Thiamethoxam's Impact on Soil Microbial Population and Metabolic Enzyme Activity

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Abstract: Thiamethoxam is widely use in north east India and Assam. However, effect on soil microorganisms isn't evidently realized. To realize the degree of the pesticide's effect on soil microbial activity, study was performed. Microbial Colony Forming units (CFU/mL), Nitrate reductase activity and dehydrogenase activity were used to estimate the microbial dynamics in soil. Soil were treated with 0.20%, 0.40% and 0.80% of Thiamethoxam and observations were recorded on 0th, 3rd, 7th, 15th and 21st days after treatment. Treated soil demonstrated a noteworthy change in number of colony per gram of soil minimum being in 0.80%. Nitrate reductase activity also showed significant decrease, minimum observed in 0.80% on the 21st day followed by 0.40% on the 15th day. Dehydrogenase activity however increased in 0.20%, 0.40% and 0.80%. Results indicated a negative effect of Thiamethoxam on soil microbial colonies and their enzyme activity and may prove to be detrimental to soil health and fertility.

KEYWORDS: Insecticide, Thiamethoxam, Microbial Colony forming units, Nitrate reductase activity, Dehydrogenase activity

I. INTRODUCTION:

Use of systematic neonicotinoids like Thiamethoxam (3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-N-nitro-1,3,5-oxadiazinan-4-imine) has gradually increased in modern Agriculture. With the advance in synthesis of such pesticides and lower prices, farmers are eagerly adopting systematic pesticides over the plant derived natural pesticides. Thiamethoxam is a second – generation neonicotinoid insecticide. It is a nitromethylene derivative with low vapor pressure (4.9×10^{-11} mmHg at 25ºC) and high-water solubility with contact, stomach, and systematic activity used for both foliar and soil applications [1]. It is mainly used for chewing and sucking insects that are known to infect various agricultural crop of rice [2], *Heteronychus arator,* (Coleoptera: Scarabaeidae) of Maize [3], Jassid (*Amarasca devastation*) of Cotton [4], Aphids (*Aphis gossypii*) and Jassids (*Amrasca biguttula*) on Okra [5], potato Leafhopper infestations in snap bean [6], Hopper (*Amritodus atkinsoni*) infesting Mango [7]. It too is efficient in preventing transmission of tomato yellow leaf curl Gemini virus (TYCLV) by white butterfly *Bemisia tabaci* [8]. Such uses are inconsiderate of its negative effects on the environment. Studies indicate that thiamethoxam persists more than 30 days in soil with a half-life varying from 46.3 to 301 days, the dispersion is faster under dry conditions and slower in moist conditions [9].

Microbial population in soil is very dynamic throughout the year and under different conditions of temperature and sunlight. Different soil types have different microbial population characteristics linked directly to the availability of nitrogen to the ecosystem [10]. Nitrate reductase a very important enzyme in the soil ecosystem as it converts nitrate (NO_3^-) to nitrite (NO_2^-) and is responsible in maintaining the nitrate reserve in the soil which are made available to plants. This reaction important because nitrate is the predominant form of nitrogen compound present in fertilized soil used by crop plants to produce several amino acids needed for protein biosynthesis [11]. The amount of nitrate in soil may often correlate to growth and development of plants. This activity can be utilized as biochemical measure for grain in production and total protein production [12, 13]. Prokaryotic nitrate reductase is a part of the sulfite oxidase family of molybdoenzymes and is responsible for the transfer of electron from NADH and NADPH to nitrate [14]. The enzyme activity is easily analysed by the rate of conversion of NO_3^- to NO_2^- such that the amount of NO_2 in a sample is immediately associated with the enzyme activity. Dehydrogenase is an enzyme of oxidoreductase group [15] which oxidizes substrate by reducing some electron acceptor like NAD^+/$NADP^+$ or flavin coenzymes [16]. Dehydrogenase activity is an important enzyme for the proper functioning of microbial electron transport chain which is directly linked to microbial respiration. Dehydrogenases remains in cells of all living microbial cells and it is linked with microbial respiratory processes [17]. Dehydrogenase activity may also help in understanding the variation in soil microbial diversity. Dehydrogenase is responsible for the production of high energy compounds like ATP in cells hence they play a significant role in the metabolism of microbial cells. The experiment was undertaken to understand the effect of the pesticide on the soil microbial population and their metabolic enzymatic activity for which we considered enzymes nitrate reductase and microbial dehydrogenase.

II. MATERIALS AND METHOD:

Collection of Soil:

The soil samples were collected from a depth of about 10 cm from botanical garden, Department of Life Sciences, Dibrugarh University located at 27.4505ºN Latitude and 94. 8913ºE Longitude. The study area Dibrugarh, Assam has a humid subtropical hot summer climate that is mild with dry winters, hot humid summers and moderate seasonality [18]. According to Holdridge life zones system of bioclimatic classification situated in or near the subtropical wet forest biome with average annual temperature 22.9

degrees Celsius (73.3 degrees Fahrenheit) and total annual precipitation averages 2758 mm (108.6 inches) which is equivalent to 2758 Liters/m² (67.65 Gallons/ft²).

Pesticide used:

Syngenta's Actara (25% WG), the commercially available formulation for thiamethoxam was used for experiment. 1% solution of pesticide was prepared and used as stock solution. The stock was then diluted to prepare 0.20%, 0.40% and 0.80% solution.

Treatment of Soil Samples:

100 g of soil from was mixed with 50 mL of the 0.20% pesticide solution, the soil was then mixed thoroughly with 900 g of untreated soil to form a homogenous mixture. The same was repeated for Treatment 2 and Treatment 3 and 0.40% and 0.80% respectively to obtain three different treated samples of 1kg each. The Control soil was left untreated (0%). The treated soils along with the control soil were then placed in 1Kg plastic bags in a room where they were kept for 21 days in unregulated temperature and moisture conditions.

Preparation of Nutrient Agar:

Nutrient media was prepared as according to with 10g of peptone, 5g LEMCO (Beef Extract) and 1g sodium chloride (NaCl) with 2% Agar in 1000 ml water. Weighed amount of peptone and LEMCO were dissolved in about 150 ml distilled water in 1000 ml conical flask. pH was adjusted to 9 by adding Sodium Hydroxide (NaOH). The solution was warmed for about 10 minutes and filtered. 1g of Sodium Chloride (NaCl) was dissolved in the solution and pH was adjusted to 7 by adding HCl. Volume was made 1000 ml by adding adequate volume of distilled water. Solution was distributed in 5 conical flasks of 250ml and 2% agar (15g) each was added. Prepared media was autoclaved for 15 minutes at 15lb pressure before plating [19].

Serial Dilution Method, Plating and Laboratory Incubation:

1 g soil was mixed in 10 mL of distilled water to prepare undiluted stock solution in a laminar airflow chamber. The stock solution was diluted by mixing 1 mL of stock solution in 9 mL of distilled water to prepare 10^{-1} concentrated solution. Further dilution was done by taking 1 mL from 10^{-1} concentration and mixed with 9 mL distilled water to prepare a 10^{-2} concentrated solution. The process was repeated till 10^{-8} concentration and each concentration was maintained separately. Same was done for the treated soil. The diluted solutions were then used for plating.
For plating, freshly prepared and autoclaved Nutrient media was cooled and 20 mL of the media was poured into sterilized petri dished inside the laminar air flow chamber. 1 mL of the stock solution was poured into the plate before the plated media was set and the petri dish was stirred to mix the solution homogenously and kept in the laminar air flow for an hour. The same was repeated for all the 8 concentrations of both untreated and treated samples, 3 replication plates for each concentration were prepared for all the samples. The dishes were then moved to a laboratory-based BOD Incubator and incubated for 2 days at 28°C.

Colony Forming Units per gram soil (CFU/g Soil):

After two days of incubation at 28°C plates were collected and the number of colonies were counted. Counting was done by dividing the plate into four equal halves and a number of colonies visible to the naked eye were counted in one of the halves. The other halves were considered to have the equal number of colonies as the counted half. Observations were made throughout a period of 21 days.

Preparation of Standard Curve for Nitrate Reductase Activity:

Griess Test was used to detect the presence of Nitrite ion in a solution [20]. Griess reagent typically contains 0.2% Naphthylethylenediamine dihydrochloride and 2% sulphanilamide in 5% phosphoric acid. When Sulphanilamide was added the nitrites form a diazonium salt, the chromophoric azo-derivative, N-alpha-naphthyl-ethylenediamine develops pink colour. Intensity of colour was spectroscopically measured at a wavelength of 543 nm [21].

Preparation of Clark's Nitrate Reduction Broth:

5 g peptone was melted 1L distilled water and solution was warmed in a hot water bath to allow the peptone to dissolve. The pH of the solution was raised to 9 by adding 0.1N NaOH, 1g NaCl and 1g $NaNO_3$ are added to the solution. The pH was then brought down to 7 by adding 0.1N HCl. The solution thus prepared was used as nutrient broth. 1g of the soil sample was poured into 20 mL of the broth. Three replications per broth were prepared. The inoculated samples are then incubation at 28°C for 1 hour [22].

Estimation of Nitrate Reductase Activity:

To inoculated nutrient broth 1ml of the Griess Reagent was added and appearance of pinkish colour was recorded. Spectrophotometric at a wavelength of 543nm was taken and recorded [20].

Preparation of Standard Curve for Dehydrogenase Activity:

Dehydrogenase Activity (DHA) was estimated by the method developed by Casida [23]. According to this method, specific dyes such as TTC (Triphenyl Tetrazolium Chloride) that can specify the flow of electrons was useful indicators of Electron Transport System (ETS) activity. By the reduction of colourless, water-soluble substrate TTC, by dehydrogenase present in the soil environment an insoluble red colour product Triphenyl Formazan (TPF) was formed. TPF can be quantified calorimetrically at the range of visible light [24]. Each test tubes TTF was filled with increasing concentration. Water was added to make volume 0.5mL. 0.5mL methanol was added to the solution after the appearance of pink colour. The solution was shaken to allow the colour to be dissolved in methanol. The coloured methanol was then used to measure the absorbance values. Absorbance was measured using a UV spectrophotometer at a wavelength of 485nm.

Estimation of Dehydrogenase Activity:

A set of 16 Test tubes were taken and arranged in 4 sets of 4 each. This was labeled for zero hour activity and for 48hrs or 2days activity for four different samples in 3 replicas of each set. To each Test tubes, 0.1g of the soil of each experimental sample was taken. In control samples, the soil was added just before centrifugation after two days of incubation of the experimental samples. To each test tubes, 2ml of 0.1 M glucose and 2ml of 0.5% TTC are added. After centrifugation, the supernatant was taken in different test tubes. To this 2 drops of concentrated H_2SO_4 was added. After addition of H_2SO_4, 3 ml of Toluene was added. The solutions were then mixed properly and the absorbance was taken at 484nm. The value for 1 O.D was found to be 504 µM of TTC.

Analysis of Data:

Factorial Analysis of variance (ANOVA) was used to determine the effect of independent factors concentration and number after treatment on the variations and also their combined effect on variation in data. If there is any significant variation for any of the factor or their combination then a variation was regarded as a positive change caused as an effect of the factor studied or their combination. The tables for the data with the mean values for each treatments were generated in Microsoft Excel ver. 2016. Analysis of the data generated from the experiment was performed in R Statistical Software Version 3.4.4 (2018-03-15) using its inbuilt ANOVA function. The scatter plots and the bar plots were also generated in R using the package ggpubR available at CERN library of package.

Table 1: Mean value of Colony Forming unit/g of soil (CFU/g Soil) of Soil Microbes observed on different days from treatment.

Treatments	CFU/g of Soil				
	0th Day	3rd Day	7th Day	15th Day	21st Day
0%	$1.81X10^6$	$1.59X10^6$	$1.49X10^6$	$1.49X10^6$	$1.71X10^6$
0.20%	$1.49X10^6$	$9.93X10^5$	$8.00X10^5$	$6.77X10^5$	$6.77X10^5$
0.40%	$6.56X10^5$	$5.62X10^5$	$6.82X10^5$	$6.71X10^5$	$6.12X10^5$
0.80%	$6.12X10^5$	$3.61X10^5$	$5.60X10^5$	$6.77X10^5$	$5.98X10^5$
Mean	$1.14X10^6$	$8.76X10^5$	$8.83X10^5$	$8.78X10^5$	899250
SD	$5.20X10^5$	$4.70X10^5$	$3.60X10^5$	$3.52X10^5$	$4.69X10^5$
Max	$1.81X10^6$	$1.59X10^6$	$1.49X10^6$	$1.49X10^6$	$1.71X10^6$
Min	$6.12X10^5$	$3.61X10^5$	$5.60X10^5$	$6.71X10^5$	$5.98X10^5$
CV	45.593	53.734	40.835	40.160	52.158

With mean values for different days, Standard Deviation for different treatments on various days (SD), Maximum value (Max), Minimum value (Min) and Coefficient of Variation (CV).

Table 2: Mean values Nitrate reductase activity of soil microbes observed on different days from treatment.

Treatments	Nitrate Reductase Activity (µg/g Soil/Hour)				
	0 Days	3rd Days	7th Days	15th Days	21st Days

0%	1.28	1.02	0.683	0.388	0.212
0.20%	1.02	0.941	0.09	0.059	0.019
0.40%	0.866	0.39	0.033	0.008	0.341
0.80%	1.023	0.278	0.038	0.018	0.004
Mean	1.047	0.657	0.211	0.118	0.144
SD	0.172	0.377	0.316	0.181	0.162
Max	1.28	1.02	0.683	0.388	0.341
Min	0.866	0.278	0.033	0.008	0.004
CV	0.164	0.574	1.496	1.532	1.124

With mean values for different days, standard deviation for different treatments on various days (SD), Maximum value (Max), minimum value (Min) and coefficient of variation (CV).

Table 3: Mean value of dehydrogenase of soil microbes observed on different days from treatment.

Treatments	Dehydrogenase Activity (µg/g Soil/Hour)				
	0 Days	3rd Days	7th Days	15th Days	21st Days
0%	9.326	8.802	7.706	6.589	4.22
0.20%	22.787	17.432	8.653	6.312	4.178
0.40%	17.796	11.519	6.912	6.299	5.615
0.80%	17.74	15.677	10.931	9.368	1.815
Mean	16.912	13.358	8.551	7.142	3.957
SD	5.584	3.921	1.739	1.490	1.576
Max	22.787	17.432	10.931	9.368	5.615
Min	9.326	8.802	6.912	6.299	1.815
CV	0.330	0.294	0.203	0.209	0.398

With mean values for different days, standard deviation for different treatments on various days (SD), maximum value (Max), minimum value (Min) and coefficient of variation (CV).

III. RESULTS AND DISCUSSION:

Effect on Microbial Colony Forming Unit per gram soil:

Effect of Thiamethoxam observed of 4 different concentrations on 5 different days' show that on the day of treatment that is to say on the 0^{th} Day Microbial population was highest for all the treatments. The Highest value among the treatments was observed in 0% Treatment followed by 0.20% and the minimum value was observed in 0.80%. On the 3^{rd} day after treatment of soil the maximum population remains that of 0% treatment however, for 0.20% treatment the CFU/g Soil decreases by 1.50 times, 1.16 times in 0.40% and 1.70 times in 0.80%. 7^{th} day showed similar trend with 1.24 times decrease in 0.20%, 0.82 times decrease in 0.40% and 0.64 times decrease in 0.80% from 3^{rd} day observations. 15^{th} day had decreased 1.18 times in 0.20%, decreased 1.02 times in 0.40% and 0.83 times decrease in 0.80% treatment. On the 21^{st} day of treatment no decrease was observed in 0.20%. However, number of colony forming units in 0.40% decreased 1.10 times from 15^{th} day observation and that of 0.80% decreased 1.13 times. Between various treatments on 0^{th} day, colony forming units decreased 1.21 times in 0.20%, 2.76 times in 0.40% and 2.96 times in 0.80%. On the 3^{rd} day, colony forming unit decreased 1.61 times in 0.20%, 2.83 times in 0.40% and 4.43 times in 0.80% when compared to 0% treatment. On the 15^{th} day, 0.20% decreased 2.20 times while 0.40% decreased 0.22 times and 0.80% decreased 2.20 times. On 21^{st} day, 0.20% decreased 2.53 times, 0.40% decreased 2.79 times and 0.80% decreased 2.86 times when compared to 0% treatment values of the same day.

Table 4: ANOVA showing variation in CFU/g Soil for different treatments and in different days from treatment with thiamethoxam (Days).

Source of variation	DF	Sum Sq	Mean Sq	F Value	Pr (>F)
Treatments	3	$1.15X10^{13}$	$3.83X10^{8}$	167.46	$1.4X10^{-28}$***
Days	4	$5.87X10^{11}$	$1.46X10^{11}$	14.79	$1.6X10^{-7}$***

Treatments x Days	12	$3.00X10^{11}$	$2.50X10^{11}$	2.52	$1.04X10^{-2}$*
Residuals	40	$1.28X10^{8}$	$2.29X10^{6}$		

Analysis of variance for both variations among different treatments and variation among different days from treatment showed significance at 99.99% confidence level. While variation in the combined effect of treatments and different days of observations showed significance at 95% confidence level. The overall coefficient of variance was highest in 0.20% with the value of 0.36, followed by 0.21 for 0.80% and lowest value i.e. 0.077 was found in 0.40% treated soil.

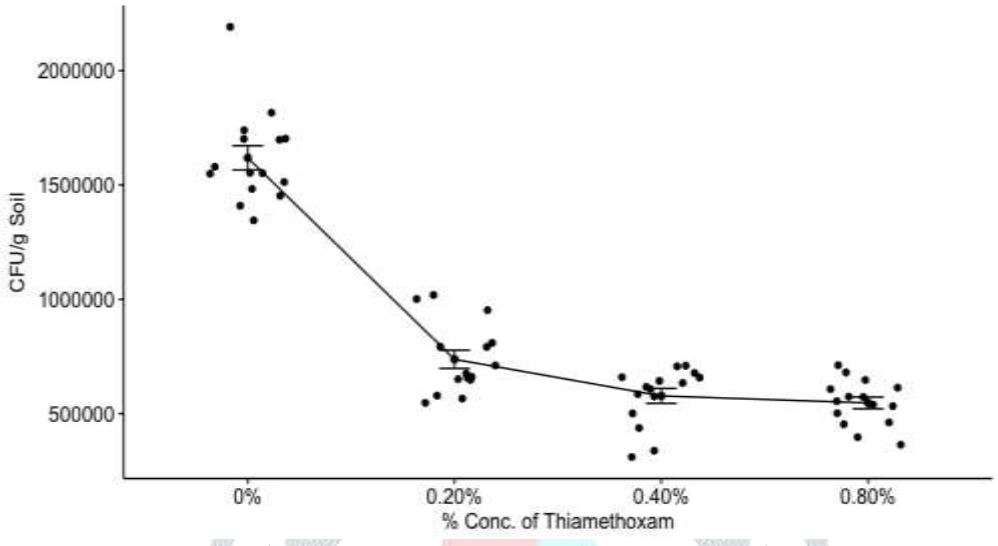

Figure 1: Scatter plot representing variation in variation in Microbial Colony Forming Units per Gram Soil CFU/g soil in Y axis and treatments in the X axis.

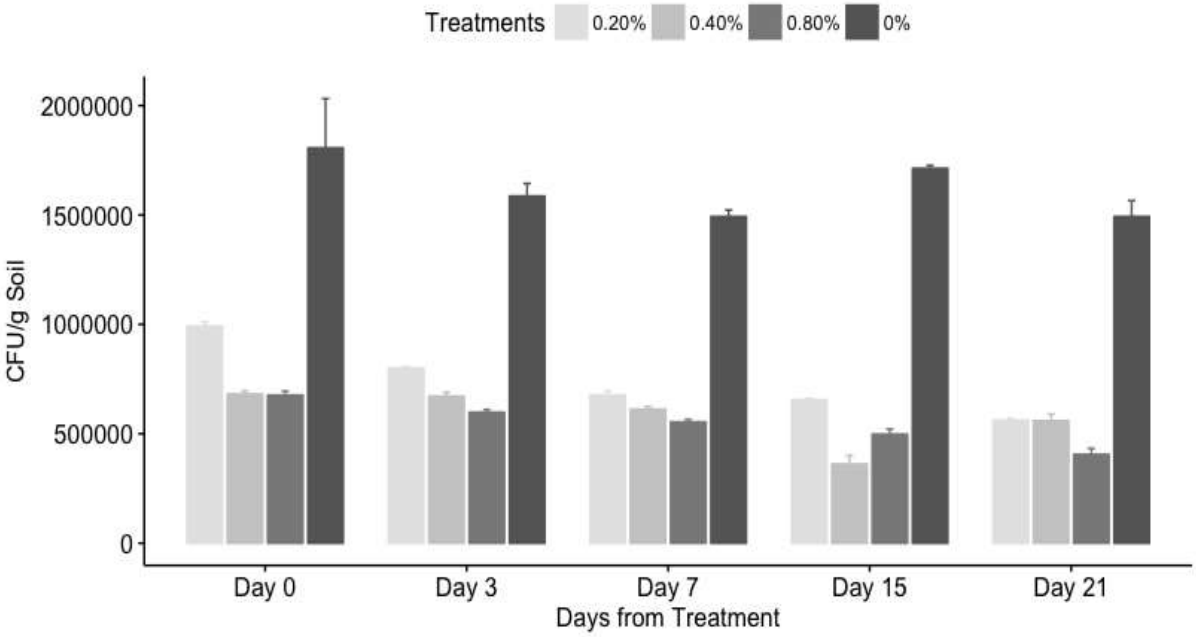

Figure 2: Bar plot representing variation in Colony Forming Microbial units present in the sample soil in different treatments studied on various days from treatment of the soil.

Effect on soil microbial nitrate reductase activity:

Nitrate reductase activity in soil microbial organisms was important for the utilization of nitrogen present in the soil in the form if nitrate (NO_3^{2-}) ion. On the 3rd day of treatment, nitrate reductase activity decreased 1.08 times in 0.20%, 2.22 times in 0.40% and 3.68 times in 0.80%. On the 7th day nitrate reductase activity decreased 10.46 times in 0.20%, 11.82 times in 0.40% and 7.30 times in 0.80%.

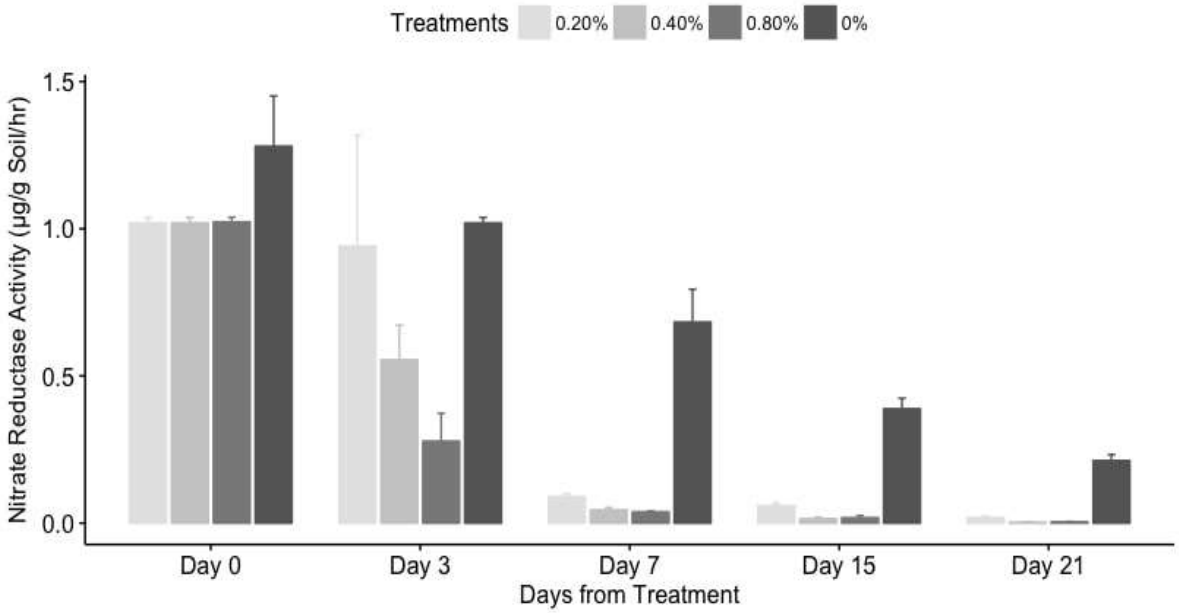

Figure 3: Bar Plot representing variation in activity of Nitrate reductase for different treatments on different days of observation from treatment.

The rate of decrease was gradually stabilized on the 15th day as 0.20% decreased only 1.52 times, 0.40% decreased 4.13 times and 0.80% decreased 2.11 times from their 7th day nitrate reductase activity. On 21st day, 0.20% treatment showed 3.11 times decrease in nitrate reductase activity, however, 0.40% showed 42.63 times increase in nitrate reductase activity and 0.80% showed 4.50 times decrease when compared to 15th day activity. When compared among treatments, 0.40% showed a decrease 1.48 times which was highest among the treatments on the 0th day followed by 1.25 times decrease in both 0.20% and 0.80% when compared to 0th day activity. On 3rd day, highest decrease i.e. 3.67 times decrease in activity was observed in 0.80% followed by 2.62 times decrease in 0.40% and 1.08 times decrease in 0.20%. On 7th day, highest decrease was observed in 0.40% treatment followed 0.80% the lowest was in 0.20%. On 15th day the decrease was very high in 0.40% with 48.50 times decrease in activity when compared to the 0% treatment followed by 0.80% and decrease was lowest in 0.20%. 0n 21st day highest decrease was observed in 0.80% which decreased 53 times in activity of nitrate reductase when compared to 0% treatment. 0.20% showed the second highest decrease on the 21st day from treatment of soil. 0.40% however showed increase 0f 1.61 times from the activity in 0% treated soil samples.

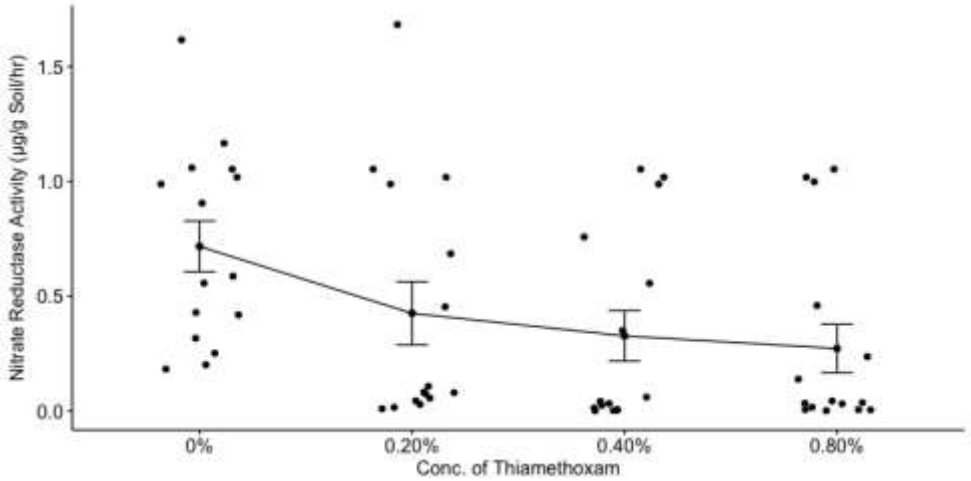

Figure 4: Scatter plot representing variation in variation in soil microbial nitrate reductase activity (µg/g soil/hour) in Y-axis and treatments in the X-axis.

Table 3: ANOVA showing significant variation in soil nitrate reductase activity (µg/g Soil/Hour) for different treatments and in different days from treatment (days)

Source of variation	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
Treatments	3	1.761	0.587	18.658	$9.98X10^{-8}$***
Days	4	9.380	2.344	74.51	$2.0X10^{-16}$***
Treatment X Days	12	0.736	0.0613	1.948	0.057
Residuals	40	9.384	0.167		

Analysis of variance suggest significant variation among different treatments and variation among different days from treatment at 99.99% confidence level. The combined effect of different treatments and days failed to show significant variation. Overall coefficient of variation for nitrate reductase activity in a span of 21 days from treatment was highest in 0.80% treated soil with a value of 1.59 followed by 0.20% with a value of 1.19 and 0.40% with value of 1.06. 0% treatment was found to have the lowest coefficient of variation among the treatments.

Effect on Soil Microbial Dehydrogenase Activity:

Dehydrogenase activity is a measure of metabolic activity of microbes in the soil. When compared to 0^{th} day activity dehydrogenase activity decreased 1.31 times in 0.20%, 1.54 times in 0.40% and 1.13 times in 0.80%. While on day 7^{th} the activity further decreased 2.01 times in 0.20%, 1.67 times in 0.40% and 1.43 times in 0.80% when compared to 3^{rd} day activity. On the 15^{th} day activity decreased further 1.17 times 1.37 times in 0.20%, 1.10 times in 0.40% and 1.17 times in 0.80%. On the 21^{st} day 0.80% showed a dramatic 5.16 times decrease from its day 15^{th} activity followed by 1.51 times decrease in 0.20% and 1.12 times decrease in 0.40%.

Table 4: ANOVA showing significant variation in soil dehydrogenase activity (µg/g Soil/Hour) for different treatments and in different days from treatment (days).

Source of variation	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
Treatments	3	173.1	57.710	178.60	$3.4X10^{-23}$***
Days	4	1221.452	305.363	944.95	$5.22X10^{-39}$***
Days X Treatments	12	296.641	24.720	76.496	$1.12X10^{-23}$***
Residuals	40	12.926	0.323		

Among the different treatments the activity showed highly significant variation between treatments and between as well as in combined effect of both treatments and days. When compared to activity of 0% treated soil samples on 3^{rd} day 0.20% showed 1.98 times increase in activity followed by 1.78 times increase in 0.80% and 1.31 times increase in 0.80%. Similar increase was observed on the 7^{th} day with 1.12 times increase in 0.20%, and 1.42 times increase in 0.80% but 0.40% registered a 1.11 times decrease in activity. On the 15^{th} day the only increase in activity was observed in 0.80% where 1.42 times increase was registered the other two treatments showed decrease in activity 1.04 times in 0.20% and 1.05 times in 0.40%. On the 21^{st} day the trend however shifted as 0.40% which showed decrease in 15^{th} day registered a 1.33 times increase in activity. While 0.20% showed almost similar activity to that of 0% treatment and 0.80% registered a drastic 2.33 times decrease in activity. The overall coefficient of variance for different treatments in a span of 21 days was observed to be highest in 0.20% with a value of 0.67. followed by 0.56 in 0.80% and 0.53 in 0.40%. The coefficient of variance for 0% treatment was 0.28.

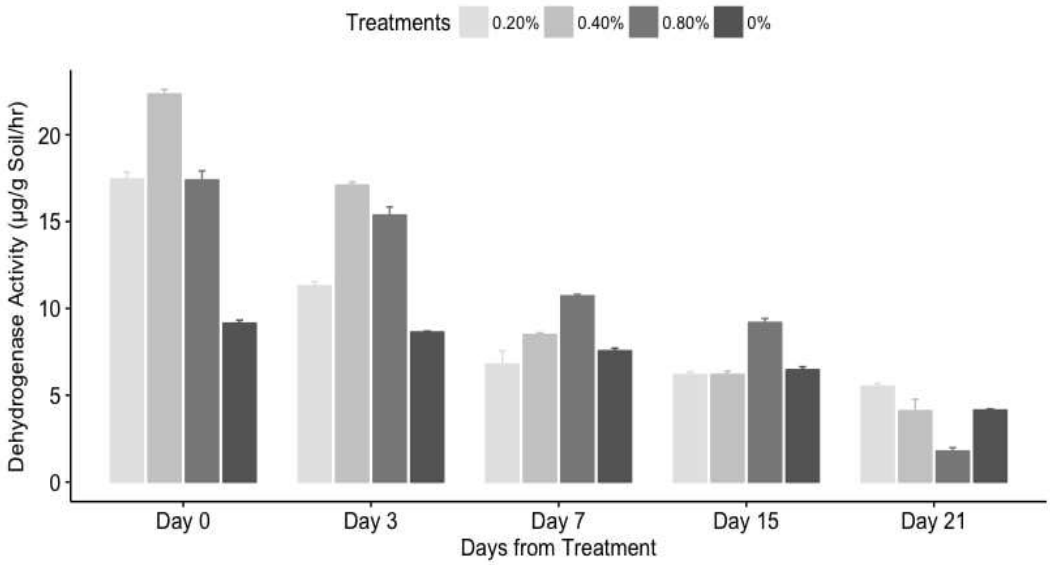

Figure 6: Bar plot representing variation in activity of dehydrogenase for different treatments on different days of observation from treatment.

Figure 5: Scatter plot representing variation in soil microbial dehydrogenase activity (µg/g soil/hour) in Y axis and treatments in the X axis

Thiamethoxam is effectively used in the treatment of aphides in many crops in 0.2% and 0.4% concentrations but tends to remain in soil for a long time. Interaction between the pesticide and soil microbes result in stressed environment in soil. This was confirmed when Serial dilution and plating showed significant decrease in all three treatments at 99% confidence level with lowest values observed in 0.80% in all the days of observation and the highest was observed in 0% treatment. This results disagree with results obtained by Satpute and other workers [4] in their experiment with Soybean (*Glycine max* (L.) Merr.) where microbial population showed no significant changes in the rhizosphere microbes.

Application of thiamethoxam shows no immediate effect. However, there was highly significant decrease in nitrate reductase activity in the 3rd day in 0.20% and 0.40% when compared to 0% (Figure 3) . Nitrate reductase activity on 7th, 15th and 21st day after application of Pesticide show highly significant decrease in enzyme activity as the minimum value was observed 0.40% on 7th and 15th day; on the 21st day the minimum value was observed in 0.80% (Figure 4). Nitrate reductase activity in soil microbial organisms is important for the utilization of nitrogen present in the soil in the form if nitrate (NO_3^{2-}) ion. Such changes may result in changes in the soil nitrate reserve resulting in decrease in soil fertility. Crops without the ability to symbiotically associate with nitrogen fixing bacteria may find it difficult to grow in such soils.

Dehydrogenase activity in thiamethoxam treated soil increased in 0 a 3rd day after treatment. However, the activity gradually decreased in the 7th, 15th and 21st day after treatment. Initial increase may result of activities of soil microbes to adopt to the chemically stressed environment. The variation in DHA was presented in Figure 5 with a scatter plot with mean as the trend line. The graph shows a gradual increase in the 0 and 3rd day after treatment in 0.20% and 0.40% however there is declination in 0.80% (Figure 6). The highest amount of variation was observed in 0.20%. This may be correlated to the decrease in microflora. Singh and Kumar [25] revealed that Acetamiprid increased dehydrogenase activity up to 22% after first insecticide application. Dehydrogenase activity in soil was an indicator of overall microbial activity of soils. Studies on the effect of organophosphate insecticide (quinalphos) on dehydrogenase activity (DHA) in soil and observed 30% inhibition in DHA after 15 days. DHA was recovered after 90 days of treatment which may be due to adaptation of soil microbes to counter the effect of chemical stress in hostile conditions. Similar observation was made with the application of other insecticides [26].

Soil Organic Matter (SOM) content is also an important factor in the soil microbial population dynamics and enzyme activity as SOM quality may increase or decrease the enzymatic activity [27, 28]. Dry soil has high water potential and low microbial activity. As the soil dries down the microbial enzymatic activity decreases [29]. In case of wet soil, there is higher microbial population and increased microbial enzymatic activity both for nitrate reductase and dehydrogenase [16].

The outcomes revelated that thiamethoxam negatively affects soil Microbial Population and enzyme activity this might be straightforwardly identified with the lessening in the fertility of the farming soil. With its utilization as a wide range bug spray in various plants, Thiamethoxam presents a significant risk to soil microflora and its metabolic capacities. Its wide use in horticultural and agricultural fields may present risks to normal growth and development of soil microflora which may result in decrease in soil nutrient cycling and hence soil fertility.

IV. ACKNOWLEDGEMENT:

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