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Relative Influence of Insecticides on Colony Forming Units (CFUs) of Bacteria and Fungi in Rice (Oryzasativa L.) Soil

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ABSTRACT: A laboratory study was conducted at Department of Entomology, Agricultural College, Bapatla to investigate the influence of five insecticides *viz.*, imidacloprid 17.8 SL, chlorantraniliprole 18.5 SC, dinotefuran 20 SG, pymetrozine 50 WG, cartap hydrochloride 4 G at recommended dose (RD) and double the recommended dose (DRD) on the colony forming units (CFUs) of bacteria and fungi in black clay and sandy clay loam soils collected from fallow rice fields of Guntur district. The results indicated that the application of imidacloprid 17.8 SL at both RD and DRD resulted in significant decline in bacterial and fungal population in both the soils with a mean value of 146.50 × 10⁻⁶ bacterial CFUs/g soil and 19.83 × 10⁻⁴ fungal CFUs/g soil at DRD. Chlorantraniliprole 18.5 SC at both RD and DRD had no significant inhibitory effect on bacterial population in both the soils. In fact, the fungal population declined slightly in both the soils (28.33 × 10⁻⁴ CFUs/g in sandy clay loam and 36.33 × 10⁻⁴ CFUs/g in black clay soil) treated with chlorantraniliprole at DRD compared to control. Cartap hydrochloride 4 G at RD had no inhibitory effect on bacteria 20 SG and pymetrozine 50 WG at DRD, resulted in greater declination in the bacterial and fungal population in both the soils compared to RD and control. The descending order of effect of insecticides total CFUs of bacteria and fungi were as follows: imidacloprid 17.8 SL > pymetrozine 50 WG > dinotefuran 20 SG > cartap hydrochloride 4 G > chlorantraniliprole 18.5 SL.

Keywords: Rice (Oryza sativa L.), Soil, Insecticides, Bacteria, Fungi, Colony forming units.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most common cereal, serving as a staple food for around half of the global population and is the major dietary energy source for most of the countries in the world. Over two billion people in Asia alone gain 80% of their energy requirements from rice, which has 80% carbohydrates, 7–8% protein, 3% fat and 3% fibre (Juliano and Goddard, 1985). It also provides the bulk of daily calories for many domesticated animals and humans (Ryan, 2011). Even though it possesses lesser antioxidant potency compared with other cereals, it can be considered as good candidate for natural sources of antioxidants and may hold the potential for the evolution of rice based functional foods, drugs, food preservatives, pharmaceuticals and cosmetic products (Chaudhari *et al.*, 2018).

In India, rice is grown in many regions and is the second leading producer in the entire world next to China (148.30 M t), producing 102.01 M t out of total food grain production of 144.52 M t (MoA & FW, 2020). In Andhra Pradesh, rice is grown in *kharif* (wet season) with 60% of total rice being cultivated during the season. Within the state, the area under rice cultivation in *kharif*, 2019-20 has increased to 15.64 lakh hectares as against 15.26 lakh hectares in *kharif*, 2018-19. Production in the state has increased to 80.13 lakh ton in *kharif*, 2019-20 as against 78.65 lakh ton in *kharif* 2018-19 (MoA&FW, 2020).

In modern agriculture, divergent groups of pesticides with different formulations, either simultaneously or in sequence are applied to save crops against weeds, insects, fungi and other pests (Quazi *et al.*, 2011). The application of pesticides in rice starts from the pre-sowing stage of crop growth and are often applied many times during one crop season and a part of applied pesticides always reaches the soil and may effect the soil environment (Xiaoqiang *et al.*, 2008).

The soil microbiota in rice cropping system is a complex entity comprising several genera of bacteria, fungi, actinomycetes and algae, whose populations are always in dynamic balance and continuously influence each other. Actinomycetes and fungi are dominant microbes and bacteria make up almost half of the soil microbial biomass (Nasreen *et al.*, 2015). These microorganisms are also involved in basic ecological processes such as recycling of essential plant nutrients, trash decomposition and humus formation, degradation of xenobiotics (Zabaloy *et al.*, 2008), nitrogen fixation, mineralization of carbon, nitrogen, phosphorus and other elements. The employment of pesticides in rice has been observed to exert detrimental effects on ecological activity of microbes. Although these pesticides are applied in low concentrations, once in the soil they can alter the physico-chemical properties of the soil like pH, salinity, alkalinity leading to unproductiveness of soil (Sarnaik *et al.*, 2006) and may lead to drop in the number of microorganisms, both qualitatively and quantitatively (Filimon *et al.*, 2015). With this background, the present study was conducted with the main objective to study the effect of insecticides on CFUs of bacteria and fungi.

MATERIALS AND METHODS

Soil Sampling: Black clay and sandy clay loam soils were collected randomly at different sites in the harvested rice fields of Guntur district of Andhra Pradesh, India. Samples were collected near the rhizosphere in empty field using spade at a depth of 0-

15 centimeters and mixed thoroughly to prepare a homogeneous composite sample (1 kg) and air dried at room temperature prior to insecticide treatment.

Preparation of Primary Stock Solution: Primary stock solutions of chlorantraniliprole 18.5 SC, imidacloprid 17.8SL, dinotefuran 20 SG, pymetrozine 50 WG at the concentration of 1850, 1780, 2000 and 5000 μ g ml⁻¹ each were prepared by adding exactly 1.0 ml of each insecticide into 100 ml of double-distilled water, respectively.

Preparation of Working Standards: Working standards of chlorantraniliprole 18.5 SC, imidacloprid 17.8 SL and dinotefuran 20 SG at the concentration of 1000, 500, 250, 100, 10 and 1 μ g ml⁻¹ were prepared by serial dilution method using 1850, 1780 and 2000 μ g ml⁻¹ primary stock solutions, respectively. Working standards of Pymetrozine at 2500, 1500, 1000, 500, 250, 100, 10 and 1 μ g ml⁻¹ concentrations were prepared by serial dilution method using 5000 μ g ml⁻¹ of pymetrozine primary stock solution.

Application of Insecticide Treatments to Soils: Generally the amount of soil present in one hectare of agricultural land is 2.24×10^{-6} kg. Based on this assumption, the amount of insecticide that has to be sprayed on 1 kg of soil was obtained by back calculating from the amount of insecticide (in terms of a.i.) that is sprayed on one hectare of Agricultural land. Cartap hydrochloride 4 G was directly applied to one kg of soil as this is soil applied insecticide.

The soils were treated with insecticides at both recommended dose (RD) and double the recommended dose (DRD) separately. Required dose of respective insecticide in μg a.i. (equivalent to one ppm of 100 ml of working standard of insecticide) was uniformly applied on one kilogram of soil using a small hand sprayer.

S. No.	Insecticide	Dose (g a.i ha ⁻¹)		Dose (µg a.i kg ⁻¹ soil)	
		RD	DRD	RD	DRD
1.	Chlorantraniliprole 18.5 SC	30	60	14	28
2.	Cartap hydrochloride 4 G	1000	2000	500	1000
3.	Imidacloprid 17.8 SL	25	50	12	24
4.	Dinotefuran 20 SG	40	80	18	36
5.	Pymetrozine 50 WG	150	300	67	134

Table 1: Details of treatments for application to soil.

Preparation of Media: Agar media were prepared separately for bacteria (nutrient agar) and fungi (potato dextrose agar) (Dhingra and Sinclair, 1985).

Preparation of Dilution Blanks and Enumeration of Bacteria and Fungi: Soil dilutions were prepared by diluting one gram of insecticide treated soil to 9 ml sterilized distilled water which makes 10^{-1} dilution. This stock solution was serially diluted to the concentrations at which the desirable organisms showed the optimum growth (the dilution blanks were labeled as 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7}). The required concentration was 10^{-6} for bacteria, and 10^{-4} for fungi. About 0.1 ml of the respective dilution was spread evenly using a sterilized glass spreader on the agar-medium plate and was inverted and incubated for 5–7 days at a temperature of 37° C. After the incubation period, the plates were removed from the incubator and the colony forming units (CFUs) were counted manually ranging between 30 and 300 in a Quebec colony counter and subjected to statistical analysis using two way ANOVA (two factorial CRD).

The total number of bacteria/fungi per mL of the original suspension / sample is as follows:

Organisms per millilitre per gram of the sample = $\frac{\text{Number of colonies (average of three replicates)}}{\text{Multiply}} \times \text{dilution}$

Amount plated

RESULTS AND DISCUSSION

Effect of insecticides on Soil Bacteria: The application of chlorantraniliprole at both recommended dose $(217.40 \times 10^{-6} \text{ CFUs/g})$ and DRD $(217.33 \times 10^{-6} \text{ CFUs/g})$ had no significant inhibitory effect on the bacterial population in sandy clay loam soil which was at par with control. In fact, in black clay soil, similar trend was noticed with no significant inhibitory effect on bacterial population at both recommended application rate $(273.33 \times 10^{-6} \text{ CFUs/g})$ and DRD $(266.85 \times 10^{-6} \text{ CFUs/g})$, which were on par with each other and also with control (Table 2). The above results were in uniformity with the findings of Sahu *et al.* (2019) where faster dissipation of chlorantraniliprole was observed in rice rhizosphere soil with 23.89 and 34.65 days dissipation half-lives for recommended dose (RD) and double the recommended dose (DRD) treatments, respectively having no considerable effect on the soil bacteria.

The application of cartap hydrochloride at recommended rates also had no significant inhibitory effect on bacterial population in sandy clay loam $(215.33 \times 10^{-6} \text{ CFUs/g})$ and black clay soil $(268.33 \times 10^{-6} \text{ CFUs/g})$ which were on par with control. However, it was observed that bacterial population decreased significantly in both the soils $(205.67 \times 10^{-6} \text{ CFUs/g})$ in sandy clay loam and $253.67 \times 10^{-6} \text{ CFUs/g}$ in black clay soil) treated with cartap hydrochloride at DRD compared to control (Table 2). The above results were corroborating with the experiments of Kumar *et al.* (2012) where the microbial biomass of soil increased with time up to 30 days in cartap hydrochloride as well as chlorpyriphos treated soil.

It was found that bacterial population declined significantly in both the soils treated with imidacloprid at recommended rates $(145.67 \times 10^{-6} \text{ CFUs/g} \text{ in sandy clay loam and } 208.33 \times 10^{-6} \text{ CFUs/g} \text{ in black clay soil} \text{ compared to control } (220.33 \times 10^{-6} \text{ CFUs/g} \text{ in sandy clay loam and } 274.33 \times 10^{-6} \text{ CFUs/g} \text{ in black clay soil} \text{ and other test molecules. Additionally, at DRD of imidacloprid, greater decrease in bacterial population was noticed in both the soils <math>(117.67 \times 10^{-6} \text{ CFUs/g} \text{ in sandy clay loam and } 175.33 \times 10^{-6} \text{ CFUs/g} \text{ in black clay soil}) compared to control (Table 2). The above results were identical with the findings of Mahapatra$ *et al.*(2017) who reported that application of imidacloprid at 25 g a.i. ha⁻¹ and 50 g a.i. ha⁻¹ had significantly affected the population and distribution of bacteria in soil. Similar studies conducted by Cycon*et al.*(2013) reported that imidacloprid applied at the recommended dose and 10 times the RD had caused significant effect on total bacterial and fungal populations in soil from the beginning of the experiment. These results were in line with our present findings.

The application of dinotefuran at recommended dose resulted in slight decline in the bacterial population in both the soils $(207.33 \times 10^{-6} \text{ CFUs/g} \text{ in sandy clay loam and } 262 \times 10^{-6} \text{ CFUs/g} \text{ in black clay soil})$ compared to control with 220.33 x 10^{-6} CFUs/g and 274.33 × 10^{-6} CFUs/g in sandy clay loam and black clay soil, respectively. Whereas at DRD, the same molecule

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resulted in greater decline in bacterial population in both the soils $(167.33 \times 10^{-6} \text{ CFUs/g in sandy clay loam and } 228 \times 10^{-6} \text{ CFUs/g in black clay soil})$ compared to control (Table 2). These results were similar with the findings of Thompson *et al.* (2020) where the application of neonicotinoids at higher doses altered the microbes and their enzymatic activities with nitrifying and nitrogen fixing bacteria being the most sensitive.

The application of pymetrozine at both recommended dose and DRD resulted in significant inhibitory effect of bacterial population in both the soils. Moreover, high declination was noticed at DRD (167.33×10^{-6} CFUs/g in sandy clay loam and 216.67 CFUs/g in black clay soil) compared to control with 220.33×10^{-6} CFUs/g and 274.33×10^{-6} CFUs/g in sandy clay loam and black clay soil, respectively (Table 2). These results were also identical with the reports of Xu *et al.* (2018) where application of pymetrozine at higher doses may result in contamination of enzymatic activites and microbial populations of soil. Among all the treatments chlorantraniliprole applied at recommended dose was found safer for soil bacteria with a mean value of 245.37×10^{-6} CFUs/g compared to other test molecules and control. Whereas, imidacloprid applied at DRD was found to decline the bacterial population of soil to a greater extent with a mean value of 146.50×10^{-6} CFUs/g compared to other test molecules (Table 2).

S. No.	Insecticide Treatments	Dose (g a.i ha ⁻¹)	Number of colony forming units (CFUs) of bacteria x 10 ⁶ /g of soil at 24 h after insecticide treatment				
			Sandy clay lo	oam Black clay soil	Mean		
1.	T ₁ : Chlorantraniliprole 18.5 SC @ RD	30	217.40 ^f	273.33 ^{ab}	245.37 ^{ab}		
2.	T ₂ : Chlorantraniliprole 18.5 SC @ DRD	60	217.33 ^f	266.85 ^{abc}	242.00 ^b		
3.	T ₃ : Cartap hydrochloride 4 G @ RD	1000	215.33 ^{fg}	268.33 ^{abc}	241.83 ^b		
4.	T ₄ : Cartap hydrochloride 4 G @ DRD	2000	205.67 ^h	253.67 ^d	229.67 ^c		
5.	T5: Imidacloprid 17.8 SL @ RD	25	145.67 ^k	208.33 ^{gh}	177.00 ^g		
6.	T6: Imidacloprid 17.8 SL @ DRD	50	117.67 ¹	175.33 ⁱ	146.50 ^h		
7.	T7: Dinotefuran 20 SG @ RD	40	207.33 ^h	262.00 ^c	234.67 ^c		
8.	T ₈ : Dinotefuran 20 SG @ DRD	80	167.33 ^j	228.00 ^e	197.66 ^e		
9.	T ₉ : Pymetrozine 50 WG @ RD	150	179.67 ⁱ	246.33 ^d	213.00 ^d		
10.	T ₁₀ : Pymetrozine 50 WG @ DRD	300	167.33 ^j	216.67 ^f	192.00 ^f		
11.	T ₁₁ : Untreated control		220.33 ^f	274.33 ^a	247.33ª		
	Mean		187.37 ^b	243.00 ^a			
For comparison of means							
	SEn		±	CD@5%	CV%		
	Treatment			5.31			
	Soil			2.26	2.12		
	Interaction			7.51			

DHA- Dehydrogenase Enzyme Activity, RD- Recommended Dose, DRD- Double the Recommended Dose

Note: Means that do not share a letter are significantly different at 5% level of significance.

Effects of Insecticides on Soil Fungi: The application of chlorantraniliprole at recommended rates had no significant inhibitory effect on fungal population in sandy clay loam $(31 \times 10^4 \text{ CFUs/g})$ and black clay soil $(38.67 \times 10^4 \text{ CFUs/g})$ which were on par with control. Surprisingly, it was observed that fungal population declined slightly in both the soils $(28.33 \times 10^4 \text{ CFUs/g})$ in sandy clay loam and $36.33 \times 10^4 \text{ CFUs/g}$ in black clay soil) treated with chlorantraniliprole at DRD compared to control (Table 3). Similar results were obtained by Ghosal *et al.* (2018) where the application of chlorantraniliprole at higher doses significantly changed the compositions of microbial communities in paddy soils initially, but the compositions of soil microbial communities recovered at later stage which may be due to utilization of the insecticide and its degraded products.

The application of cartap hydrochloride at recommended rates had no significant inhibitory effect on fungal population in sandy clay loam $(30.33 \times 10^4 \text{ CFUs/g})$ and black clay soil $(38.67 \times 10^4 \text{ CFUs/g})$ which were on par with control. Notably, it was observed that fungal population declined slightly in both the soils $(29.00 \times 10^4 \text{ CFUs/g})$ in sandy clay loam and $38.33 \times 10^4 \text{ CFUs/g}$ in black clay soil) treated with cartap hydrochloride at DRD compared to control (Table 3). These results were in uniformity with the outcome of Karnatak *et al.* (2007) where the application of cartap hydrochloride singly in rice ecosystem resulted in increase of fungal populations during the study period.

It was found that fungal population declined significantly in both the soils treated with imidacloprid at recommended rates (19.33 $\times 10^{4}$ CFUs/g in sandy clay loam and 27 $\times 10^{4}$ CFUs/g in black clay soil) compared to control (32 $\times 10^{4}$ CFUs/g in sandy clay loam and 40.67 $\times 10^{-4}$ CFUs/g in black clay soil) and other test molecules. In fact, at DRD of imidacloprid, greater decline in fungal population was noticed in both the soils (16.33 $\times 10^{-4}$ CFUs/g in sandy clay loam and 23.33 $\times 10^{-4}$ CFUs/g in black clay soil) compared to control (Table 3). This inhibitory effect of imidacloprid might be due to its longer persistence in soil. Studies conducted by Wang *et al.* (2014) on the effect of imidacloprid on soil microbes reported that the inhibitory effect of imidacloprid is due to its longer persistence in soil which restricts the growth of microbes.

It was found that application of dinotefuran at recommended dose had slight inhibitory effect on the fungal population in both the soils $(29.00 \times 10^4 \text{ CFUs/g} \text{ in sandy clay loam and } 36.33 \times 10^4 \text{ CFUs/g} \text{ in black clay soil})$ compared to control with $32.00 \times 10^4 \text{ CFUs/g}$ and $40.67 \times 10^4 \text{ CFUs/g}$ in sandy clay loam and black clay soil, respectively. But at DRD, the same molecule resulted in significant decline in fungal population in both the soils $(25.67 \times 10^4 \text{ CFUs/g} \text{ in sandy clay loam and } 29.33 \times 10^4 \text{ CFUs/g} \text{ in black clay soil})$ compared to control (Table 3). The findings of Yu *et al.* (2020); Zhang *et al.* (2015) with application of dinotefuran and other neonicotinoids at recommended rates to soil increased the activity of microbes and high application rates decreased the community diversity added strength to our results.

The application of pymetrozine at both field and recommended doses resulted in significant inhibitory effect of fungal population in both the soils. But more declination was observed at DRD (23.67×10^{-4} CFUs/g in sandy clay loam and 29.67×10^{-4} CFUs/g in black clay soil) compared to control (Table 3). Meena *et al.* (2020) proposed the use of pesticides at higher doses may result in decrease in the total microbial populations of soil which supported our findings.

Among all the treatments, chlorantraniliprole applied at RD was found to be safe for soil fungi with a mean value of 34.83×10^{-4} CFUs/g compared to other test molecules. Whereas, imidacloprid applied at DRD was found to decline the fungal population of soil to a greater extent with a mean value of 19.83×10^{-4} CFUs/g compared to other test molecules (Table 3).

Table 3: Effect of insecticides on f	fungal population in so	oil.
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S. No.	Insecticide Treatments	Dose (g a.i ha ⁻¹)	Number of colony forming units (CFUs) of fungi $\times 10^{-4}$ /g of soil at 3 days after insecticide treatment		
			Sandy clay loam soil	Black clay soil	Mean
1.	T ₁ : Chlorantraniliprole 18.5 SC @ RD	30	31.00 ^{ef}	38.67 ^{ab}	34.83 ^{ab}
2.	T ₂ : Chlorantraniliprole 18.5 SC @ DRD	60	28.33 ^{gh}	36.33°	32.33°
3.	T ₃ : Cartap hydrochloride 4 G @ RD	1000	30.33 ^{efg}	38.67 ^{ab}	34.50 ^b
4.	T ₄ : Cartap hydrochloride 4 G @ DRD	2000	29.00 ^{fgh}	38.33 ^{bc}	33.67 ^{bc}
5.	T5: Imidacloprid 17.8 SL @ RD	25	19.33 ¹	27.00 ^{hi}	23.17 ^f
6.	T ₆ : Imidacloprid 17.8 SL @ DRD	50	16.33 ^m	23.33 ^k	19.83 ^g
7.	T ₇ : Dinotefuran 20 SG @ RD	40	29.00 ^{fgh}	36.33°	32.67 ^e
8.	T ₈ : Dinotefuran 20 SG @ DRD	80	25.67 ^{ij}	29.33 ^{fg}	27.50 ^e
9.	T ₉ : Pymetrozine 50 WG @ RD	150	24.33 ^{jk}	34.00 ^d	29.17 ^d
10.	T10: Pymetrozine 50 WG @ DRD	300	23.67 ^{jk}	29.67 ^{fg}	26.67 ^e
11.	T ₁₁ : Untreated control		32.00 ^{de}	40.67 ^a	36.33ª
Mean			26.27 ^b	33.85 ^a	
		For comparis	son of means		
		SEm±	С	D@5%	CV%
Treatment		0.57		1.63	
Soil		0.24		0.70	4.67
Interaction		0.81		2.31	

DHA- Dehydrogenase Enzyme Activity, RD- Recommended Dose, DRD- Double the Recommended Dose Note: Means that do not share a letter are significantly different at 5% level of significance.

However, in all the treatments bacterial and fungal populations were significantly higher in black clay soil compared to sandy clay loam soil at both recommended dose and DRD. This might be due to due to presence of higher organic matter and soil moisture which provide enough substrate to support higher microbial biomass in black clay soil. Similar results were propounded by Uhlirova *et al.* (2005), who attributed that availability of moisture and organic matter strongly influenced the metabolism and survival of microorganisms in soil as the moisture level was less in sandy clay loam compared to black clay soil.

CONCLUSION

Chlorantraniliprole 18.5 SC at both recommended rate and DRD had no inhibitory effect on bacterial population in both the soils. Surprisingly, fungal population declined slightly in both the soils at DRD. This indicated that chlorantraniliprole was a safer molecule among all the test molecules except for fungi at DRD. But cartap hydrochloride 4 G at DRD decreased the bacterial and fungal population significantly in both the soils compared to control indicating that its application at higher rates is not favourable to bacterial and fungal colonies. Imidacloprid 17.8 SL at both recommended and DRD, resulted in greater drop in the bacterial and fungal populations in both the soils. This indicated that it is not favourable for bacterial and fungal colonies. On the other hand, dinotefuran 20 SG and pymetrozine 50 WG at both RD and DRD, resulted in significant decline in bacterial and fungal populations in both the soils indicating that both were not favourable to bacterial and fungal colonies even at recommended doses.

FUTURE SCOPE

Based on the results obtained from the study, for better understanding of impact of insecticides on soil microbes, a thorough study on interaction between soil environment, soil enzymes (dehydrogenase especially) and soil microorganisms is strongly felt necessary for maintaining soil health.

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Conflict of Interest. The authors have not declared any conflict of interest.

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