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Investigation on the response of rhizosphere microbiota to insecticide application in rice production system

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ABSTRACT

In modern agriculture application of pesticides which plays a pivotal role in agricultural fields is an unnecessary evil for non-target microorganisms in rhizosphere. A field experiment was carried out to determine the effect of different insecticides namely triflumezopyrim, flubendiamide, thiamethoxam, cartap hydrochloride, pymetrozine and acephate on soil microflora in rice ecosystem during *kharif*, 2023. Experimental results showed that cartap hydrochloride showed stimulatory effect on bacterial population with an increase of 28.33 %, whereas the other insecticides showed negative effect. Pymetrozine showed a 14.43 % reduction in bacterial population when compared to check followed by thiamethoxam (14.22) and acephate (12.14). Similarly, cartap hydrochloride recorded a 21.18 % increase in fungal population, whereas other insecticides showed inhibitory effect. Pymetrozine recorded 30.82 % decrease in fungal population in comparison to untreated check followed by thiamethoxam (29.16).

Key Words: *Bacteria, Fungi, Insecticide, Rice and Rhizosphere microbiota.*

Rice, (*Oryza sativa* L.) is the world's second most important cereal crop. In 2023, over 513 million metric tons of milled rice were produced worldwide. Historically, Asian countries have dominated the world rice production market. However, pest and disease issues pose significant challenges to increasing rice yields (Dale, 1994). The primary method used to control insect pests is the application of insecticides (Reissig *et al.*, 1986). Despite its effectiveness in combating insect pests, insecticide treatment can have detrimental effects on non-target organisms, such as soil microflora, leading to changes in soil fertility and hindering plant growth and development. Soil microorganisms play a vital role as indicators of soil fertility, facilitating the breakdown and release of nutrients that support essential ecosystem functions (Vannette and Hunter, 2009). The impact of pesticide application on micro flora is influenced by the interaction between microorganisms and the active substance and formulations. Comprehensive knowledge of the impact of insecticides on non-target microorganisms will empower scientists to choose safer molecules that align with pest management strategies and have minimal adverse effects on soil microflora. Hence, the present investigation is carried out with six insecticides namely triflumezopyrim, flubendiamide, thiamethoxam, cartap hydrochloride, pymetrozine and acephate along with untreated check to assess their effect on soil microflora.

MATERIAL AND METHODS

The study was carried out in southern block, field number 49, Agricultural College Farm, Bapatla, which is situated at 15055'23" N latitude and 80028'50" E longitudes with an elevation of 5 meters above MSL. The field experiment was laid out in Randomized Block Design with three replications. The size of the plot was 5 x 5 m^2 and popular variety Samba Mashuri (BPT-5204) was taken as test cultivar in the present investigation during *kharif* season, 2023 in direct sown method. The treatments evaluated are as indicated in Table 1.

Various insecticides from distinct groups and with unique modes of action were employed in the research to assess their impact on the soil microbial population present in the rhizosphere. The insecticides were administered three times at 30, 60, and 90 days after sowing (DAS) and soil samples from the rhizosphere were gathered at intervals of 0, 5, 10, 15, 30, 45, and 60 days subsequent to the third

Table 1 : . List of Insecticides used for the study

Tr.no.	Active ingredient	Strength of the formulation	Recomm-ended dose (a.i. ha^{-1})
T_1	Flubendiamide SC	39.35%	24g
T_2	Triflumezopyrim SC	10%	20g
T_3	Thiamethoxam WG	25%	25g
T_4	Cartap hydrochloride G	4%	1000g
T_5	Pymetrozine WG	50%	150g
T_6	Acephate SP	75%	750g

application of insecticides in order to analyze the population of microflora using the standard serial dilution technique.

Preparation of media

For the preparation of nutrient agar media about 5 g of peptone and 3 g of beef extract were dissolved in 500 ml of boiling water. In a separate beaker, around 20 g of agar was dissolved in 500 ml of distilled water. The dissolved agar was then added to the chemical solution, bringing the total volume to one litre. For the preparation of PDA, about 200 g of peeled and sliced potatoes were utilized and combined with 500 ml of distilled water in a one-litre beaker, then boiled until they reached a soft consistency. The resulting mixture was filtered through a muslin cloth, with all liquid thoroughly squeezed out. Then, approximately 20 g of dextrose was introduced to the extract. In a separate beaker, 20 g of agar was dissolved in 500 ml of distilled water, then added to the potato-dextrose extract to reach a final volume of one litre. About 0.1g of streptomycin sulphate was added to the solution to inhibit the growth of bacteria. Subsequently, five 250 ml conical flasks were filled with approximately 200 ml of chemical solution in each flask. The flasks were sealed with non-absorbent cotton and sterilized in a pressure cooker at 15 pounds of pressure for 15 minutes. After cooling, agar plates were prepared by pouring 20-25 ml of media into each petri dish under aseptic conditions, allowing the media to solidify. Soil samples were diluted by mixing one gram of soil with 9 ml of sterilized distilled water, resulting in a 10^{-1} dilution. The initial solution was subsequently diluted in a sequential manner to achieve concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, which were designated as dilution blanks. The selected concentrations were based on the optimal growth range of the target organisms, with a count between 30 and 300 colony-forming units (CFUs). Notably, a concentration of 10^{-4} was found to support the optimal growth of both bacteria and fungi. Around 0.1 ml of 10^{-4} dilution of each soil sample was evenly spread on an agar-medium plate using a sterilized spreader, with three replications maintained for each treatment. Subsequently, the plates were inverted and placed in an incubator set at 37°C for a duration of 1-3 days. After the specified incubation period, the plates were retrieved from the incubator, and the number of CFUs was manually enumerated using a colony counter.

RESULTS AND DISCUSSION

Influence of insecticides on bacterial population in rice ecosystem during *kharif*, 2023

The impact study of insecticides on soil bacteria in rice ecosystem revealed both stimulatory and inhibitory effects based on enumeration of colony forming units. Compared to control, cartap hydrochloride exhibited a stimulatory effect, while pymetrozine and thiamethoxam showed inhibitory effects on bacterial growth. Acephate initially had no significant effect, but later displayed inhibitory effects. Flubendiamide and triflumezopyrim showed partial inhibition only in the initial days after application. Effect of flubendiamide lasted up to 15 days post-application before recovery, while triflumezopyrim exhibited inhibition of bacterial population on the 0^{th} and 5^{th} days post-application. Thiamethoxam and pymetrozine had adverse effects on bacterial populations, lasting up to 60 days post-application. Cartap hydrochloride, on the other hand, showed stimulatory effects up to 15 days post-application, remaining on par with the untreated check thereafter. Cartap hydrochloride had the highest stimulatory effect on bacterial populations, with a 28.33% increase compared to the untreated check. Pymetrozine had the highest inhibitory effect, with a 14.43% reduction, followed by thiamethoxam, acephate, flubendiamide, and triflumezopyrim compared to the untreated check as shown in the Table 2. The day-wise effects of insecticides on bacterial populations are illustrated in Figure 1.

Influence of insecticides on fungal population in rice ecosystem during *kharif*, 2023

Table 2: Mean value of Colony Forming unit/g of soil (CFU/g Soil) of soil bacteria observed on different days from treatment.

Insecticide Treatment	Dose (g. a.i ha^{-1})	Number of colony forming units (CFUs) of bacteria x 10^4/g of soil.								% Reduction/ Increase over control
		0^{th} day	5^{th} day	10^{th} day	15^{th} day	30^{th} day	45^{th} day	60^{th} day	Mean	
T_1: Flubendiamide 39.35 SC	24	96.00^c	54.33^e	178.66^c	184.33^d	233.00^a	291.00^a	287.33^a	189.23	-9.15
T_2: Triflumezopyrim 10 SC	20	95.66^c	59.33^e	193.66^b	214.33^b	233.33^a	286.33^a	289.00^a	195.95	-5.92
T_3: Thiamethoxam 25 WG	25	80.00^d	79.00^d	177.66^c	197.00^c	212.66^b	267.33^b	237.00^c	178.67	-14.22
T_4: Cartap hydrochloride 4 G	1000	364.33^a	206.00^a	257.33^a	233.33^a	231.66^a	293.00^a	285.33^a	267.29	28.33
T_5: Pymetrozine 50 WG	150	83.33^d	105.00^c	164.66^d	189.00^{cd}	188.33^c	253.00^c	264.33^b	178.24	-14.43
T_6: Acephate 75 SP	750	118.66^b	108.66^c	128.33^e	187.00^{cd}	208.66^b	261.33^b	268.33^b	183	-12.14
T_7: Control	--	112.67^b	119.33^b	196.00^b	215.00^b	228.33^a	290.00^a	296.66^a	208.29	--
SE(m)		2.44	2.8	3.7	3.69	2.29	2.66	3.77	--	--
CV%		3.12	4.66	3.46	3.15	1.8	1.66	2.37	--	--

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

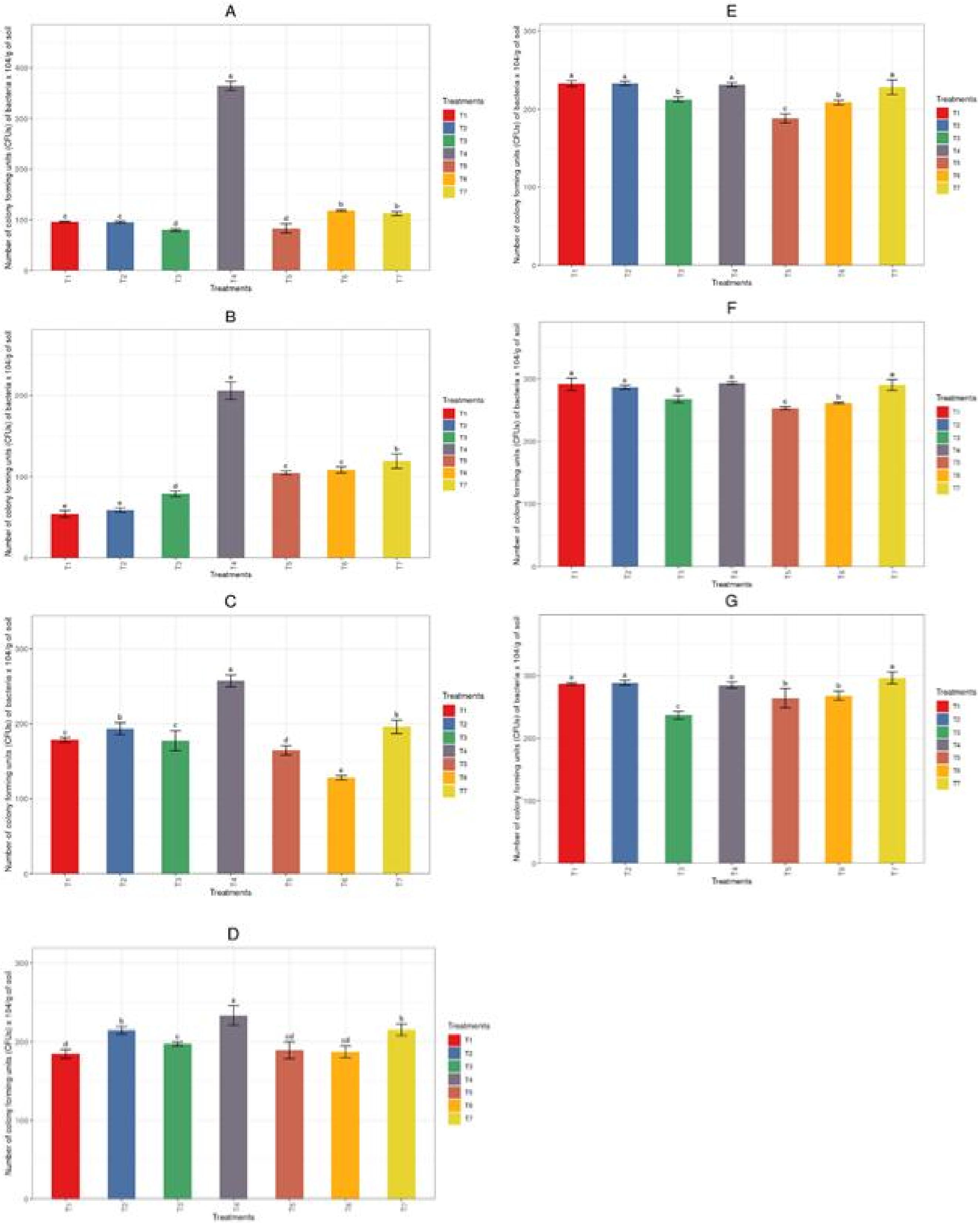

Figure 1 : Effect of insecticides on soil bacterial population. A) 0th day. B) 5th day. C) 10th day. D) 15th day. E) 30th day. F) 45th day. G) 60th day. T1: Flubendiamide, T2: Triflumezopyrim, T3: Thiamethoxam, T4: Cartap hydrochloride, T5: Pymetroznie, T6: Acephate, T7: Untreated check.

***Treatments sharing common alphabet are not significant.**

The impact of insecticides on the fungal population exhibited both stimulatory and inhibitory effects as assessed from number of colony forming units. Thiamethoxam displayed complete inhibition of the fungal population as against control, while flubendiamide, triflumezopyrim, pymetrozine, and acephate showed partial inhibition followed by recovery. Cartap hydrochloride, on the other hand, had a stimulatory effect on the fungal population. Inhibitory effect of flubendiamide persisted until the 30th day post-application before recovery, whereas triflumezopyrim's inhibitory behavior lasted until the 15th day post-application. Acephate showed inhibitory effect until the 30th day post-application, while pymetrozine's inhibitory effect lasted until the 15th day post-application. Among all treatments, cartap hydrochloride applied at the recommended dose resulted in a 21.28% increase in the fungal population compared to the control plot. Conversely, pymetrozine applied at the recommended dose led to a significant reduction in the fungal population, with a 30.82% decrease compared to the control, followed by thiamethoxam, acephate, triflumezopyrim, and flubendiamide as indicated in Table 3. The day-to-day effects of insecticides on bacterial populations are depicted in Figure 2.

Flubendiamide showed decline in the population of microorganisms upto 15th day and 45th day after application with respect to bacteria and fungi, respectively. These results are in partial conformity with the results of Jaffer *et al*., 2015 where they described reduction in enzyme concentration may be correlated to decline in microbial population. Bacteria in the treated plot with flubendiamide recovered after 15th day *i.e.* from 30th day on wards (233.00 x 10^4 CFUs / g of soil) and was on par with the control (228.33 x 10^4 CFUs / g of soil), whereas fungal population recovered from 45th day after application (9.00 x 10^4 CFUs / g of soil) and was on par with the control (12.33 x 10^4 CFUs / g of soil). This indicates that it took nearly 15 days and 45 days for bacteria and fungi, respectively to recover its population which was almost double. The result could be related to the half-life of the chemical which is about 37.62 to 60.21 days in different soils according to Paramasivam and Banerjee (2012). In the present study, Triflumezopyrim, showed decline in bacterial and fungal population till 5th day after application and 10th day after application, respectively when compared to the untreated check. According to Mishra *et al*. (2022) dissipation of triflumezopyrim occurred at 3.78 to 4.79 days in soil. Hence, the decrease in the bacterial population may be attributed to the presence of parent compound till the 5th day. According to Venkateswarlu and Sethunathan (1984) Itoh (1991) Bhuyan *et al.*, (1992) microorganisms have the potential to adapt and utilize applied pesticides for energy and growth. Furthermore, due to the intricate fungal structure, nutrient requirements, metabolic rates, the recovery in growth of the fungi might be delayed. The application of thiamethoxam showed adverse effects on both soil bacterial and fungal population upto 60th day after application. Thus obtained results are in conformity with the findings of Rahman and Bhattacharjee (2018) who reported that there was a negative effect on soil microbial colonies and their enzyme activity. According to Li *et al*. (2018) the persistence of thiamethoxam in soil ranged from 65 to 170 days, which might be the cause of decline in the microbial population. Cartap hydrochloride showed stimulatory effect on both soil fungal and bacterial population upto 30th day and 15th day after application which are similar to the findings of Kumar *et al* (2012) who reported that cartap hydrochloride had stimulatory effect on the microbial biomass carbon when applied at recommended dose *i.e*., 1 kg ha^{-1}. There is a partial conformity with the above findings to the study of Singh *et al.* (2014) who reported that cartap hydrochloride at lower concentrations had stimulatory effect on cyanobacteria which might be due to the utilization of the nitrogen present in the insecticide. Pymetrozine showed a significant inhibitory effect on soil fungi and bacteria upto 15th day and 60th day after application. Acephate had an inhibitory effect on soil bacteria and fungi until 60th day after application and 30th day after application on bacteria and fungi, respectively, which corroborated the results of Du *et al.* (2013) who reported that there was a decline in the quantity of bacteria and fungi in soil. Maddela and Venkateswarlu, 2018 reported that there was an inhibitory effect of acephate on soil enzymes like phosphatases, urease and protease which could be correlated to decline in the soil microbial population.

CONCLUSION

Thus, the present study clearly showed that cartap hydrochloride had stimulatory effect on the soil microbiota which favors the beneficial microorgansims. The insecticides like flubendiamide and triflumezopyrim

Table 3: Mean value of Colony Forming unit/g of soil (CFU/g Soil) of soil fungi observed on different days from treatment.

Insecticide Treatment	Dose (g a.i ha)	Number of colony forming units (CFUs) of fungi x 10^4/g of soil.								% Reduction/ Increase over control
		0^{th} day	5^{th} day	10^{th} day	15^{th} day	30^{th} day	45^{th} day	60^{th} day	Mean	
T_1: Flubendiamide 39.35 SC	24	6.33^{d}	8.33^{c}	8.00^{c}	11.33^{c}	9.00^{c}	11.66^{c}	13.00^{cd}	9.66	-15.41
T_2: Triflumezopyrim 10 SC	20	6.33^{d}	2.00^{f}	7.66^{c}	12.33^{bc}	10.33^{b}	11.66^{c}	14.33^{a}	9.23	-19.18
T_3: Thiamethoxam 25 WG	25	9.66^{b}	5.00^{d}	7.66^{c}	9.66^{d}	6.00^{d}	6.00^{d}	12.66^{d}	8.09	-29.16
T_4: Cartap hydrochloride 4 G	1000	3.66^{e}	17.33^{a}	11.00^{a}	17.66^{a}	20.66^{a}	13.33^{a}	13.33^{bcd}	13.85	21.18
T_5: Pymetrozine 50 WG	150	3.00^{e}	3.33^{e}	5.33^{d}	8.00^{e}	9.66^{bc}	12.00^{bc}	14.00^{ab}	7.9	-30.82
T_6: Acephate 75 SP	750	8.66^{c}	3.00^{e}	6.00^{d}	9.66^{d}	5.66^{d}	13.00^{ab}	13.66^{abc}	8.52	-25.39
T_7: Control	--	11.33^{a}	10.00^{b}	9.33^{b}	13.33^{b}	10.00^{b}	12.33^{abc}	13.66^{abc}	11.42	--
SE(m)		0.31	0.21	0.25	0.44	0.25	0.33	0.28	--	--
CV%		7.63	5.4	5.56	6.45	4.28	5.05	3.6	--	--

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

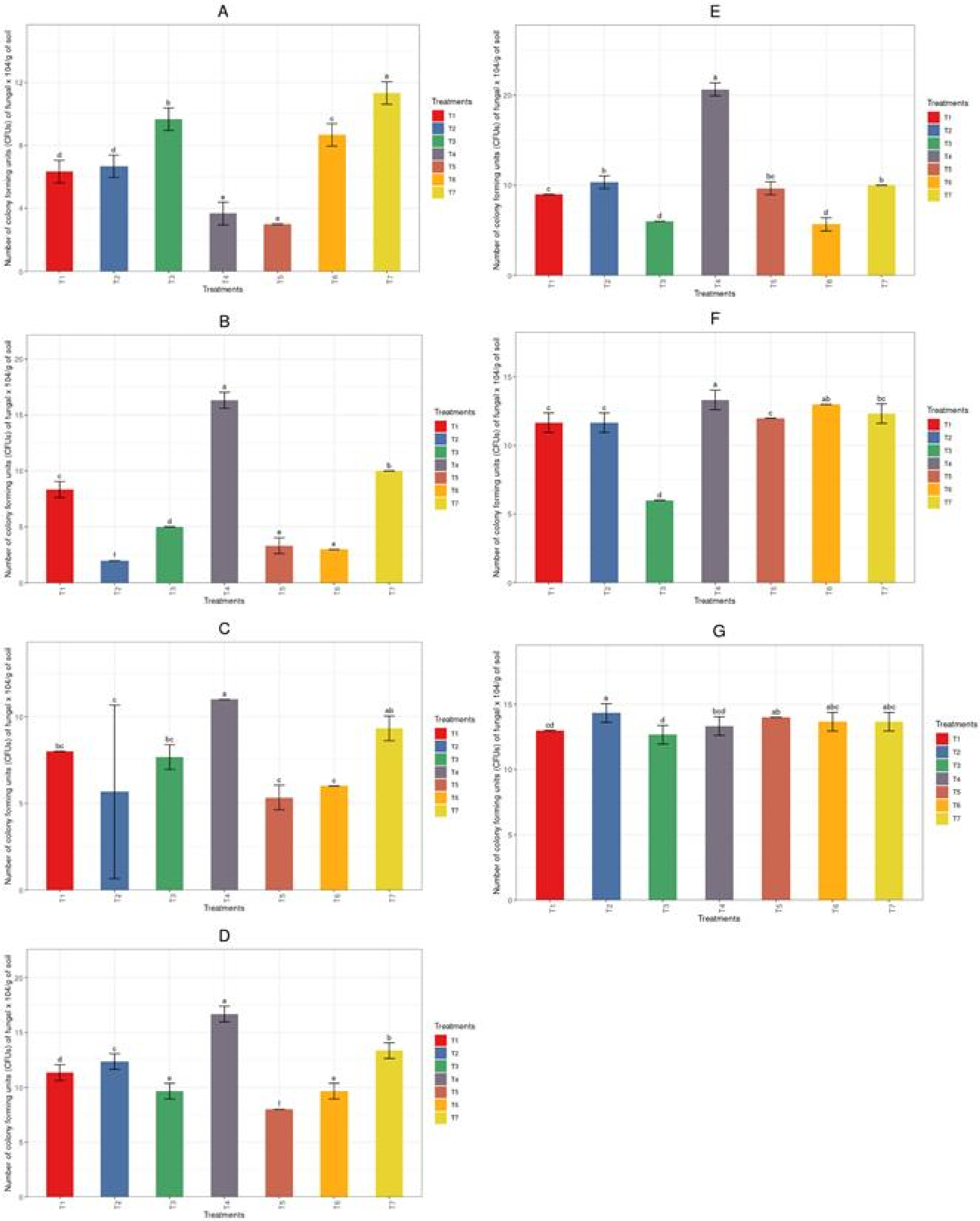

Figure 2 : Effect of insecticides on soil fungal population. A) 0th day. B) 5th day. C) 10th day. D) 15th day. E) 30th day. F) 45th day. G) 60th day. T1: Flubendiamide, T2: Triflumezopyrim, T3: Thiamethoxam, T4: Cartap hydrochloride, T5: Pymetroznie, T6: Acephate, T7: Untreated check.

***Treatments sharing common alphabets are not significant at 5% LOS.**

even though had an inhibitory effect had less detrimental effect. Hence, these insecticides when used judiciously may not pose threat to the soil microbial diversity. Pymetrozine, thiamethoxam and acephate had adverse effects on soil microorganisms.

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