha (5.75), chlorfenapyr 200 g a.i./ha (5.67), chlorpyrifos 400 g a.i./ha (5.66) and indoxacarb 150 g a.i./ha (5.33), respectively.

The effect of different insecticide treatments on soil fungus population are presented in the Table 2. The results showed that the mean log CFU/g population of soil fungus after the first spray was maximum in indoxacarb 75 g a.i./ha (6.10) followed by chlorpyrifos 100 g a.i./ha (5.99), chlorpyrifos 200 g a.i./ha (5.98), indoxacarb 150 g a.i./ha

(5.55), chlorfenapyr 100 g a.i./ha (5.45), chlorfenapyr 200 g a.i./ha (5.32) and control (5.25), respectively. After the 3 days of second spray, the mean log CFU/g population of soil fungus were highest in the chlorpyrifos 200 g a.i./ha (5.54) followed by indoxacarb 75 g a.i./ha (5.49), chlorpyrifos 100 g a.i./ha (5.32), indoxacarb 150 g a.i./ha (5.29), control (5.25), chlorfenapyr 100 g a.i./ha (5.20) and chlorfenapyr 200 g a.i./ha (5. 04), respectively.

In the present experiment, it was found that indoxacarb at recommended dose had less negative impact on soil bacterial population whereas chlorpyrifos had more detrimental effect on soil bacteria as compared to control. The double dose of all tested insecticides recorded less number of bacterial populations. The findings of present study are in close conformity with results of Ahmed and Ahmad (2006) who reported that among the insecticides (chlorpyrifos, imidacloprid, cypermethrin, endosulfan, carbofuran, cypermethrin and bifenthrin), chlorpyrifos treated petri plates showed reduction of bacterial colonies and proved to be most destructive on soil bacteria however, effects in field experiment disappeared after 21 days of application. The present results are in agreement with the study of Sultan et al. (2010) who showed that treatment of soil with chlorpyrifos at different concentrations brought about a reduction in bacterial population in all concentrations and the plate treated with higher concentration (10000 ppm) showed the low bacterial population as compared to control plate. The lower dose of pesticides increased the population counts of soil bacteria and actinomycetes; though, at the higher levels of pesticides may alter or depressed the microbial population in soil (Fletcher 1960; Omar and Abdel-Sater, 2001). Organophosphate pesticides have effect on some groups of soil microorganisms like nitrogen fixers, nitrifiers, heterotrophic bacteria and fungi (Mitra and Raghu, 1998; Singh et al. 1999; Das and Mukherjee, 2000). Indiscriminate application of chlorpyrifos may lead to the microbial imbalance (Adak et al. 2016; Kumar et al. 2012; Sasikala et al. 2012; Gupta et al. 2013). In the present study, higher population of fungi was observed in normal dose of indoxacarb and chlorpyrifos but double dose of all tested insecticides recorded less number of fungal populations. Similar results reported by Pandey and Singh (2004) who revealed that the fungal populations were significantly enhanced after chlorpyrifos treatment. Bhagabati and Sharma (2011) reported that the soil treated with pesticides dichlorvos and carbofuran had some effect on population of soil micro flora as compared with control. The presence of pesticide residues in soil had short term inhibitory effects on soil microbial as well as toxicity effects to the soil microflora and microfauna (Hua et al. 2009; Babendreier et al. 2015; Singh et al. 2015; Sahoo et al. 2016). However, information on long-term effect of pesticides on non-target soil microflora and fauna is scanty (Smith et al. 2000).

From the present experiment it was found that the higher populations of soil bacteria were recorded in the normal dose indoxacarb and chlorfenapyr whereas populations of soil fungi were higher in the normal dose of indoxacarb and chlorpyrifos. Therefore, it may be concluded that indoxacarb is relatively safer insecticide towards soil bacteria and fungi.

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Table 1. Population of soil bacteria after 1st and 2nd spray of insecticides

Treatments	Dose (g a.i./ha)	Population after 1st spray (cfu/g of soil)	Population after 2nd spray (cfu/g of soil)
Indoxacarb 14.5 SC	75	9.9×10^{5} $(5.99)^{c}$	6.2×10^{5} $(5.77)^{a}$
Indoxacarb 14.5 SC	150	3.7×10^{5} $(5.57)^{a}$	3.4×10^{5} $(5.53)^{a}$
Chlorfenapyr 10SC	100	6.2×10^{5} $(5.79)^{b}$	7.5×10^{5} $(5.83)^{a}$
Chlorfenapyr 10SC	200	5.5×10^{5} $(5.75)^{b}$	4.3×10^{5} $(5.67)^{a}$
Chlorpyrifos 20EC	200	5.3×10^{5} $(5.72)^{a}$	6.4×10^{5} $(5.75)^{a}$
Chlorpyrifos 20EC	400	3.4×10^{5} $(5.52)^{a}$	3.4×10^{5} $(5.66)^{a}$
Control	-	6.7×10^{5} $(5.82)^{b}$	1.40×10^{6} $(6.14)^{b}$

Figures in parentheses are mean log CFU/g soil

Table 2. Population of soil fungus after 1st and 2nd spray of insecticides

Treatments	Dose (g a.i./ha)	Population after 1st spray (cfu/g of soil)	Population after 2nd spray (cfu/g of soil)
Indoxacarb 14.5 SC	75	2.0×10^{5} $(6.10)^{b}$	3.1×10^{5} $(5.49)^{c}$
Indoxacarb 14.5 SC	150	3.6×10^{5} $(5.55)^{a}$	2.0×10^{5} $(5.29)^{b}$
Chlorfenapyr 10SC	100	2.9×10^{5} $(5.45)^{a}$	1.6×10^{5} $(5.20)^{b}$
Chlorfenapyr 10SC	200	2.1×10^{5} $(5.32)^{a}$	1.1×10^{5} $(5.04)^{a}$
Chlorpyrifos 20EC	200	9.6×10^{5} $(5.99)^{b}$	2.1×10^{5} $(5.32)^{b}$
Chlorpyrifos 20EC	400	9.4×10^{5} $(5.98)^{b}$	3.5×10^{5} $(5.54)^{c}$
Control	-	1.8×10^{5} $(5.25)^{a}$	1.8×10^{5} $(5.25)^{b}$

Figures in parentheses are mean log CFU/g soil