EVALUATION OF THE EFFECT OF MALATHION PESTICIDE ON MICROBIAL POPULATIONS AND ENZYMATIC ACTIVITIES IN VEGETABLE CULTIVATED SOIL

[1]Niti Chawla and [2]Pratibha
[1]Assistant Professor, [2]Post graduate student
[1]Department of Biotechnology,
Chaudhary Bansi Lal University, Bhiwani, Haryana, India.

Abstract: In the present study, the effect of different concentrations of Malathion (0.1ppm, 1ppm, 10ppm and 100ppm), an organophosphate pesticide was evaluated in the laboratory in the soil collected from vegetable (okra) cultivated field of Bawani khera, Bhiwani. Microbial populations (bacterial and actinomycetes populations) and enzymatic activities (amylase, invertase, acidic phosphatase and alkaline phosphatase) were assessed after every seven days of incubation with a total period of 21 days. Concentration upto 1ppm enhanced the bacterial and actinomycetes populations and thus found to be beneficial but higher concentrations i.e.10 ppm and 100 ppm showed reduction in the microbial populations, as well as in the activities of invertase, acidic phosphatase and alkaline phosphatase enzymes. Actinomycetes population was found to decrease by 11%, with the concentration of 10 ppm and bacterial populations was observed to be slightly affected by that concentration. Concentration of 100 ppm affected bacterial as well as actinomycetal populations which were decreased by 30% and 39% respectively. Similar trend of reduction was also observed in the enzymatic activities. Invertase, alkaline phosphatase and acidic phosphatase activities showed overall reduction of 19% 30%, 23% with the application of 10 ppm and 45% 64%, 49% with the application of 100 ppm respectively. Amylase activity was found not to be affected by even higher concentrations.

Key words: *Pesticide, Malathion, Bacteria, Actinomycetes, Amylase, Invertase, Phosphatase*.

I. INTRODUCTION

Wide varieties of pests all over the world are responsible for causing destruction especially in the agricultural crops. Estimated annual losses of crops due to pests in India are nearly US$ 42.66 million (Sushil, 2016). To control these pests, use of pesticides has become a routine practice. Pesticides refer to the substances that kill any type of pest such as insect, rodent, weed and are classified as insecticides, herbicides, fungicides, rodenticides, weedicides etc. The major problem associated with the pesticides is that besides controlling pests they also alter the originality of environment generally in the negative way. Their long term persistence in the environment is responsible for causing several diseases. Their residues have been detected in the soil, water and even in the food items which is a matter of high concern. They have also affected the soil health by altering the beneficial microbes and their biochemical activities present in the soil. Overall, pesticides come in the category of most dangerous persistent organic pollutants in the environment. Several studies on effect of pesticides on soil microbial diversity and their biochemical processes have already been carried out by many researchers (Niewiadomska, 2004; Ingram *et al*., 2005; Wang et al., 2006 Littlefield-Wyer *et al*., 2008). Some of the microbes use pesticides as a source of carbon and energy to grow and divide. On the other hand, for others these are toxic. They may lead to enhancement or depletion of the microbial diversity and activities (Wang *et al*., 2006; Pampulha and Oliveira, 2006). But the actual fate of pesticides cannot be predicted without carrying out studies. In view of that the present study has been carried out on Malathion, an organophosphate insecticide that was first time registered for use in the United Stated in 1956. Till now, thousands of products containing it have been registered all over the world with different brand names. Physical formulations that are marketed are in the form of liquids, dust or wet able powders or emulsions. Besides used in controlling agricultural pests, it is also used in the public health and mosquito control programs.

In the present study, the impact of malathion pesticide on soil health has been depicted by throughout evaluation in terms of analyzing populations of bacteria and actinomycetes as well as enzyme activities of amylase, invertase, acidic phosphatase and alkaline phosphatase with the application of different doses of malathion in the soil procured from vegetable (okra; *Abelmoschus esculentus*) cultivated field.

II. MATERIALS AND METHODS

2.1 Chemicals

Malathion (50EC), Shivalik Crop Sciences (P) Ltd. was purchased from the local pesticide supplier. All other chemicals used AR grade from Hi-media, research laboratories.

2.2 Media

Ken-Knight & Munarier's Medium and Nutrient Agar Medium were prepared by dissolving the ingredients in distilled water and sterilized at 15 psi (121^{0}C) pressure for 20 min after adjusting the pH.

2.3 Soil samples

Soil samples were taken from rhizospheric region (at the depth of 0-10 cm) from the vegetable (Okra) cultivated field of Bawani Khera, Bhiwani (Haryana, India). Soil samples were then mixed properly, partially air dried overnight and then sieved through 2mm mesh sieve.

2.4 Physiochemical analysis of soil

Soil pH was determined by pH meter. Water holding capacity was determined by using filter paper. Soil texture and micronutrients content were determined by Hi-media test kits.

2.5 Treatments

Fifty gram soil was kept in each petri-plate and treated with different concentrations of malathion i.e. 0.1 ppm (T1), 1 ppm (T2), 10 ppm (T3) and 100 ppm (T4) respectively. Multiple plates were kept of each treatment. Control was also kept without the treatment of malathion. Stock solution of malathion was prepared in acetone. After treatment, soil samples were homogenized to evenly distribute the malathion, and enough distilled water was added to maintain at 50-60% water holding capacity (WHC). Sterilized distilled water was added after every two days of incubation to compensate for the loss of water by evaporation.

2.6 Effect of different concentrations of malathion on microbial populations

The effect of different concentrations of malathion was determined on microbial populations in the soil, in triplicates at 1st, 7th, 14th, 21st day after treatment with malathion by using standard dilution technique and spread plate method. For bacterial colonies, plating was done on nutrient agar media and plates were incubated at 30°C for 24 hrs. For actinomycetes colonies, plating was done on Ken-Knight & Munarier's Medium and plates were incubated at 30°C for 3days. Counts were expressed as the colony forming units (CFU) per gram of soil (dry weight basis).

2.7 Effect of different concentrations of malathion on enzymatic activities

For estimation of the enzyme activities, duplicates of each treatment were withdrawn at 1st, 7th, 14th, 21st day and enzymatic activities were determined in triplicates using the following methods:

a. Amylase and Invertase activities were determined by Dinitrosalicylic acid (DNS) colorimetric method

b. Acidic and Alkaline Phosphatase activities were determined by using PNPP (Paranitrophenyl phosphate) colorimetric method

III. RESULTS AND DISCUSSION

In the present study, the effect of different concentrations of malathion pesticide was evaluated on microbial populations and enzymatic activities in the soil at different days of incubation.

3.1 Physiochemical properties of the soil

Physiochemical properties of the soil used in the study have been represented in the **Table 1**.

Table1. physiochemical properties of the soil used in the study

S. No.	Name of the Property	Value
1.	Clay (<=2.00mm) (%)	35
2.	Silt (<=2.00mm) (%)	15
3.	Sand (<=2.00mm) (%)	50
4.	Soil textural class	Sandy clay loam
5.	Soil pH	5.2
6.	Water holding capacity (%)	56
7.	Iron (Fe)(ppm)	More than 6.0
8.	Manganese (Mn) (ppm)	0.2 - 2.0
9.	Copper (Cu) (ppm)	0.0 - 0.5
10.	Molybdenum (Mo) (ppm)	0.0 - 0.1
11.	Zinc (Zn) (ppm)	0.5 - 2.0
12.	Boron(B) (ppm)	0.1 - 1.0

3.2 Effect of Malathion on microbial populations

3.2.1 Bacterial population

Bacterial population showed significant increase of 9.3% at 1ppm concentration of malathion (Table 2 and Fig. 1a). At 10ppm the population was almost similar to the control. Thereafter, at 100ppm significant decrease was observed of about 30%. Malathion with the concentration of 100 ppm (T_4) showed lowest bacterial count (54.00 CFUx10^7/gm soil). It was followed by counts with the malathion concentration of 10 ppm (T_3) and 1 ppm (T_2) which were 76.50 CFUx10^7/gm soil and 84.50 CFUx10^7/gm soil, respectively. Interaction studied between treatments and days of incubation was found to be statistically significant (CD= 1.402; P=0.05). There was observed slight increase in the population in the first week and thereafter decrease in the 2nd and 3rd week of observation.

Similar studies have shown higher concentration of pesticides decreased the number of bacteria after every week starting from the first week (Hindumathy and Gayathri., 2013). Contradictory evidence is also there that have shown slight increase in the bacterial population at 250 ppm malathion due to the degradation of pesticide in soil and also the persistence in soil was upto 2 weeks (Das and Mukherjee 1998; Das et al., 2003; Kay Lynn Newhart., 2006). Haleem *and his coworkers in* 2013 also observed the inhibition of soil bacteria by 16%, 24% and 40% after 24, 48 and 72 hours of application of malathion at 250ppm. Similarly, Congregado *et al.*, 1979 studied the effect of dimethoate and malathion at 10 and 100 ug/g in the soil which caused initial stimulation of population of phospholipases producing bacteria from first week of application until fourth week and thereafter population returned to the original levels. Manikar *et al*., 2013 also studied the effect of malathion pesticide on the cyanobacterial biofertilizer; *Anabaena variabilis* applied in the paddy field. They observed that with increasing concentration of malathion the test organism showed increased oxidative stress due to the production of free radicals which in turn caused the reduction in growth.

3.2.2 Actinomycetes Population

The results of varying concentrations of malathion (0-100ppm) on actinomycetes population showed significant increase upto 1ppm of malathion when compared with control (Table 3 and Fig1b). Thereafter, at higher concentrations (10 and 100ppm) significant decrease in the populations was observed i.e. by 11% and 39% respectively. Malathion with the concentration of 100 ppm (T_4) showed lowest actinomycetes count (6.62 CFUx10^6/gm soil). It was followed by counts with the concentration of 10 ppm (T_3) and 1 ppm (T_2) which were 9.60 CFUx10^6/gm soil and 13.11 CFUx10^6/gm soil, respectively. Interaction between treatments and days of incubation also found to be statistically significant (CD= 0.639; P=0.05). The population was observed to increase slightly up to 1st week of incubation but thereafter slight decrease was observed in week 2nd and 3rd respectively.

Similar to our results, Haleem and his coworkers in 2013 also observed decrease in the actinomycetes population by 72% with the application of higher concentration of malathion i.e.250 ppm. According to another study (Phillip et al., 2014) actinomycetes population was found to decrease by the application of different pesticides. Gundi *et al*., (2005) also observed the effect of other pesticides (monochrotophos, quinalphos, and cypermethrin) on microbial populations and observed positive effects at the lower doses and adverse effects at the higher doses. Similarly, some other studies indicates that the application of pesticides preferred the actinomycetes growth in the soil at low concentration (Supreeth *et al*., 2016) and higher concentration sight the reduction in actinomycetes populations (Shan *et al*., 2006). Toxic effect of higher concentration of malathion may be due to the quick breakdown of the pesticide to more toxic substance called malaxon, by the action of water and bacteria present in the soil (Magar and bias, 2013).

Table2: effect of malathion on bacterial counts* per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control	86.50	84.00	71.00	68.00	77.30
T1 (0.1 ppm malathion)	87.50	84.00	70.50	69.00	77.70
T2(1 ppm malathion)	91.00	94.00	82.00	71.00	84.50
T3(10 ppm malathion)	79.00	78.00	76.00	73.00	76.50
T4(100 ppm malathion)	57.00	65.00	54.00	40.00	54.00
Mean	80.20	81.00	70.70	64.20	

Factors	*C.D.at 5%*	*SE(d)*	*SE(m)*
Treatments	*0.70*	*0.34*	*0.24*
Days	*0.62*	*0.31*	*0.22*
Treatments x Days	*1.40*	*0.69*	*0.49*

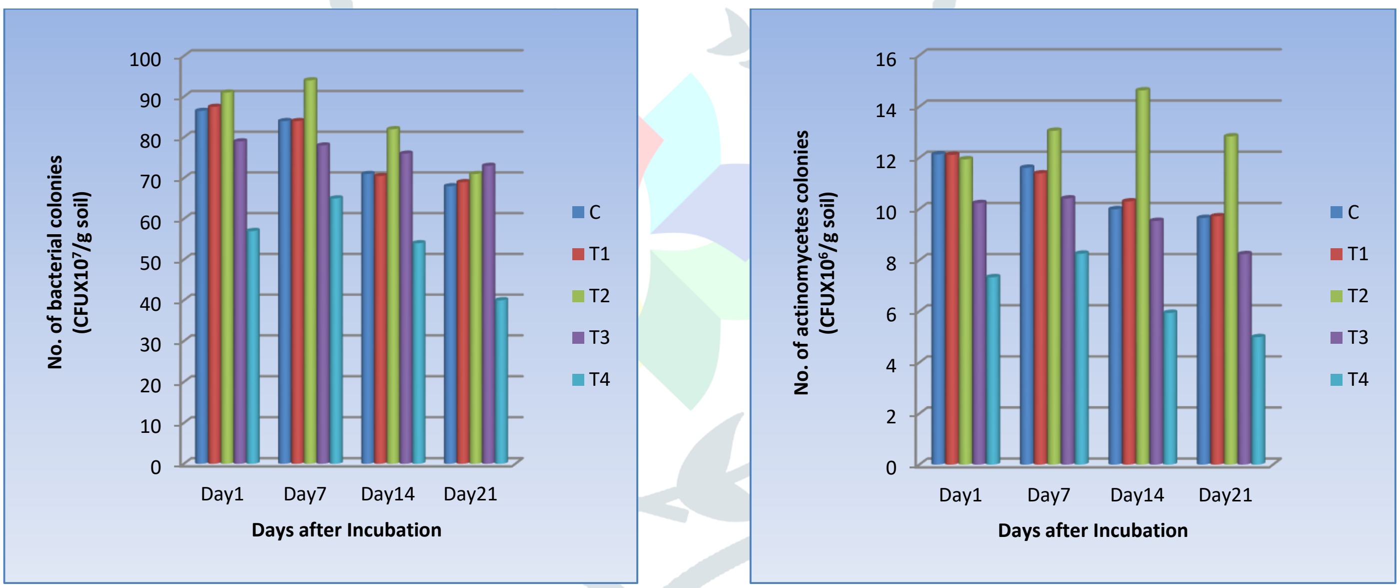
*Number of colonies per gram soil= Colony forming units x dilution factor
Dry weight of soil

Table3: effect of malathion on actinomycetes count* per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control	12.13	11.61	9.99	9.65	10.84
T1 (0.1 ppm malathion)	12.11	11.39	10.29	9.71	10.88
T2(1 ppm malathion)	11.94	13.05	14.63	12.83	13.11
T3(10 ppm malathion)	10.23	10.41	9.53	8.23	9.60
T4(100 ppm malathion)	7.33	8.25	5.94	4.99	6.62
Mean	10.75	10.94	10.07	9.08	

Factors	*C.D.at 5%*	*SE(d)*	*SE(m)*
Treatments	*0.32*	*0.15*	*0.11*
Days	*0.29*	*0.14*	*0.10*
Treatments x Days	*0.64*	*0.31*	*0.22*

*Number of colonies per gram soil= Colony forming units x dilution factor
Dry weight of soil



a. Bacterial Population **b. Actinomycetes Population**

fig1. effect of malathion on microbial populations

3.3 Effect of malathion on enzyme activities

3.3.1. Amylase Activity

Starch hydrolysing enzyme, amylase was found not much affected even at higher concentrations of malathion i.e. 10 ppm and 100 ppm (Table 4.and Fig.2a). The effect of interaction between days of incubation and treatments was found to be statistically significant (CD= 0.69; P=0.05). Activity was found to increase up to 7 days of incubation. Thereafter, continuous decrease was observed up to 21 days of observation. Similar to our results Walia et al., 2014 also observed decrease in the amylase activity only at higher concentrations of 500ppm and 1000ppm whereas lower concentrations upto 250ppm, not much affected the activity. Recently, Madella and Venkateswarlu., 2018 found the stimulation on amylase activity by the addition of pesticides at lower concentration while higher concentrations showed opposed effects. El-Nahhal *et al*., 2017 also studied the effects of bromacil, malathion and thiabendazole on cyanobacterial mats. They observed significant negative effects in treatments containing bromacil and thiabendazole but malathion showed increased population growth and not affected the ammonium production.

3.3.2 Invertase Activity

The results (Table 5 and Fig.2b) showed that 1ppm concentration of malathion favoured the invertase activity. Here, the increase in the activity was of 13.9% as compared to control. Thereafter at higher concentration decrease in activity was observed which was 19% and 45% at 10 and 100 ppm respectively. The interaction between treatment and days of incubation was found to be statistically significant (CD= 1.04; P=0.05). Activity increased in the first week, decreased in the 2nd week and attained equal to control and thereafter significant decrease was observed after the 3rd week of incubation. Our results are well justified as recently, Ataikiru and his co-workers in 2019 also observed reduction in invertase activity in response to higher concentrations of these pesticides, Paraquat and Carbofuran in the soil.

3.3.3 Alkaline Phosphatase Activity

The results of varying concentrations of malathion (0-100ppm) on alkaline phosphatase activity showed significant increase in the activity at 1ppm of malathion which was about 10% (Table 6 and Fig.2c). Thereafter, 10 and 100ppm concentrations significantly decreased the activity by 30%and 64% respectively. Interaction between days and treatment were also observed of producing the statistically significant effect on the activity (CD= 0.61; P=0.05). Activity increased in the first week, decreased in the 2nd week and attained equal to control and thereafter significant decrease was observed after the 3rd week of incubation. Similarly, Lethbridge *et al.*, 1981 studied the effect of four herbicides and one insecticide (malathion) on 1, 3-β-glucanase and urease activities in the soil. Lower doses showed no effect on the activity of either enzyme whereas high dosages disrupted the enzyme activities. Our results are also well justified with Walia and his coworkers in 2018 who observed malathion affected P-solubilizing bacteria very less in the initial two weeks of observation but in last week, P-solubilizing bacterial population decreased significantly. Similarly, Mahanta et al., 2014 also reported malathion treatment slowly elevate alkaline phosphatase activity in soil on 10th day of application and decreased thereafter.

3.3.4 Acidic Phosphatase Activity

The results showed that concentrations upto 1ppm not much affected the activity but at higher concentrations i.e. 10 and 100 ppm decreased the activity by 23%and 49% respectively as compared to control (Table 7 and Fig 2d). Interaction between days and treatments was observed to be statistically significant. Activity decreased continuously from 1st to 21st day of observation. Studies carried out by other researchers on effect of insecticides on phosphatase activity revealed variable effects i.e. sometimes inhibitory and some-times stimulatory depending upon the concentrations and days of incubation (Defo *et al*. 2011, Jastrzebska 2011). Ataikiru and his coworkers in 2019 found significant effect of Paraquat and Carbofuran on phosphatase activity w.r.t days of incubation in soil. Phosphatase activity decreased upto 3rd week and thereafter increased due to application of paraquat. Baćmaga *et al.* (2012) reported the stimulatory effect of carfentrazone-ethyl on acid phosphatase and alkaline phosphatase. Thus, effect of different concentration of the pesticides exerted variable effect on acid phosphatase activity of soil microbes during different periods of time.

Table.4: effect of malathion on amylase activity per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	**Day7**	**Day14**	**Day21**	
Control	33.62	51.04	15.35	16.04	29.01
T1 (0.1 ppm malathion)	26.73	39.49	18.45	22.93	26.90
T2(1 ppm malathion)	27.76	40.29	28.45	16.55	28.26
T3(10 ppm malathion)	33.11	41.55	20.00	28.11	30.69
T4(100 ppm malathion)	30.86	46.04	15.69	20.35	28.24
Mean	30.42	43.68	19.59	20.80	

Factors	***C.D.***	***SE(d)***	***SE(m)***
Treatments	*0.35*	*0.17*	*0.12*
Days	*0.31*	*0.15*	*0.11*
Treatments x Days	*0.69*	*0.34*	*0.24*

Table.5: effect of malathion on invertase activity per gram of control and treated soil at different days of incubation

Treatments	**Days of Incubation**				**Mean**
	Day1	**Day7**	**Day14**	**Day21**	
Control	176.73	301.21	150.81	64.80	173.39
T1 (0.1 ppm malathion)	177.24	301.73	147.42	65.00	172.85
T2(1 ppm malathion)	209.03	334.49	161.41	85.66	197.65
T3(10 ppm malathion)	51.56	276.38	107.59	125.18	140.18
T4(100ppm malathion)	49.51	183.28	66.382	82.07	95.31
Mean	132.81	279.42	126.72	84.54	

Factors	*C.D.*	*SE(d)*	*SE(m)*
Treatments	*0.52*	*0.26*	*0.18*
Days	*0.46*	*0.23*	*0.16*
Treatments x Days	*1.04*	*0.51*	*0.36*

Table.6: effect of malathion on alkaline phosphatase activity per gram of control and treated soil at different days of incubation

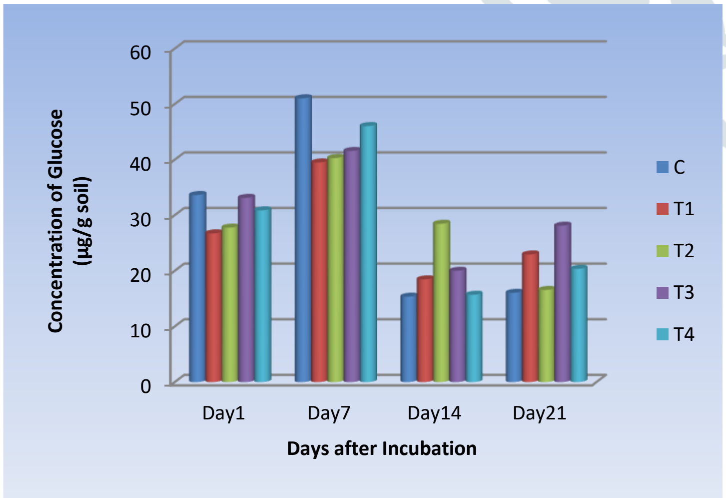
Treatments	**Days of Incubation**				**Mean**
	Day1	**Day7**	**Day14**	**Day21**	
Control	34.40	36.25	33.85	26.11	32.65
T1 (0.1 ppm malathion)	36.89	35.63	32.84	26.36	32.93
T2(1 ppm malathion)	38.27	39.79	37.91	28.64	36.15
T3(10 ppm malathion)	26.11	24.84	23.21	17.23	22.84
T4(100ppm malathion)	19.15	17.74	6.72	3.43	11.64
Mean	27.13	30.85	26.90	20.35	

Factors	*C.D.*	*SE(d)*	*SE(m)*
Treatments	*0.30*	*0.15*	*0.11*
Days	*0.27*	*0.13*	*0.09*
Treatments x Days	*0.61*	*0.30*	*0.21*

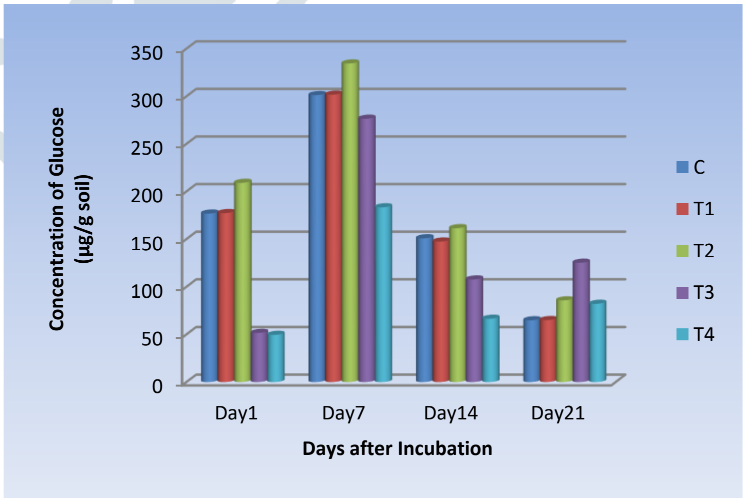
Table7: effect of malathion on acidic phosphatase activity per gram of control and treated soil at different days of incubation

Treatments	**Days of Incubation**				**Mean**
	Day1	**Day7**	**Day14**	**Day21**	
Control	78.28	78.27	73.55	49.32	69.85
T1 (0.1 ppm malathion)	78.27	77.31	74.31	48.94	69.71
T2(1 ppm malathion)	82.41	80.53	74.89	44.22	70.51
T3(10 ppm malathion)	60.60	57.38	54.21	42.55	53.68
T4(100 ppm malathion)	50.07	39.92	35.19	18.11	35.82
Mean	70.32	66.68	62.43	40.62	

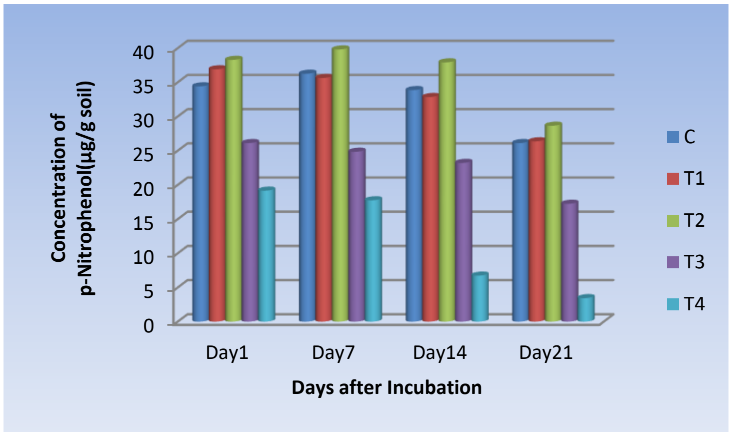
Factors	*C.D.*	*SE(d)*	*SE(m)*
Treatments	*2.47*	*1.22*	*0.86*
Days	*2.21*	*1.09*	*0.77*
Treatments x Days	*4.93*	*2.43*	*1.72*



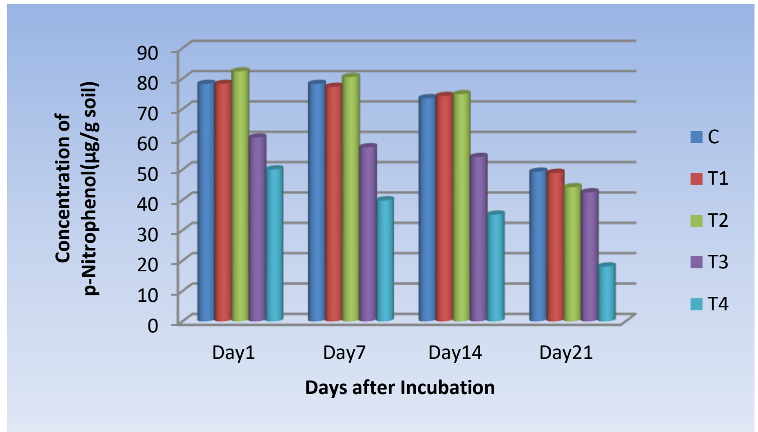
a. Amylase Activity



b. Invertase Activity



c. Alkaline Phosphatase Activity



d. Acidic Phosphatase Activity

Fig 2. effect of malathion on enzyme activities

IV. CONCLUSION

This study has come out with the conclusion that malathion at lower concentrations is beneficial for the microbial populations and enzymatic activities. Higher concentration i.e. 10 ppm has not much affected the microbes. The reason behind may be the microbes were having the potential of degrading the malathion pesticide. Harmful effect was observed at 100 ppm in all the parameters of observation except amylase activity. It is suggested that these finding should be validated by carrying out long term studies using new advanced technologies.

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