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Impact of chlorantraniliprole insecticide on microbial activity in paddy soils of Kerala

Greeshma Suresh and Thomas George

Abstract
Chlorantraniliprole, a systemic insecticide coming under carbanilic diamide group, acting on insect ryanodine receptors, is effective against the lepidopteran, coleopteran and dipteran pests of crops including rice [1]. The presence of Chlorantraniliprole residues in soil is reported to have adverse effect on microbial population and enzymatic activity in soil [2] and the studies on enzyme activities are important since they indicate the potential of soil to support biochemical processes ultimately deciding the biological reactions as well [3]. The study was conducted in paddy fields with the objective of assessing soil microbial activity consequent to the application of different formulations of chlorantraniliprole.
The field experiment and soil enzyme assays were carried out in-order to understand the metabolic activity of soil micro-organisms before and after application of chlorantraniliprole formulations (Ferterra 0.4 GR and Coragen 18.5 SC).
The urease and dehydrogenase activity were estimated in order to assess the effect of residues on soil microbes after single, double and triple application of both the formulations. Soil application of chlorantraniliprole (Ferterra 0.4 GR) had more significant effect than foliar application (Coragen 18.5 SC) in rice on soil microbes. The control field had higher microbial activity than all the treatment plots indicating reduction in microbial activity during the exposure period. However, the soil microbial activity was regained 60 days after the last application.

Keywords: Urease activity, dehydrogenase, soil enzyme, chlorantraniliprole, rice

Introduction
Rice is the staple food of more than 80% population in India and is highly prone to infestation with a wide array of pests and diseases during the development stages and even during storage, necessitating timely adoption of needed pest and disease management practices in order to improve the yield and economy [4-8]. Chlorantraniliprole, is recommended for the control of various lepidopteran pests of rice and hence is a widely used insecticide [9, 10]. However, no information is available on build-up of residues in rice soil and alteration of soil biochemical properties subsequent to its use.
Chlorantraniliprole residues may remain in soil for a longer period acting as a sink for chemical contamination. Soils with varying physicochemical properties have profound influence in transformation of pesticides [11] and these molecules can impact nutrient turnover and soil quality by altering soil microbial activities [12-14]. Studies relating the soil properties and degradation of Chlorantraniliprole are also limited. These chemicals might alter the native microbial species in soil depending on their frequency and application rate. In order to understand the scenario, the study was initiated.

Materials and Methods
The experiment was conducted at the Research fields of Integrated Farming System Research Station, Kerala Agricultural University, Karamana (8.4736 °N; 76.914 °E). Two different formulations of chlorantraniliprole Coragen 18.5 SC (C) and Ferterra 0.4 % GR (F) each at 30 and 40 g a.i per ha, respectively were applied to the treatment plots. The frequency of application was single (25 DAT), twice (25 and 50 DAT) and thrice (25, 50 and 75 DAT) applications. The treatment details are as follows:
T1 - Single application - Coragen 18.5 SC@25 DAT
T2 - Two application - Coragen 18.5 SC @25, 50 DAT
T3 - Three application - Coragen 18.5 SC @25, 50 DAT
T4 - Single application - Ferterra 0.4 GR@25 DAT
T5 - Two application - Ferterra 0.4 GR@25, 50 DAT

T6 - Two application - Ferterra 0.4 GR@25, 50, 75DAT

The effect of these chlorantraniliprole formulations on soil microbes were assessed indirectly using enzyme assays *viz.* dehydrogenase and urease activity estimation. Soil samples for the purpose were collected from the same treatment field plots subjected to different treatment applications at an interval of 0th day, 7th day, 15th day, 30th day and 45th day after the last application. The data regarding enzyme activities for the three different schedules of application *viz.* single application; double application; triple application for both the formulations are given in the following table 1 (Dehydrogenase activity) and table 2 (Urease activity).
The soil physico-chemical properties like pH, EC, PD, BD, CEC, OC, OM, major and secondary nutrients were estimated prior to the microbial study using standard procedures. The soil enzyme assays for urease and dehydrogenase were used to measure the metabolic activity of microorganisms in the soil indirectly in order to assess the effect of chlorantraniliprole residues on soil microbes [15]. Soil samples were collected from the experimental fields of rice in coastal alluvium soil where chlorantraniliprole formulations were applied. The soil samples were collected at an interval of 0, 7, 15, 30 and 45 days after the last application of chlorantraniliprole and the effect of residues on the microflora were assessed through standard detection methods for dehydrogenase [16] and urease activity [17] in the soil.

Determination of dehydrogenase activity in soil
Dehydrogenase activity in soil was determined using standard procedures [16]. About six grams of air dried soil was taken and added 0.2 g $CaCO_3$ to it. The tubes were added with 1mL of 3 per cent aqueous solution of 2,3,5- triphenyl tetrazolium chloride (TTC) and 2.5 mL distilled water, mixed thoroughly with a glass rod and incubated at 37 ℃ for 24 hours in a biological oxygen demand (BOD). The dehydrogenase activity was expressed as microgram of Triphenyl formazan (TPF) formed per gram of soil per hour at 37 ℃. The TPF formed was then extracted from each tube separately into 50 mL volumetric flasks by transferring soil into a funnel using non-absorbent cotton. The soil was washed with enough quantity of methanol till filtrate run free of colour. The filtrate was then diluted with methanol and the volume made was up-to 50 mL and the intensity of pink colour was determined in a spectrophotometer at 485 nm against methanol as a blank. The TPF formed from TTC reduction was determined by referring to a standard graph and expressed as microgram of TPF formed per gram of soil per hour at 37 ℃.

Determination of urease activity in soil
One gram soil was taken in a 50 mL conical flask. Urea solution (20 mL of 500 ppm) added to each tube and shaken well for mixing urea solution with soil and incubated at 37 ℃ in BOD incubator. Then added 0.1gram calcium sulphate and filtered through Whatman No. 1 filter paper. Filtrate from each tube was collected in a separate 50 mL volumetric flask. Later 10 mL of colouring agent was added to it. Colouring agent was prepared by using p-dimethyl amino-benzaldehyde, ethyl alcohol and concentrated HCl in 1:50:5 ratio.
Urease enzyme activity was measured by reading the intensity of color developed at 420nm against blank in a spectrophotometer. Blank receive only distilled water and colouring agent. The quantity of urea hydrolyzed was calculated by referring to a standard curve and expressed as microgram of urea hydrolyzed per gram of soil per hour at 37 ℃. Different concentrations of Urea solutions were prepared and calibration curve was prepared using optical density values at 420nm.

Results
Physico-chemical properties of soil
The particle density and bulk density of coastal alluvial soil was 2.57 and 1.36 Mg m^{-3}. The estimated pH was 5.2 (strongly acidic) and EC 0.28 dS m^{-1} for coastal alluvium soil. The cation exchange capacity was 5.18 meq $100g^{-1}$. The organic matter contents of coastal alluvial soil was 1.07. Available P and K was 14.48 kg ha^{-1} and 121 kg ha^{-1} for coastal alluvium soil, and the secondary nutrients Ca, Mg and S were 230 ppm, 132 ppm and 98 ppm.

Effect of chlorantraniliprole on dehydrogenase enzyme activity
Dehydrogenase enzyme activity in soil from treated plots are represented in table 1. The dehydrogenase enzyme activity is expressed in µg TPF hydrolysed g^{-1} soil 24 h^{-1}.
The highest dehydrogenase enzyme activity was recorded in the control plot on the 0th day after application (83.49) and ranged from 81.57 to 83.49 µg TPF hydrolysed g^{-1} soil 24 h^{-1}. Samples collected from the treatment plots on the 0th day after application revealed that T6 had the lowest dehydrogenase enzyme activity and among the treatments T1 had the highest enzyme activity. From the data, it is evident that foliar application (Coragen 18.5 SC) had lower effect on the soil microbial activity compared to soil application of chlorantraniliprole (Ferterra 0.4 G).
In the case of foliar application, T3 had significant effect on the soil microbial community. The dehydrogenase activity recorded for T3 on 0th day, 7th day, 15th day, 30th day and 45th day was 57.77, 62.81, 65.07, 68.38 and 70.01 µg TPF hydrolysed g^{-1} soil 24 h^{-1} respectively. The activity recorded for T1 on 0th day, 7th day, 15th day, 30th day and 45th day was 70.73, 71.40, 70.92, 72.99 and 71.93µg TPF hydrolysed g^{-1} soil 24 h^{-1} respectively. Whereas, dehydrogenase activity recorded for T2 was 69.48, 70.01, 70.44, 70.39, 71.45µg TPF hydrolysed g^{-1} soil 24 h^{-1}.

Table 1: Effect of Chlorantraniliprole on Dehydrogenase enzyme activity

Treatment	Dehydrogenase activity (µg TPF hydrolysed g-1 soil 24 h-1)				
	0th DAY	7th DAY	15th DAY	30th DAY	45th DAY
T1	70.73 b	71.40 b	70.92 b	72.99 b	71.93 b
T2	69.48 c	70.01 c	70.44c	70.39 c	71.45 c
T3	59.26 e	62.81 e	65.07d	68.38 d	70.01d
T4	62.57 d	65.07 d	62.81e	65.07 e	68.38 e
T5	48.51 f	54.22 f	55.90f	58.11 f	59.17 f
T6	27.59 g	33.25 g	41.75g	48.90 g	51.73 g
Control	83.49 a	82.05 a	81.96 a	81.57 a	82.28 a
CD (0.05)	0.409	0.391	0.193	0.278	0.232
SE(m)	0.138	0.132	0.065	0.094	0.078
CV	0.457	0.419	0.203	0.281	0.230

In the case of soil application, T6 had significant effect on the soil microbial community. The dehydrogenase activity recorded for T6 on 0th day, 7th day, 15th day, 30th day and 45th day was 27.59, 33.25, 41.75, 48.90 and 51.73µg TPF hydrolysed g^{-1} soil 24 h^{-1} respectively. The activity recorded for T4 on 0th day, 7th day, 15th day, 30th day and 45th day was 62.57, 65.07, 62.81, 65.07 and 68.38µg TPF hydrolysed g^{-1}

soil 24 h^{-1} respectively. Whereas, dehydrogenase activity recorded for T5 was 48.51, 54.22, 55.90, 58.11 and 59.17 µg TPF hydrolysed g^{-1} soil 24 h^{-1}.

Effect of chlorantraniliprole on urease enzyme activity

Urease enzyme activity in soil from treated plots are represented in table 2. The enzyme activity is expressed in ppm of urea hydrolyzed g^{-1} soil h^{-1} at 37 °C. The highest urease enzyme activity was recorded in the control plot on the 0th day after application (190.09) and treatments ranged from 62.47 to 119.62 ppm of urea hydrolyzed g^{-1} soil h^{-1}.

3.3. Effect of chlorantraniliprole on urease enzyme activity

Urease enzyme activity in soil from treated plots are represented in table 2. The enzyme activity is expressed in ppm of urea hydrolyzed g^{-1} soil h^{-1} at 37 °C. The highest urease enzyme activity was recorded in the control plot on the 0th day after application (190.09) and treatments ranged from 62.47 to 119.62 ppm of urea hydrolyzed g^{-1} soil h^{-1}.

Table 2: Effect of Chlorantraniliprole on Urease enzyme activity

Treatment	Urease activity (ppm of urea hydrolysed g^{-1} soil h^{-1} at 37 °C)				
	0th day	7th day	15th day	30th day	45th day
T1	119.62 b	141.36 b	165.14 b	190.09 a	189.13 a
T2	95.17 c	111.5 c	127.03 c	143.73 b	165.14 b
T3	78.427 e	90.422 e	112.62 e	128.87 c	144.82 d
T4	89.548 d	105.04 d	122.95 d	142.61 b	155.35 c
T5	74.137 f	87.382 f	103.63 f	120.87 d	138.2 e
T6	62.475 g	77.802 g	86.715 g	90.422 e	111.21 f
Control	190.09 a	189.72 a	188.09 a	189.97 a	189.67 a
Cd (0.05)	2.713	2.718	2.737	2.46	2.497
SE(m)	1.250	1.251	0.921	1.165	1.177

Samples collected from the treatment plots on the 0th day after application revealed that T6 had the lowest urease enzyme activity (62.475) and among the treatments T1 had the highest enzyme activity (119.62). From the data, it is evident that foliar application (Coragen 18.5 SC) had lower effect on the soil microbial activity compared to soil application of chlorantraniliprole (Ferterra 0.4 G) as observed in the case of dehydrogenase enzyme activity.

In the case of foliar application, all the treatments were significantly different. Triple application of Coragen (T3) had significant effect on the soil microbial community when compared to single (T1) and double (T2). The urease activity recorded for T3 on 0th day, 7th day, 15th day, 30th day and 45th day was 78.427, 90.422, 112.62, 128.87 and 144.82 ppm of urea hydrolysed g^{-1} soil h^{-1} respectively. The effect of T1 and T2 on microbial community was also significantly different. The activity recorded for T1 on 0th day, 7th day, 15th day, 30th day and 45th day was 119.62, 141.36, 165.14, 190.09 and 189.13 ppm of urea hydrolysed g^{-1} soil h^{-1} respectively. Whereas, urease activity recorded for T2 was 95.17, 111.5, 127.03, 143.73 and 165.14 ppm of urea hydrolysed g^{-1} soil h^{-1}.

In the case of soil application, T6 had drastic effect on the soil microbial community. The urease activity recorded for T6 on 0th day, 7th day, 15th day, 30th day and 45th day was 62.475, 77.802, 86.715, 90.422 and 111.21 ppm of urea hydrolysed g^{-1} soil h^{-1} respectively. The urease activity recorded for T4 on 0th day, 7th day, 15th day, 30th day and 45th day was 89.548, 105.04, 122.95, 142.61 and 155.35 ppm of urea hydrolysed g^{-1} soil h^{-1} respectively. Whereas, urease activity recorded for T5 was 74.137, 87.382, 103.63, 120.87 and 138.2 ppm of urea hydrolysed g^{-1} soil h^{-1}. Data reveals that soil application had more adverse effect on microbial activity.

Discussion

Enzymes play an important role in the life processes of microorganisms in the soil [18]. The data given in Table 1 reveals that soil application of Ferterra had more significant effect on dehydrogenase activity than foliar application of Coragen. As well as residual effect of chlorantraniliprole increased with frequency of application.

On 45th day of sampling, the dehydrogenase activity was in the order Control > T1 > T2 > T3 > T4 > T5 > T6 compared to 0th day – Control > T1 > T2 > T4 > T3 > T5 > T6. This might be due to the persistence of chlorantraniliprole in soil for longer period owing to higher frequency of application.

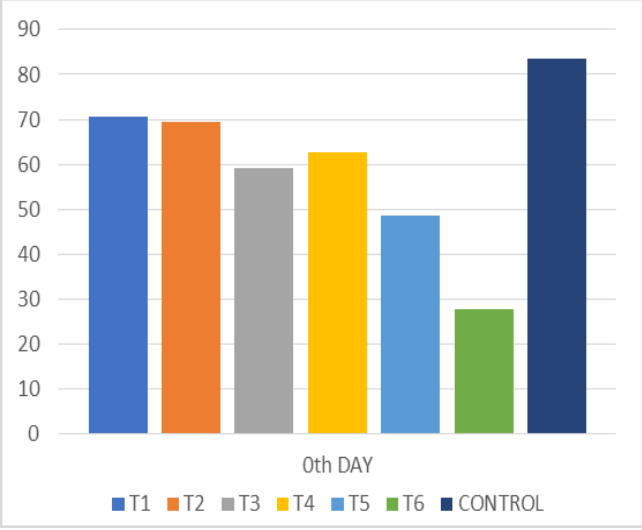

Fig 1: Effect of chlorantraniliprole residues on dehydrogenase activity- 0th day

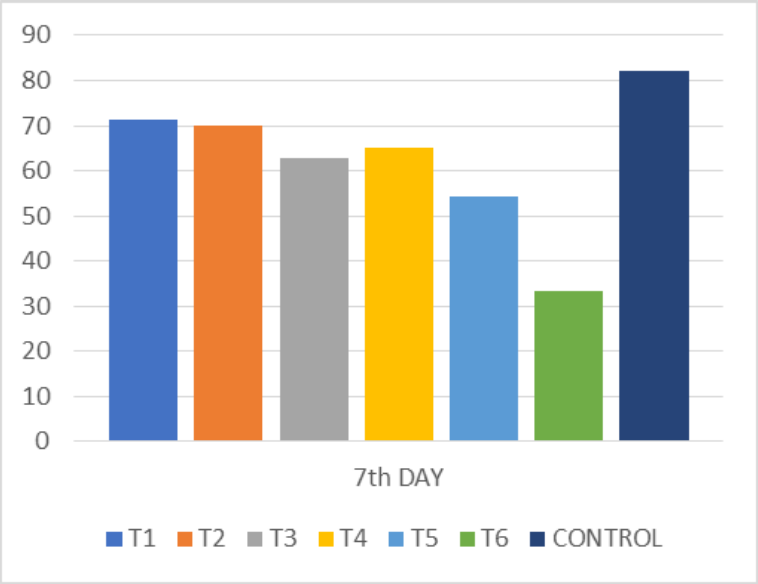

Fig 2: Effect of chlorantraniliprole residues on dehydrogenase activity-7th day

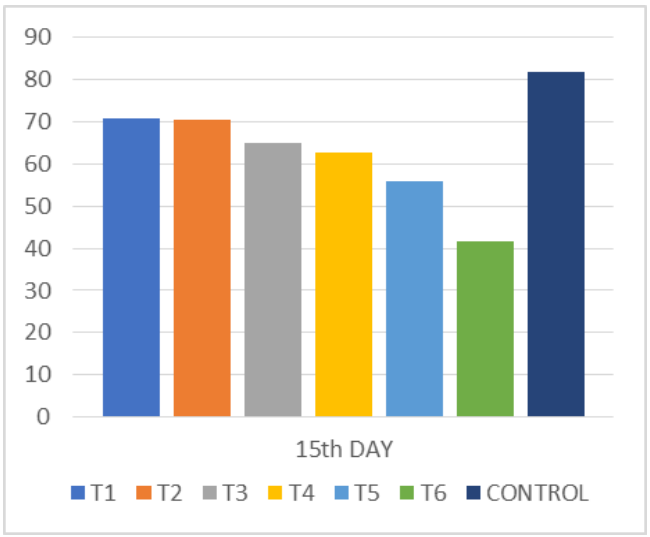

Fig 3: Effect of chlorantraniliprole residues on dehydrogenase activity-15th day

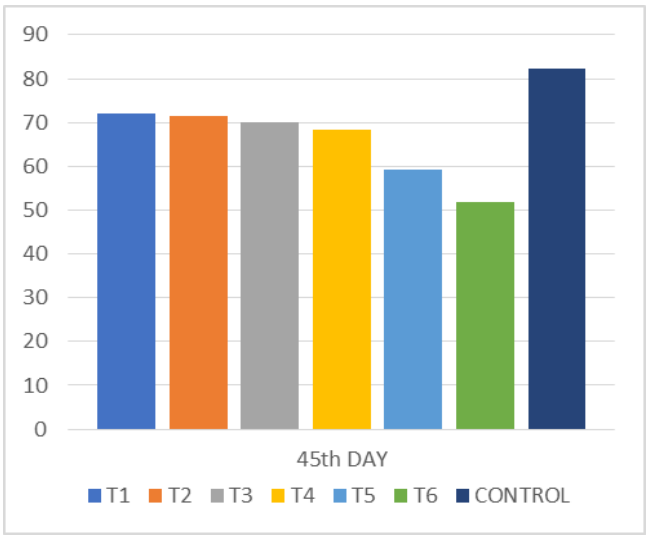

Fig 5: Effect of chlorantraniliprole residues on dehydrogenase activity-45th day

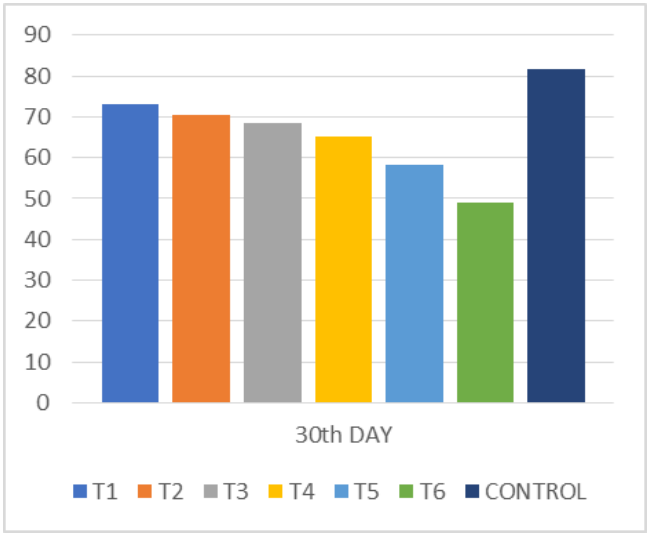

Fig 4: Effect of chlorantraniliprole residues on dehydrogenase activity-30th day

On the 30th and 45th day of sampling, the dehydrogenase activity of T1 (C-Single), T2 (C-double), T3 (C-triple) and T4 (F-single) to found to stabilize towards equilibrium. But the Treatments T5 (F-double) and T6 (F-triple) still has lower dehydrogenase activity.

Similarly, data in Table 2 shows that urease activity is also altered by the varied application of chlorantraniliprole. On 45th day of sampling, the urease activity was in the order Control = T1 > T2 > T4 > T3 > T5 > T6 compared to 0th day – Control > T1 > T2 > T4> T3 > T5 > T6.

Unlike dehydrogenase activity, the urease activity of control and T1 (Coragen-single application) were on par, and other treatments were significantly different. Foliar application had minimal residual effect on soil microbial activity than soil application of chlorantraniliprole.

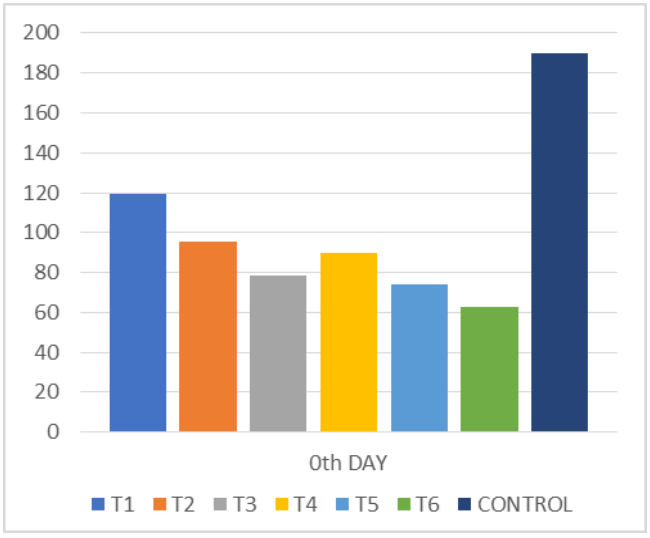

Fig 6: Effect of chlorantraniliprole residues on urease activity - 0th day

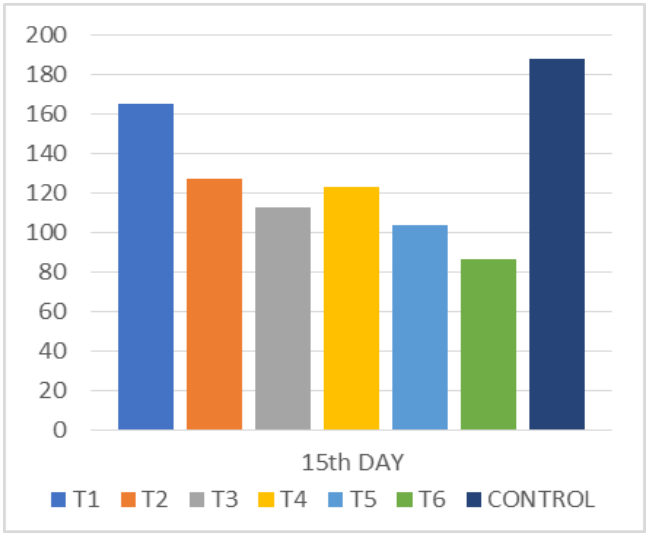

Fig 8: Effect of chlorantraniliprole residues on urease activity - 15th day

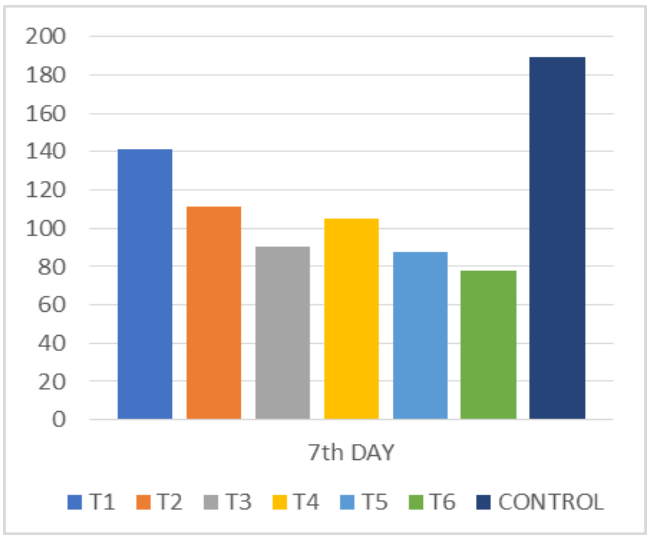

Fig 7: Effect of chlorantraniliprole residues on urease activity - 7th day

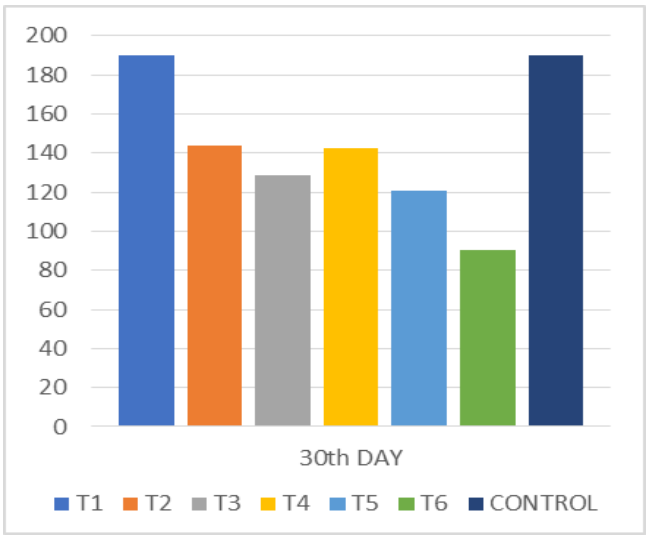

Fig 9: Effect of chlorantraniliprole residues on urease activity - 30th day

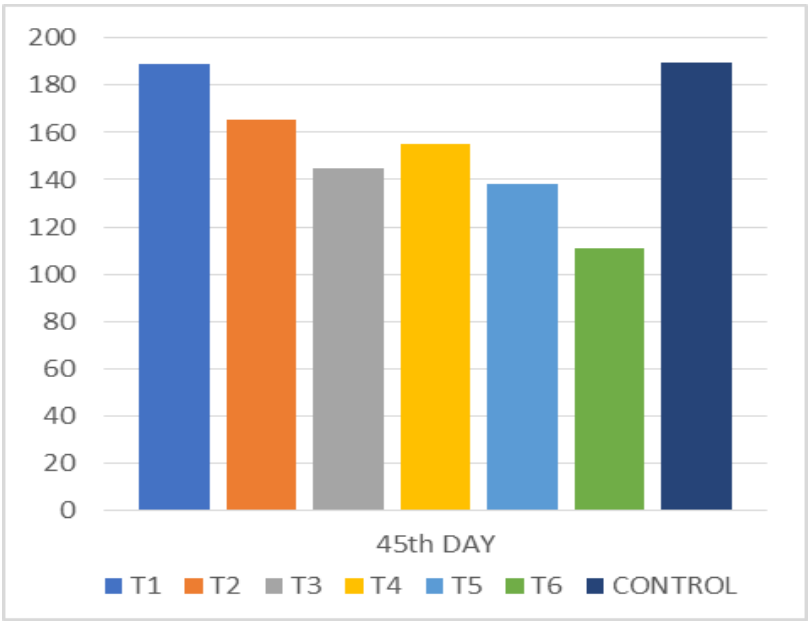

Fig 10: Effect of chlorantraniliprole residues on urease activity - 45th day

The paddy soils of Karamana had higher clay and organic matter content which might have ensured the faster recovery of the treated soils in terms of microbial activity. Clay content played critical role in dissipation of chlorantraniliprole [19, 20]. The residue levels Pesticide degradation is influenced by soil properties namely, pH, clay content and organic carbon (OC) content as well as the environmental factors that influence soil temperature and moisture content [21].

Conclusion

Soil application of chlorantraniliprole (Ferterra) had more significant effect than foliar application (Coragen) in rice on soil microbes. The control field had higher microbial activity than all the treatment plots indicating reduction in microbial activity during the exposure period. During the first two weeks the soil microbial activity was lower but soon was found to stabilize towards equilibrium condition by after 45-50 days. Recommended dose of chlorantraniliprole did not have long term negative impact on soil microbes. Even the residual effect of double and triple application was neutralized after 60 days. The soil microbial activity was regained 60 days after the last application for both the soil enzymes *viz.* dehydrogenase and urease. But unscientific and higher dosages of Chlorantraniliprole results in development of resistance [22]. Hence, the study reveals that the use of chlorantraniliprole on paddy doesn't hold any long-term impact on the soil microbial community and the use of chlorantraniliprole is safe for optimum use.

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