Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 40-45

Received: 21-11-2019
Accepted: 25-12-2019

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Impact of sprayable and granular formulations of insecticides on population of rhizospheric micro flora in rice ecosystem

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Abstract
Field and laboratory experiments were conducted to investigate twelve insecticides comprising granular and spray formulations of imidacloprid, cartap hydrochloride, fipronil, chlorantraniliprole along with carbofuran, phorate and monocrotophos as insecticidal checks for their effects on soil micro flora in rice ecosystem during *kharif* and *rabi* 2015. The populations of micro flora were clearly dependent on the type of insecticide formulation and the number of days after application. Insecticidal applications at 20 and 50 DAT demonstrated a decline in soil microbial populations at 7 days after application, which gradually recovered with a gradual built up at 14 and 21 days after. Granules in general showed a greater negative effect on soil micro flora compared to their spray counterpart. At initial stage of application of insecticides the population of micro flora reduced in an exponential manner, although it was seen to recover after 21 days after application of insecticides. The data collected during *kharif* '2014 visibly indicated that carbofuran 3G and phorate 10G were the most favourable granules for augmentation of microbial population. The granular insecticides that helped in boosting up the population of bacteria and actinomycetes were carbofuran and phorate along with sprayable imidacloprid and chlorantraniliprole during both *kharif* and *rabi* seasons. All the granular treatments were detrimental on fungal population and amongst the sprayable ones; cartap, fipronil and chlorantraniliprole were the ones having less adverse effect on fungal population.

Keywords: Soil micro flora, bacteria, fungi, actinomycetes, sprayable and granular insecticides

Introduction
Rice is grown in many regions across India and is the second leading producer (111.0 million tonnes) of rice in the entire world next to China (148.87 million tonnes) during 2017-18. In India, out of total food grain production of 277.49 million tones, rice contributes to 111.01 million tonnes. (Ministry of Agriculture & Farmer's welfare, 2017-18). An estimated 18-26 % of annual crop production worldwide has been caused by insects. The losses have been heaviest in developing countries with a 13-16% loss in field condition [1]. Insecticidal application though considered as first line of defense against insect pests, has some adverse effect on non-target organisms including soil micro flora which may affect several soil functions altering the soil fertility and influencing the growth and development of plants. Plants interacting with beneficial microbes can also benefit from increased tolerance to herbivory [2]. A thorough understanding on effects of insecticides on non target organisms will therefore, enable the scientists in selection of a safer formulation of insecticide which fits into pest management strategies as well as has less deleterious effect on soil micro flora. Hence the present investigation has been taken up to evaluate insecticides comprising of granular and spray formulations for their effects on soil micro flora in rice ecosystem. Twelve insecticides *viz*. granular and spray formulations of imidacloprid, cartap hydrochloride, fipronil, chlorantraniliprole along with carbofuran, phorate and monocrotophos as insecticidal checks were taken up for assessment of their effects on soil micro flora. The insecticides were applied at recommended doses and for the evaluation of micro flora population, soil samples were collected from rhizosphere of rice field. The soil samples were analysed in laboratory using the serial dilution technique. The detail of this study has been presented below.

Materials and Methods
Field experiment was taken up in rice field of Central Agricultural Research Farm of Odisha University of Agriculture and Technology, Bhubaneswar to study the effect of sprayable and

granular formulations of insecticides on the population of soil micro flora.

The field experiment was laid out in randomized complete block design with popularly grown variety "Swarna" replicated thrice in sub plots of 20 m^2 at a spacing of 20x15 cm. The treatments evaluated were as follows.

Table 1: Insecticide and formulation

Treatment No.	Insecticide and formulation	Dose (Per ha)
T_1	Imidacloprid 0.3 G	12.5 kg
T_2	Cartap 4 G	20.0 kg
T_3	Fipronil 0.3 G	12.5 kg
T_4	Chlorantraniliprole 0.4 G	10.0 kg
T_5	Carbofuran 3 G (check)	33.0 kg
T_6	Phorate 10 G (check)	12.5 kg
T_7	Imidacloprid 17.8 SL	150 ml
T_8	Cartap hydrochloride 50SP	750 g
T_9	Fipronil 5 SC	1.0 lit
T_{10}	Chlorantraniliprole 18.5 SC	150 ml
T_{11}	Monocrotophos 36 SL (Check)	1.0 lit
T_{12}	Untreated control	--

The test insecticides belonging to four different groups and with separate mode of actions were used primarily to observe their impact on soil microbial population inhabiting the rhizosphere. The insecticides were applied twice at 25 and 50 DAT and soil sample from rhizosphere were collected at 7, 14 and 21 days after second application of insecticides for studying micro flora population following standard serial dilution technique. The soil extracts were subjected to step wise dilution keeping the dilution factor constant resulting in dilution in a geometric progression leading to concentrations in a logarithmic fashion. A ten-fold serial dilution was made resulting in 1 M, 0.1 M, 0.01 M, 0.001 M concentrations. The microbial population assessments were made by using growth media for bacteria, fungi and actinomycetes following standard procedures.

Preparation of diluents

In a conical flask 420 ml of distilled water was taken and 4.53g of phosphate buffer was added to it for preparation of diluent. The diluent was then transferred to 36 test tubes, out of which 12 test tubes contained 10 ml phosphate buffer solution and 24 test tubes contained 9 ml of phosphate buffer solution each. Then the test tubes containing diluents were autoclaved at 121^{0}C at 15lb pressure for 15-20 minutes. Then 1g of soil from each treatment were added to the test tubes containing 10 ml of diluents and shaken well. (10^{-1} dilution). In order to dilute the concentrated microbial load, the solutions prepared from 10^{-1}dilution were transferred to the other test tubes containing 9 ml of the diluent. Thus making it 10^{-2} and 10^{-3} dilution and reducing the number of microbes present so as to make the population countable. For isolation and enumeration of the microbial isolates the microbiological medium was procured from HI MEDIA Ltd., Mumbai and prepared as per the manufacturer's instructions. For bacteriological analysis Nutrient Agar, for fungal study Potato Dextrose Agar and Actinomycetes isolation agar (AIA) for actinomycetes was used. As per the instructions provided by the manufacturer the above media were prepared, sterilized and poured in to plates for conducting microbial study.

Preparation of media

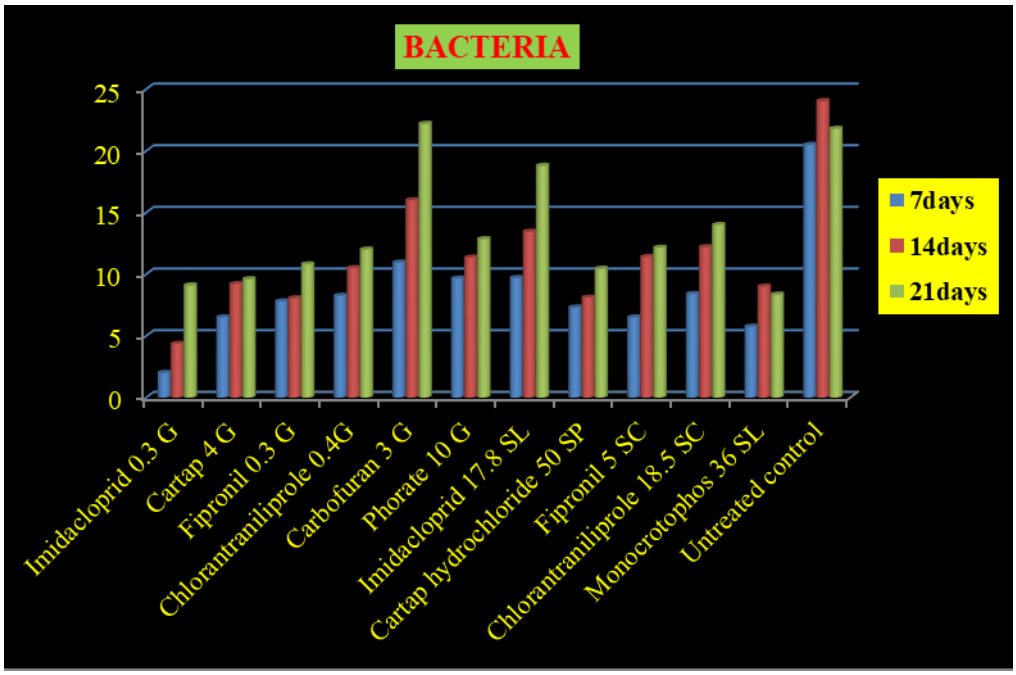
500 ml of distilled water was taken in three different conical flasks. For bacteria 18.5 g of Nutrient Agar, for fungi 19.5 g of Potato Dextrose Agar and for actinomycetes 15g of Actinomycetes Isolation Agar was added to the distilled water and shaken well. Then the media was sterilized at 121 ^{0}C at 15 lb pressure for 15-20 minutes. Prior to sterilization glycerol was added to AIA media as supplement for better growth of actinomycetes. While preparing media for fungi, anti bacterial agent (Streptomycin @ 35mg/l) was added to restrict the growth of bacteria; both anti bacterial (Streptomycin @ 35mg/l) and anti fungal agent (Nystatin @ 50mg/l) were added for actinomycetes. After sterilization of the media, it was allowed to cool down for 15-20 minutes. Then poured to the petridishes inside the laminar airflow and kept for 30 minutes till the media was solidified. For each of the experiment 24 petridishes were taken; two replications for each treatment. After solidification of the media, 10 micro litres of the diluents from each treatment were transferred on to the media with the help of a micropipette. Using a sterilized L- shaped spreader the diluents were spread onto the media. The observations were taken after 24, 48 and 72 hours for bacteria, fungi and actinomycetes population respectively. The count for the micro-organisms was taken as colony forming unit (cfu).

$$\text{cfu/g of soil} = \frac{\text{Mean cfu of plate}}{\text{Quantity taken x dilution factor}}$$

Results

Effect of insecticides on bacterial population collected from rhizospheric soil of rice ecosystem during *kharif* and *rabi* 2015

The bacterial population study provides a clear cut indication that the population of bacteria in the rhizospheric soil was found to be influenced by the type of insecticides and the days after application of insecticides. As per the days of treatments, it was observed that both the bacterial population was adversely affected within 7 days of application of the insecticides. The population did ultimately recover and increase from the first observation on i.e. the 7th day after the application of the insecticide to the last day of observation on 21st day. The experimental result of the bacterial load with respect to the insecticide application indicates that all the insecticides had adverse effect on the bacterial population within seven days. Whereas, carbofuran 3G was found to be less toxic as compared to untreated check. Imidacloprid 17.8 SL and phorate 10 G were found to have less adverse effect on bacterial population as compared to untreated check. Imidacloprid 0.3G on the other hand, unlike its sprayable formulation was found to be detrimental to bacterial population as compared to other treatments (Figure 1). The data obtained from samples collected during *rabi* '2015 showed a greater similarity to that of the soil samples collected during *kharif* '2015. Again the plots treated with granular carbofuran and phorate proved to bear maximum number of bacteria next to untreated plots. The sprayable formulation of imidacloprid was seen to have less deleterious effect on bacteria among the sprayable treatments.



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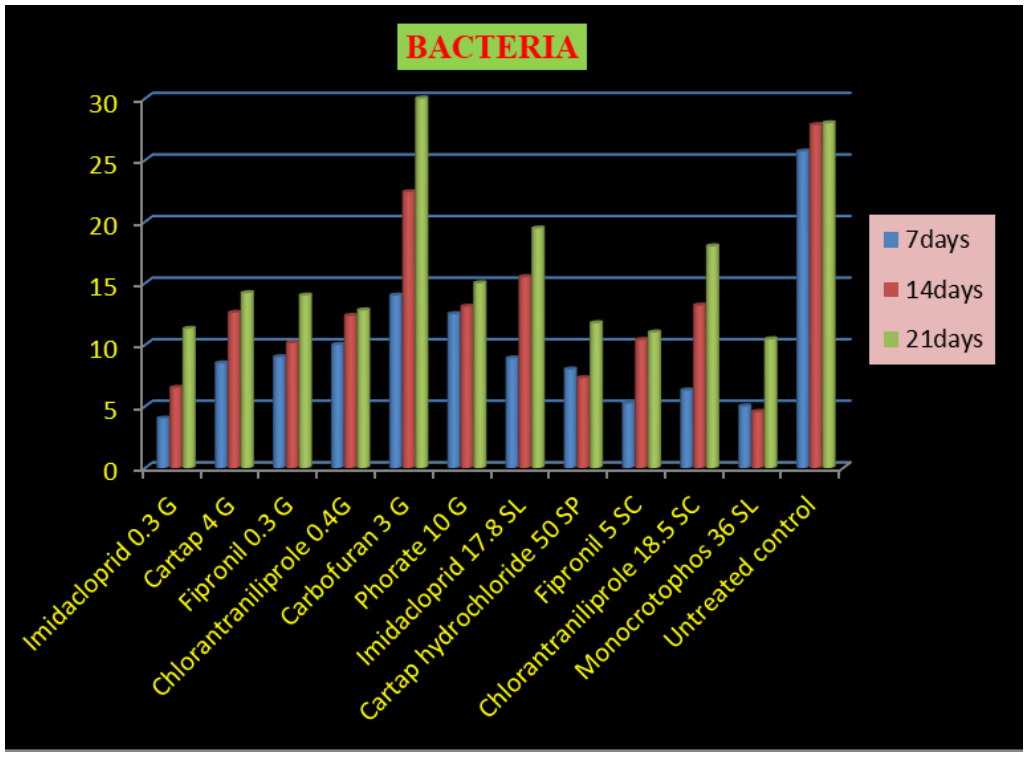
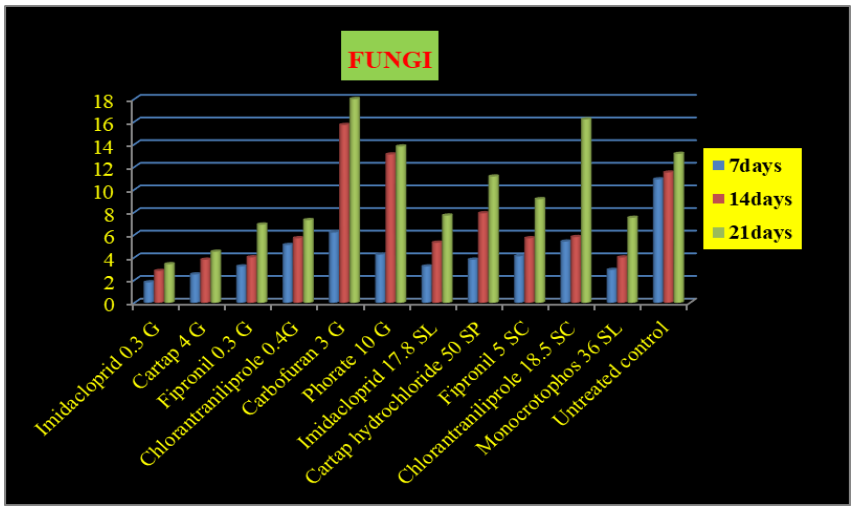


II

Fig 1: Graphical presentation of bacterial population present in rhizospheric soil of rice ecosystem during: i. *Kharif* season, ii. *Rabi* season

Effect of insecticides on fungal population collected from rhizospheric soil of rice ecosystem during *kharif* and *rabi* '2015

The soil fungal population though affected after the treatments of the insecticides, but in case of carbofuran 3G, it was found to be beneficial as it favoured in augmentation of the population of fungi. During *kharif*, it was noticed that the soil collected from carbofuran 3G treated plots were having high population of fungi as compared to untreated check. Phorate 10G and cartap hydrochloride 50SP were found to be having less deleterious effect as compared to the population in untreated check. While analyzing the fungal population load with respect to the mode of application of the insecticides, it was observed that the granular insecticides are more harmful to the fungal population of the soil than that of the foliar application. Just like in case of bacteria, imidacloprid 0.3G was found to be unfavourable for colonisation of fungal population (Figure 2). The computation of fungal colony formation units during *rabi* season presented in figure 2 showed that all the granular insecticides had deleterious effect and amongst the sprayable formulations, cartap hydrochloride, fipronil and chlorantraniliprole were having less adverse effect on fungal population. Although all the granular insecticides had deleterious effect, imidacloprid 0.3 G was seen to have more negative effect as compared to all the other treatments.



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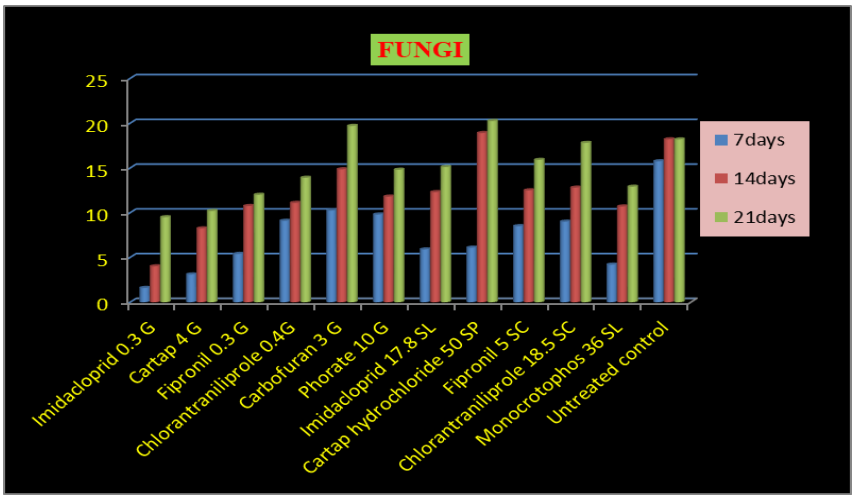

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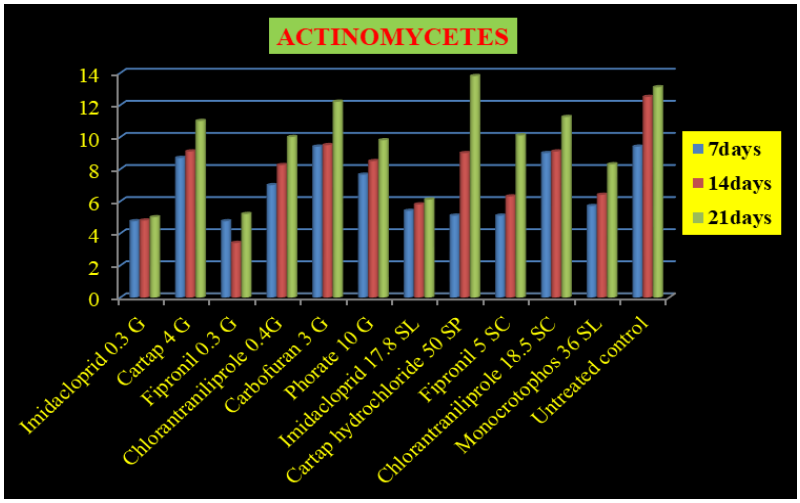
Fig 2: Graphical presentation of fungal population present in rhizospheric soil of rice ecosystem during: i. *Kharif* season, ii. *Rabi* season

Effect of insecticides on actinomycetes population collected from rhizospheric soil of rice ecosystem during *kharif* and *rabi* 2015

The study of actinomycetes population provides a clear cut indication that the population of actinomycetes in the rhizospheric soil was found to be influenced by the type of insecticides and the days after application of insecticides. From figure 3, it is seen that among granular insecticides, carbofuran 3G followed by phorate 10G and chlorantraniliprole 18.5 SC amongst the sprayable formulations were proved to have less deleterious effect on actinomycetes population as compared to the population in control. Granular formulations of fipronil and imidacloprid were having least of colonisation of actinomycetes which indicates their negative effect on the microbe.



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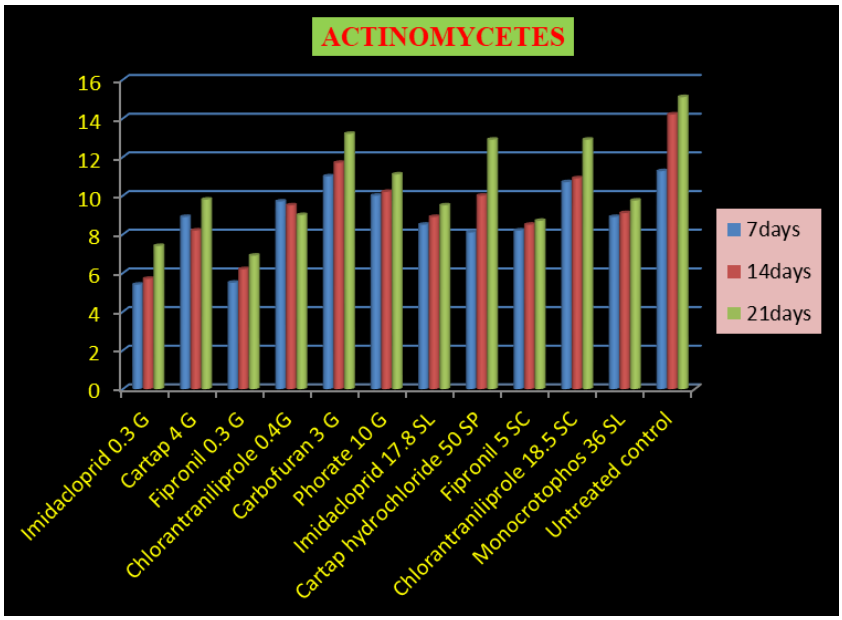

II

Fig 3: Graphical presentation of actinomyctes population present in rhizospheric soil of rice ecosystem during: i. *Kharif* season, ii. *Rabi* season

During the study in *rabi* season, it is noticed (figure 3) that initially the population is reduced but gradually the population recovers during later stages, that is, 21 days after application. The treatments having less deleterious effect on actinomycetes population were granular carbofuran and phorate again along with sprayable chlorantraniliprole. These three insecticides have been seen to have less toxic effect as compared to other insecticidal treatments. Imidacloprid 0.3 G was high detrimental as compared to all others.

Discussion

Several plant growth-promoting fungi and rhizobacteria help the plants to deal with biotic and abiotic stress by using mechanisms like induced resistance and plant growth promotion. Such beneficial soil inhabiting microbes have tritrophic interaction via plants with above ground insects such as herbivores, their natural enemies and pollinators [3]. In order to evaluate the pesticide risk assessment, it is important to understand the effect of pesticides on soil micro flora and their beneficial activities [4]. It has been reported that the impact of pesticide application on micro flora depends on interaction between microorganisms and active substance and formulations. The microorganisms can develop the ability to use an applied pesticide as a source of energy and growth.

The plant growth promoting effects of soil borne microbes has been publicized to affect plant-insect interactions, resulting in benefit for the insects and for the plants. Enhanced plant growth results in increased food supply for herbivores which affects the insect performance at several trophic levels [5]. The interaction of plants with beneficial microbes can also help the plant in promoting tolerance to herbivory [6, 2]. The regrowth of tissues after herbivory is as a result of higher nutrient uptake by beneficial microbes, thus promoting plant tolerance, which is reflected in compensation of the loss of plant biomass or yield in the presence of herbivore [7-9].

The adverse effects of pesticides are manifested only at higher concentration and have no adverse effects on the population of bacteria in soil except at concentrations exceeding recommended dose [10]. However, in this study insecticidal applications in *kharif* season at 20 and 50 DAT demonstrated marked decline in soil microbial populations at 7 days after application, which gradually recovered with a gradual built up at subsequent periods. Negative impact of insecticides on microbes has earlier been reported [11]. Agrochemical compounds and/or their degradation products showing negative effects to the environment at large and microbes in particular was also reported [12, 13]. However, in this study the population did recover to a greater extent with time, which may be due to the fact that the degradation of insecticides might have helped in resuming the microbial activity. Maximum population registered in untreated check samples were 24.05 x 10^4, 13.15x 10^3 and 13.1 x 10^3 cfu of bacteria, fungi and actinomycetes per gram of soil respectively indicating the dominance of bacteria in soil rhizosphere. Bacteria population which constitute more than 50 % of entire microbial population had a maximum decline in plots receiving imidacloprid granule (2x10^4 cfu/ g soil) compared to its spray formulation(9.7 x 10^4 cfu/ g soil) as against a population record of 20.5 x 10^4 cfu/ g soil in control at 7 days after application. However, the population rapidly built up at 14 and 21 days attaining a maximum population of 9.1 x 10^4 and 18.8 x 10^4 in granule and spray treatments of this molecule respectively as against 21.8 x 10^4 cfu/ g in control (Figure 1). Similar results were reported earlier [14, 15]. The effect of spray formulation of this compound almost got nullified at 21 days after second spraying. The insecticidal check, carbofuran 3G on the other hand had minimal adverse impact on bacterial population. Earlier findings suggest carbofuran has a phytotonic effect on plants [16] which might be attributed to greater colonization of a plant growth promoting rhizobacteria. Induced systemic resistance by fluroscent pseudomonas against various pests is considered as the most desirable approach in crop protection [17]. An increased accumulation of defence molecule such as chitinase and proteinase inhibitors due to fluroscent pseudomonas (combined strain Pf1, TDK1 and PY15) was also reported earlier which can play a vital role in management of *Cnaphalococis medinalis* in rice [18, 19]. Chlorantraniliprole, which showed a better performance against insect pests had favoured a greater colonization of bacteria (10-18 x 10^4 cfu/g) compared to that of cartap (8-11.75 x 10^4 cfu) and fipronil

(5.25 - 11 x 10^4 cfu/g) as against 25.7-28 x 10^4 cfu/g bacterial population in control at various stages of observations.

During *rabi* season similar trends in population built up of bacteria were recorded as in *kharif* season. The microbial population in this season was relatively higher compared to *kharif* one, which may be due to alternate drying and wetting of rice field providing a greater aerobic condition that are favourable for microbial multiplication. During this season also imidacloprid was the worst and chlorantraniliprole the best performer in favouring bacterial multiplications with spray formulation better than their granular formulations.

Compared to bacteria, the fungal and actinomycetes population were very low in experimental plots. A maximum of 13.15 x 10^3 and 13.1 x 10^3 cfu of fungi and actinomycetes respectively per gram of soil could be recorded in control plots at final observation in comparison to 21.8 x 10^4 cfu/g bacteria. In both the cases granular formulations showed a greater degree of toxicity compared to their spray counterparts because of the obvious reason that in spray insecticide has less chance of reaching to microbes compared to granules. Like bacteria, these two groups of microbes were most adversely affected by both formulations of imidacloprid with a record of lowest (3.4- 7.7 x 10^3 fungal and 5 - 6.1 x 10^3 cfu/g actinomycetes) population. On the other hand chlorantraniliprole proved safer to fungi (7.3-16.3 x 10^3) and actinomycetes (10 -11.25 x 10^3) compared to other test molecules but remained inferior to insecticidal check carbofuran (18 x 10^3 and 12.2 x 10^3 fungi and actinomycetes cfu/g soil respectively). Cartap was the next best chemical with comparatively lesser adverse effects on both fungi (7.3-11.2 x 10^3) and actinomycetes (10 - 13.8 x 10^3 cfu/g).

Thus, the present finding clearly demonstrated the ability of insecticides to alter the soil biological activity by directly affecting the microbial population. Compounds like chlorantraniliprole, cartap and carbofuran may be picked up as preferred compounds having better insecticidal activities and safer to microbes in soil for better management of insect pest in rice ecosystem.

Acknowledgement

The authors are very grateful to Dr. S. Dash, Assistant Professor, Department of Microbiology, CPGS, OUAT for his help in successfully conducting the laboratory assessment of soil micro flora population.

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