

Incubation Study of microbial inoculation in Enriched vermicompost on Nutrient status.

Abstract

An experiment was conducted to study – We add different type of microbes species in different vermicompost treatments and we use different quantity of microbes. then 25 mL of each inoculants were applied per kg of VC (wet base) (Busato et al., 2012). Vermicompost samples (one kg per each treatment) were incubated in a chamber with optimum aeration at 28 and 41 °C for 80 days and during incubation moisture of VC samples were maintained about 60 percent by distilled water. Enriched VC treatments were analyzed for chemical and biological characteristics at 0, 20, 40,60 and 80 days. An incubation study carried out in the laboratory through incubating prepared enriched vermicompost (T1 to T7 treatments) with biofertilizers (T8 to T14 treatments) for a period of 80 days. The N, P, K and Zn content were analyzed at 0, 20, 40, 60 and 80 days after Incubation (DAI). Among these enriched vermicompost treatment , the highest total Nitrogen was observed in enriched vermicompost after 80 DAI T11 (4.5 % respectively) followed by T9 (4.4 %) and T 14 (4.3% respectively) and Highest total phosphorus (4.9%, 4.7% and 4.2 % respectively). Among the enriched vermicompost treatment, highest K and Zn content (4.8% and 97.5 ppm, respectively) was observed in enriched vermicompost. **Key words:** Incubation, Biofertilizers, Enriched , inoculants, Vermicompost.

INTRODUCTION

Modern agricultural practices have a major impact on the environment. Excessive use of fertilizers such as urea, nitrate, phosphorous along with many other pesticides has affected air, water, and soil quality. Climate change, deforestation, loss of biodiversity, irrigation problems, pollutants, soil degradation and waste s are some of the concerns that are connected with agriculture. The Green revolution was all about higher productivity to feed the growing population and we achieved it. But to achieve this we followed the wrong road of un-sustainability. 'Modern Farming' has

affected our world. There are four important considerations i.e. what happen to the land, the food it produces, the people who eat it and the communities which lose out were over looked. On one hand tropical soils are deficient in all necessary plant nutrients and on the other hand large quantities of such nutrients contained in domestic wastes and agricultural by-products are wasted. In nature, there are a several organisms that can to convert organic waste into valuable resources. This helps to maintain nutrient flow and minimize environmental degradation. One such organism is earthworms, which could be able to recycle the organic wastes into valuable manure i.e. vermicompost. Vermicompost has several advantages when used in agriculture.

In recent times, there has been growing emphasis on the large-scale use of organic manures given escalating fertilizer prices, deteriorating soil health and environmental quality, and poor purchasing power of small and marginal farmers. Efficient recycling of locally available bio-wastes as nutrient sources is important for supplementing chemical fertilizers and maintaining soil fertility and crop productivity (Singh et al., 2020). Vermi-composting is an advantageous organic waste conversion technology due to its easy adoption, faster degradation and enhanced macro- and micro-nutrients, beneficial microorganisms along with plant growth-promoting substances.

In general, organic manures like FYM, compost , and vermicompost contain an average 0.5 to 1.5% N, 0.2 to 0.8% P_2O_5 and 0.5 to 1.2% K_2O , which is not sufficient to meet the crop demand in low doses. For supplying N at 100 kg ha^{-1} , the organic manure should be applied at 07 to 20 t ha^{-1} . These demerits of manures can be overcome to a certain extent through the preparation of enriched Vermicompost by adding natural or biological sources of nitrogen, phosphorus, potassium, sulfur and micronutrients either alone or in combination. Moreover, waste with different nutrient rich substances can open new direction of technological up gradation for improving the quality and nutrient status of vermincompost. Modification of vermicompost either by microbial enrichment or fortifying with nutrient rich rock minerals and agricultural waste may help in enriching the nutrient content.

The enrichment of vermi-compost through iron refuse makes it a rich source of total and available iron (Hashemimajd and Golchin, 2009), others confirmed that the enrichment of cow manure with rock phosphate could significantly improve the P availability, addition of biofertilizers and microorganisms including N_2 -fixing and P-

solubilizing bacteria to the Vermicomposting (Kaushik et al., 2008; Karmegam and Rajasekar, 2012; Ashwin et al., 2013). By studying the viability of biofertilizer bacteria in enriched Vermicomposting, it was demonstrated that the biofertilizer inoculants could not only survive in enriched vermicompost for long periods of time, they could also significantly improve the nutrient content in plant and soil fertility status (Kappuraj et al. 2012). Despite the clear role of biofertilizer bacteria in improving the nutrient reserves of Vermicomposting.

Material and Methods

a. Location and Place of working:

Enriched vermicompost was prepared at the animal husbandry farm, RVSKVV Gwalior (M.P) and laboratory experiment will be carried out in the Department of Soil Science and Agriculture Chemistry College of Agriculture, RVSKVV Gwalior (M.P).

C. Experimental setup and vermicomposting:

Vermicompost was prepared by using (*Eisenia fetida*) earthworm species using with different organic materials and minerals, for the enrichment of the compost for N,P,K,S,Zn,Ca and Mg. Cemented vermicompost pits were used for compost preparation. These pits were already available in Dairy Farm, RVSKVV Gwalior.

Microbial inoculation in vermicompost – We add different type of microbes species in different vermicompost treatments and we use different quantity of microbes. Then 25 mL of each inoculant were applied per kg of VC (wet base) (Busato et al., 2012). Vermicompost samples (one kg per each treatment) were incubated in a chamber with optimum aeration at 28 and 41 °C for 80 days and during incubation moisture of VC samples were maintained about 60 percent by distilled water. Enriched VC treatments were analyzed for chemical and biological characteristics at 0, 20, 40, 60 and 80 days.

D. Physico-chemical analysis of Microbes inoculated enriched vermicompost :

The physico-chemical analysis of Before enrichment of microbes and After enrichment vermicomposts in different day were done as follows.

For pH and EC, a substrate to water ratio of 1:10 (w/v) was used. Five grams of sample were mixed with 50 ml of distilled water and kept in a shaker for 45 minutes

for proper mixing. The mixture was then filtered through the Whatman filter paper, and the pH and EC were determined using a digital pH and conductivity meter (Systronics 2551). Total Kjeldahl nitrogen (TKN) was measured by the standard method of Kjeldahl digestion and distillation using Kjeldahl apparatus (Kelplus) (Tandon 2005). Total organic carbon (TOC) was estimated by loss on ignition method as described by Tandon (2005). Total potassium (K) was estimated using a flame photometer, whereas total phosphorus (P) was estimated using the vanado-molybdate method (Tandon 2005).

Available nitrogen was estimated by alkaline KMnO_4 method. The amount of NH_3 trapped was estimated by titrating with standard acid (Subbaiah and Asija, 1956). The available phosphorus in the extract was determined by ascorbic acid reductant method using spectrophotometer at 660 nm wave length (Jackson, 1973). The soil was extracted with neutral normal ammonium acetate and the content of potassium in the extract was estimated by flame photometer (Jackson, 1973). The available sulphur was extracted by 0.15 percent CaCl_2 solution and the concentration of sulphur was determined by the turbidimetric method using spectrophotometer (Chesnin and Yien 1951).

The concentrations of Cu, Zn, Fe, and Mn were analyzed using the Atomic Absorption Spectrophotometric method (DTPA Extract AAS method, Lindsay and Norvell, 1978). The C/N ratio was calculated by dividing the TOC by the total nitrogen content. All reagents and chemicals used during analytical work were of AR grade.

Treatment Details

T₈: EV₁ + *Azotobacter chroococume* + *Bacillus polymixa* (PSB) + *Bacillus firmus* (K releasing bacteria) + *Trichoderma viridae* (Cellulolytic)

T₉ : EV₂ + *Azotobacter chroococume* + *Bacillus polymixa* (PSB) + *Bacillus firmus* (K releasing bacteria) + *Trichoderma viridae* (Cellulolytic)

T₁₀: EV₃ + *Azotobacter chroococume* + *Bacillus polymixa* (PSB) + *Bacillus firmus* (K releasing bacteria) + *Trichoderma viridae* (Cellulolytic)

T₁₁: EV₄ + *Rhizobium* + *Bacillus polymixa* (PSB) + *Bacillus firmus* (K releasing bacteria) + *Trichoderma viridae* (Cellulolytic)

T₁₂: EV₅+ *Rhizobium* + *Bacillus polymixa* (PSB)+*Bacillus firmus* (K releasing bacteria) +*Trichoderma viridae* (*Cellulolytic*)

T₁₃: EV₆+*Azotobactor chroococume* + *Rhizobium* +*Bacillus firmus* (K releasing bacteria) +*Trichoderma viridae* (*Cellulolytic*)

T₁₄: Conventional Vermicompost+ *Azotobactor chroococume* + *Rhizobium* +*Bacillus polymixa* (PSB)+*Bacillus firmus* (K releasing bacteria)+*Trichoderma viridae* (*Cellulolytic*)+ Zn solubilizing Bacteria.

T₁₅: Control (Conventional Vermicompost)

Results And Discussion

Periodic release of macro and micro nutrients from prepared enriched vermicompost when incubated with biofertilizers:

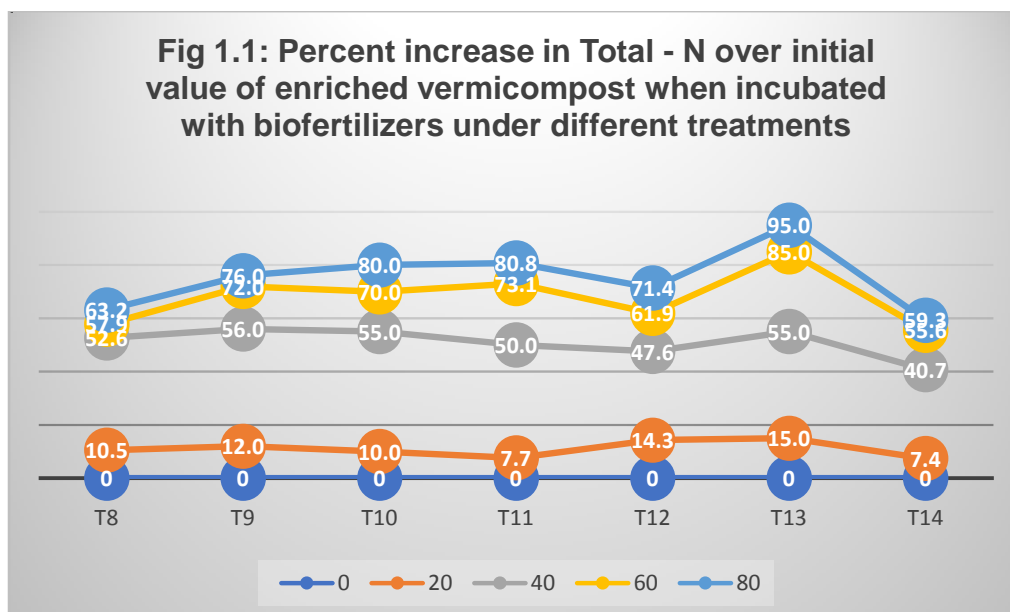
An incubation study carried out in the laboratory through incubating prepared enriched vermicompost (T1 to T7 treatments) with biofertilizers (T8 to T14 treatments) for a period of 80 days. The N, P, K and Zn content were analyzed at 0, 20, 40, 60 and 80 days after Incubation (DAI) and data are presented in Table 1.1 through 1.5 and Fig 1.1 through Fig 1.5.

The data pertaining to periodic release pattern of Nitrogen from enriched vermicompost when incubated with biofertilizers are presented in Table 1.1 and Fig.1.1. It is evident from the data that the at 0 DAI the highest N content (2.6%) was recorded in the treatment T11 and T14. Treatment T11 comprises of Sesamum cake + Groundnut Cake + *Gliricidia* leaves + Legume waste +Poultry manure + Rock phosphate + Potassium feldspar + Limestone + *Rhizobium* + *Bacillus polymixa* (PSB)+*Bacillus firmus* (K releasing bacteria) +*Trichoderma viridae* (*Cellulolytic*) while, the treatment T14 comprises of Enrichment of VC through Mustard cake+ Binola cake+ Neem cake + Groundnut cake + sesame cake + Linseed cake + RP + Potassium feldspar + *Azotobactor chroococume* + *Rhizobium* + *Bacillus polymixa* (PSB) + *Bacillus firmus* (K releasing bacteria) +*Trichoderma viridae* (*Cellulolytic*) + Zn solubilizing Bacteria. These two treatments were closely followed by the treatments T9 (2.5%), and T12 (2.1%). The total-N content increased with the enhancement of the incubation period up to 80 DAI. In all the treatments the highest Total -N was found in the treatment T11 at all the incubation periods i.e. at 20, 40, 60 and 80 DAI followed by T14, T9, T12, T13, T10 and lowest in T8.

Table 1.1: Periodic release of Total-N from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	1.9	2.1	2.9	3.0	3.1
T9	2.5	2.8	3.9	4.3	4.4
T10	2.0	2.2	3.1	3.4	3.6
T11	2.6	3.1	3.9	4.4	4.5
T12	2.1	2.4	3.1	3.4	3.6
T13	2.0	2.3	3.1	3.7	3.9
T14	2.6	3.0	3.8	4.2	4.3

Data presented in Fig 1.1 shows per cent increase in total-N over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.1 it is evident that per cent release was ranged from 7.4 % to 15% over initial value up to 20 DAI. The maximum rate of percent release was from 20 to 40 DAI and the range was 40.6% to 56% and further rate of percent release of total -N was decreased from 40 DAI to 80 DAI. The maximum percent release over initial value was up to 95% in case of T3 and minimum was in T14 (59.3%). The results clearly emphasized that the release was bit rapid up to 40 DAI and it slowed down after 40 DAI.

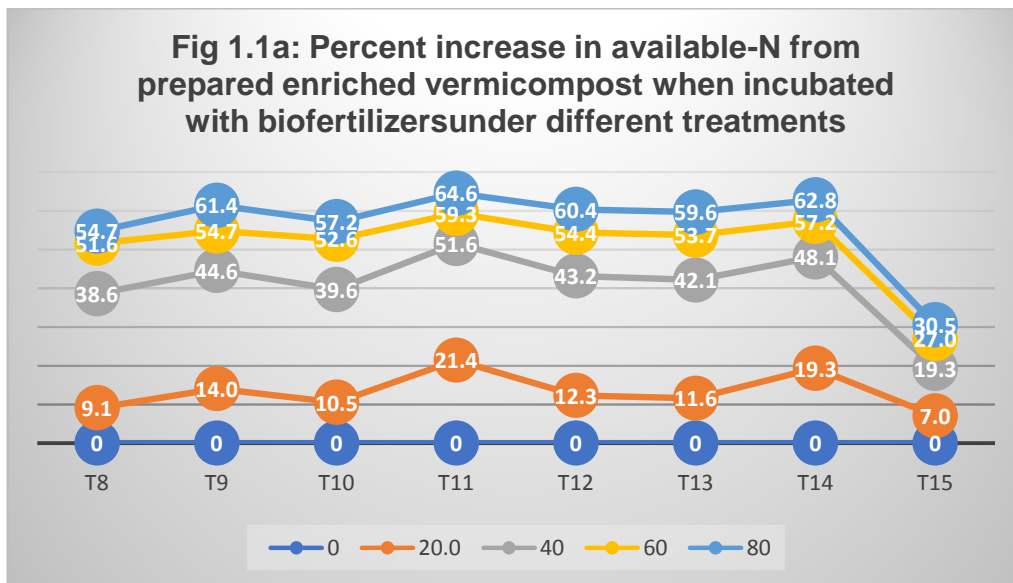


Data presented in Fig 1.1a shows per cent increase in available-N (Kgha^{-1}) over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig1.1a it is evident that per cent release was ranged from 7.0% to 21.4% over initial value up to 20 DAI. The maximum rate of percent release was from 20 to 40 DAI and the range was 19.3% to 51.6% and further rate of percent release of total-N was decreased from 40 DAI to 80 DAI. The maximum percent release over initial value was up to 64% in case of T11 and minimum was in T15 (30.5%). The results clearly emphasized that the release was bit rapid up to 40 DAI and it slowed down after 40 DAI. The results revealed that the application of biofertilizers to soil along with enriched vermicompost enhanced nitrogen availability considerably and the release pattern collaborate with the crop demand at different growth periods.

Table 1.2: Periodic release of available-N (kg ha^{-1}) from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	185	211	295	332	341
T9	185	225	312	341	360
T10	185	215	298	335	348

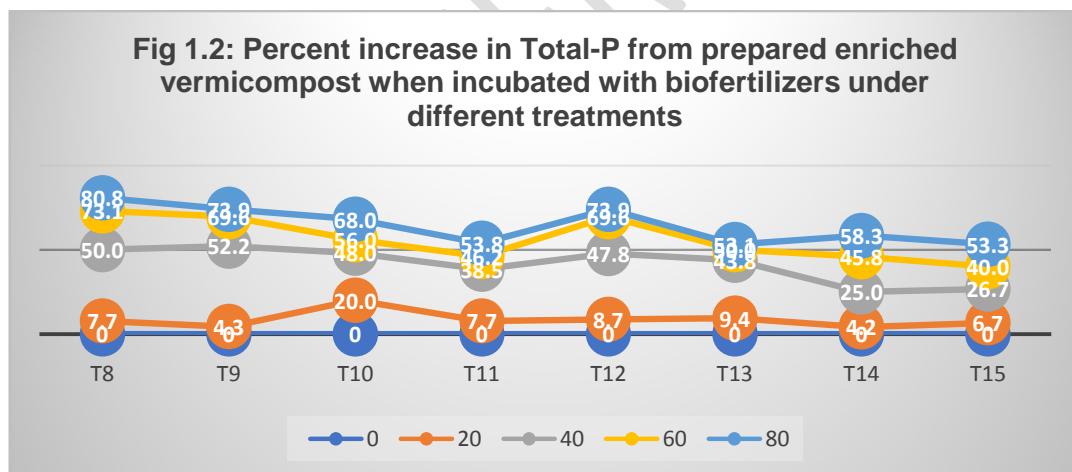
T11	185	246	332	354	369
T12	185	220	308	340	357
T13	185	218	305	338	355
T14	185	240	322	348	364



The data on periodic release of total-P₂O₅ from prepared enriched vermicompost when incubated with biofertilizers are presented in Table 1.2 and the data presented in Fig 1.2 shows per cent increase in total-P₂O₅ over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.2 it is evident that per cent release of total P₂O₅ over initial total P₂O₅ was ranged from 4.2 % to 20% up to 20 DAI. The maximum rate of percent release was from 20 to 40 DAI and the range was 25.0 % to 52.2% and further rate of percent release of total P₂O₅ was decreased from 40 DAI to 80 DAI. The maximum percent release over initial value was up to 80.8% in case of T8 and minimum was in T15 (53.3%). The results clearly emphasized that the release was bit rapid up to 40 DAI and it slowed down after 40 DAI.

Table 1.3: Periodic release of Total-P₂O₅ from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	2.6	2.8	3.9	4.5	4.7
T9	2.3	2.4	3.5	3.9	4
T10	2.5	3	3.7	3.9	4.2
T11	2.6	2.8	3.6	3.8	4.0
T12	2.3	2.5	3.4	3.9	4
T13	3.2	3.5	4.6	4.8	4.9
T14	2.4	2.5	3	3.5	3.8
T15	1.5	1.6	1.9	2.1	2.3

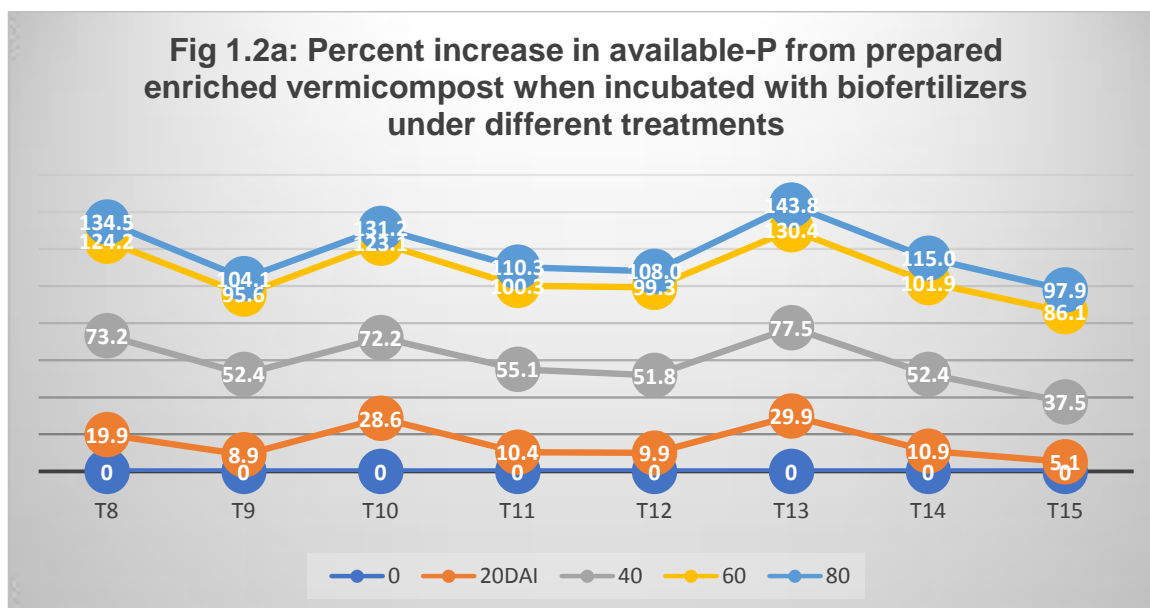


The data on periodic release of available-P from prepared enriched vermicompost when incubated with biofertilizers are presented in Table 1.4 and the data presented in Fig 1.2a shows per cent increase in available-P over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.2a it is evident that per cent release of total P₂O₅ over initial total P₂O₅ was ranged from 5.1% to 28.6% up to 20 DAI. The maximum rate of percent release was from 20 to 60 DAI and the range was 37.5 % to 77.5% at 40 DAI and

86.1% to 130.4% up to 80 DAI. The rate of percent release of total P₂O₅ was decreased from 60 DAI to 60 DAI. The maximum percent release over initial value was up to 143.8% in case of T13 and minimum was in T15 (97.9%). The results clearly emphasized that the release was bit rapid up to 60 DAI and it slowed down after 60 DAI. The nutrient status (carbon and nitrogen contents) of enriched organic manures might have supported the better growth of FNF, PSB and Trichoderma sp. Kumar and Singh (2001) reported that the population of FNF and PSB were highest in FNF, PSB and rock phosphate enriched vermicompost. The nutrient status of enriched organic manures have supported the better establishment of population of PSB and FNF in enriched vermicompost. The population of inoculated organisms (PSM and FNF) were highest in microbially enriched organic manures due to microbial and rock phosphate enrichment as reported by Rao et al. (2012).

Table 1.4: Periodic release of Available-P (kg ha⁻¹) from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	10.51	12.6	18.2	23.56	24.65
T9	10.51	11.45	16.02	20.56	21.45
T10	10.51	13.52	18.1	23.45	24.3
T11	10.51	11.6	16.3	21.05	22.1
T12	10.51	11.55	15.95	20.95	21.86
T13	10.51	13.65	18.66	24.21	25.62
T14	10.51	11.66	16.02	21.22	22.6
T15	10.51	11.05	14.45	19.56	20.8

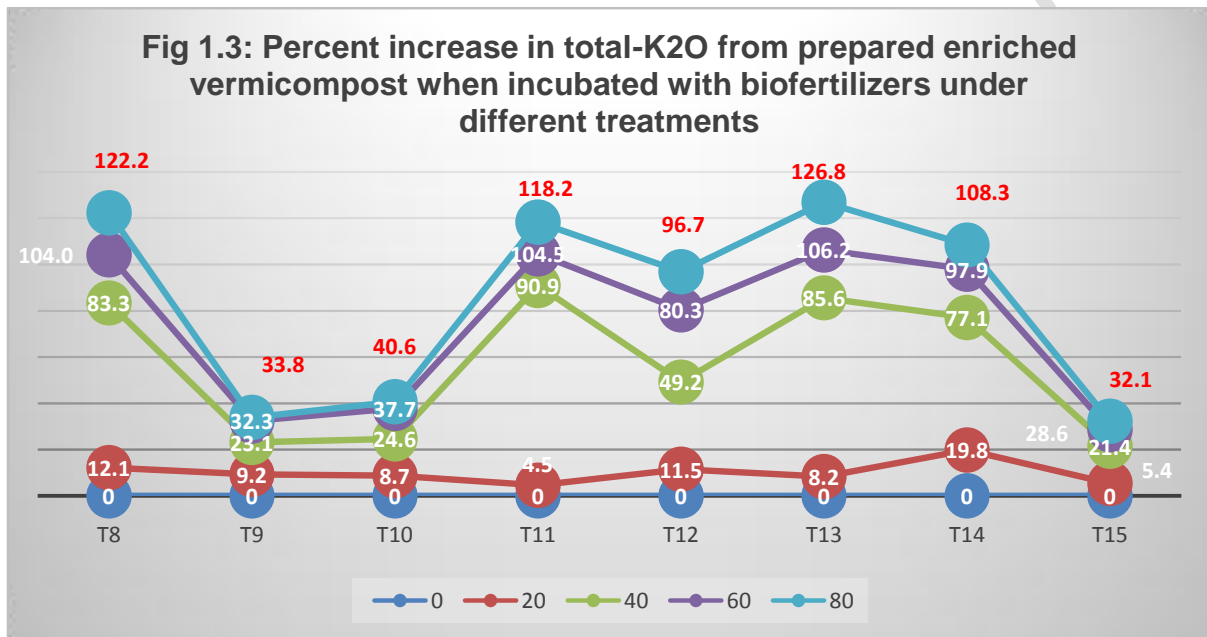


The data on periodic release of Total-K₂O from prepared enriched vermicompost when incubated with biofertilizers are presented in Table 1.5 and the data presented in Fig 1.3 shows per cent increase in total-K₂O over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.3 it is evident that per cent release of total K₂O over initial total K₂O was ranged from 5.4% to 19.8% up to 20 DAI. The maximum rate of percent release was from 20 to 40 DAI and the range was 21.4 % to 90.9 % at 40 DAI. The rate of percent release of total-K₂O was decreased from 40 DAI to 80 DAI. The maximum percent release over initial value was up to 126.8% in case of T13 and minimum was in T15 (32.1%). The results clearly emphasized that the release was bit rapid up to 40 DAI and it slowed down after 40 DAI. Addition of enriched vermicompost along with K solubilizing bacteria (*bacillus firmus*) helped to release considerable amount of K during different incubation periods.

Table 1.5: Periodic release of Total-K₂O (%) from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	1.98	2.21	3.63	4.04	4.4
T9	0.65	0.67	0.80	0.86	0.89
T10	0.69	0.72	0.86	0.95	1

T11	2.2	2.3	4.2	4.5	4.8
T12	0.61	0.71	0.91	1.1	1.2
T13	1.94	2.1	3.6	4	4.4
T14	0.96	1.2	1.8	2.1	2.3
T15	0.64	0.65	0.7	0.71	0.76

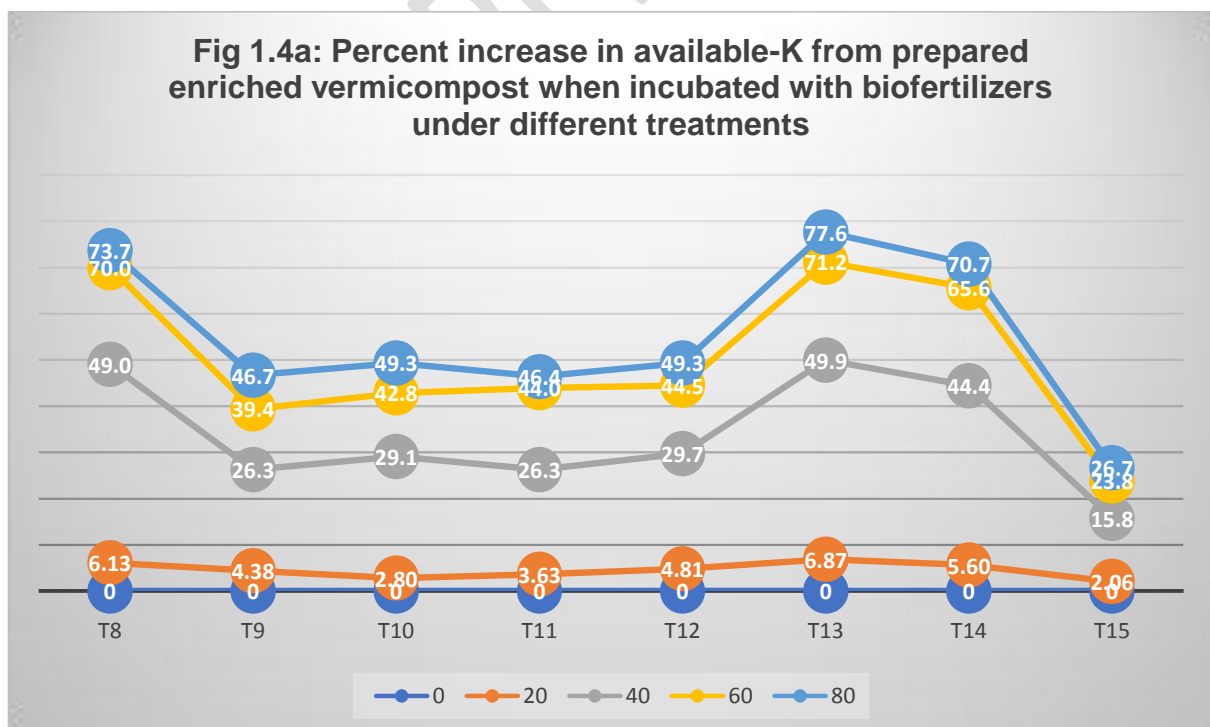


The data on periodic release of available-K from prepared enriched vermicompost when incubated with biofertilizers are presented in Table 1.6 and the data presented in Fig 1.4a shows per cent increase in available-K over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.4a it is evident that per cent release of available-K over initial available-K was ranged from 2.06% to 6.87% up to 20 DAI. The maximum rate of percent release was from 20 to 40 DAI and the range was 15.8 % to 49.9% at 40 DAI. The rate of percent release of available-K was decreased from 40 DAI to 80 DAI. The maximum percent release over initial value was up to 77.6% in case of T13 and minimum was in T15 (26.7%). The results clearly emphasized that the release was bit rapid up to 40 DAI and it slowed down after 40 DAI. Addition of enriched vermicompost along with K solubilizing bacteria (*Bacillus firmus*) helped to release considerable amount of available-K during different incubation periods it shows the

release of K is sufficient to meet out the crop requirement of K at different growth stages of crops.

Table 1.6: Periodic release of available-K (kg ha^{-1}) from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	228.5	242.5	340.5	388.4	396.8
T9	228.5	238.5	288.5	318.5	335.2
T10	228.5	234.9	295	326.4	341.2
T11	228.5	236.8	288.6	329	334.5
T12	228.5	239.5	296.4	330.2	341.2
T13	228.5	244.2	342.6	391.1	405.9
T14	228.5	241.3	329.9	378.4	390.1
T15	228.5	233.2	264.5	282.9	289.5

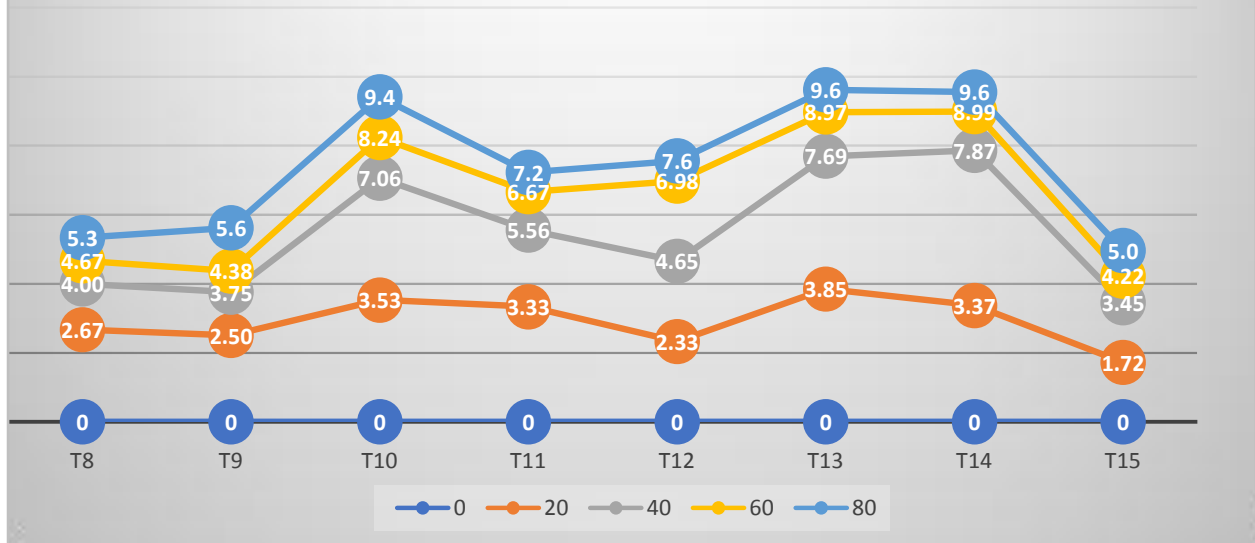


The data on periodic release of Total-Zn from prepared enriched vermicompost when incubated with biofertilizers are presented in Table 1.7 and the data presented in Fig 1.5 shows per cent increase in total-Zn over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.5 it is evident that per cent release of total-Zn over initial total-ZN was ranged from 1.72% to 3.53% up to 20 DAI. The maximum rate of percent release was from 20 to 40 DAI and the range was 3.45 % to 7.87% at 40 DAI. The rate of percent release of total Zn was decreased from 40 DAI to 80 DAI. The maximum percent release over initial value was up to 9.6% in case of T13 and T14. Minimum was in T15 (5.0%). The results clearly emphasized that the release was bit rapid up to 40 DAI and it slowed down after 40 DAI. Addition of enriched vermicompost along with Zn solubilizing bacteria helped to release considerable amount of Zn during different incubation periods.

Table 1.7: Periodic release of Total-Zn (ppm) from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	75	77	78	78.5	79
T9	80	82	83	83.5	84.5
T10	85	88	91	92	93
T11	90	93	96	96	96.5
T12	86	88	90	92	92.5
T13	78	81	84	85	85.5
T14	89	92	96	97	97.5
T15	58	59	60	60.45	60.88

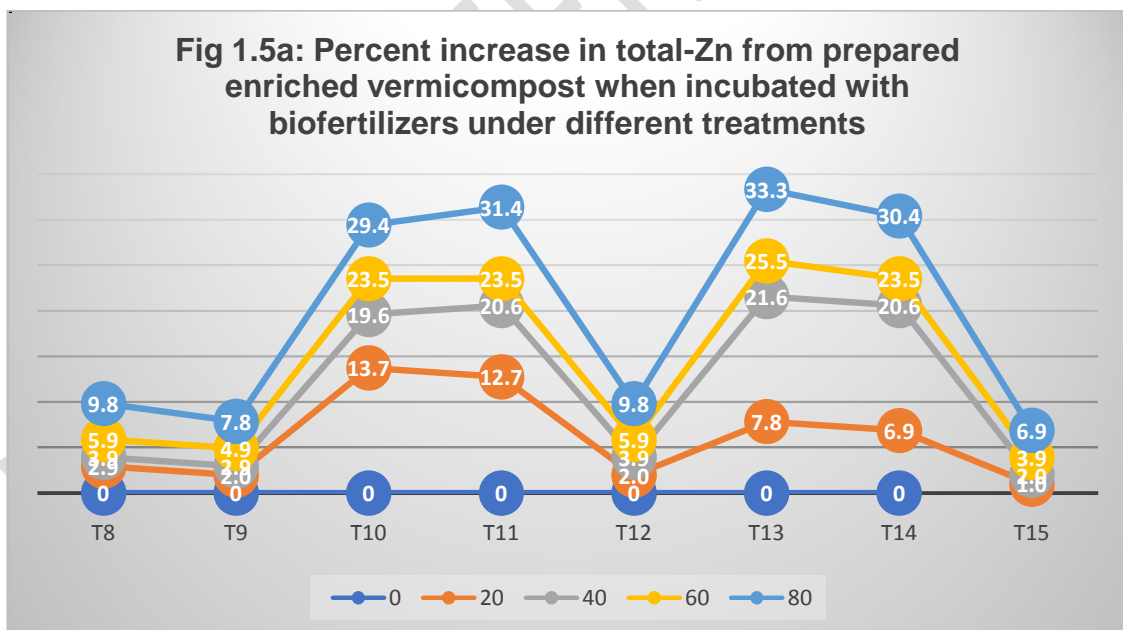
Fig 1.5: Percent increase in total-Zn from prepared enriched vermicompost when incubated with biofertilizers under different treatments



The data on periodic release of available-Zn from prepared enriched vermicompost when incubated with biofertilizers are presented in Table 1.8 and the data presented in Fig 1.5a shows per cent increase in available-Zn over initial value (i.e. at 0 DAI) of enriched vermicompost when incubated with biofertilizers under different treatments. From the Fig 1.5a it is evident that per cent release of available-Zn over initial available-Zn was ranged from 1.0% to 13.7% up to 20 DAI. The rate of release of available -Zn was higher in the treatments T10, T11, T13 and T14 in these treatments Zn solubilizing bacteria was added along with enriched vermicompost. The maximum percent release over initial value was up to 33.3% in case of T13 and minimum was in T15 (6.9%). The results clearly emphasized that the release in the treatments where we applied Zn soluble bacteria in these treatments available-Zn changed from deficient level (0.51ppm) to sufficiency level i.e. > 0.6 ppm (treatments T10, T11, T13 and T14) Table 1.8. Thus results revealed that by the application of enriched vermicompost along with Zn solubilizing bacteria can mitigate the Zn deficiency in soils.

Table 1.8: Periodic release of available-Zn (ppm) from prepared enriched vermicompost when incubated with biofertilizers:

Treatments	Days after Incubation				
	0	20	40	60	80
T8	0.51	0.525	0.53	0.54	0.56
T9	0.51	0.52	0.525	0.535	0.55
T10	0.51	0.58	0.61	0.63	0.66
T11	0.51	0.575	0.615	0.63	0.67
T12	0.51	0.52	0.53	0.54	0.56
T13	0.51	0.55	0.62	0.64	0.68
T14	0.51	0.545	0.615	0.63	0.665
T15	0.51	0.515	0.52	0.53	0.545



In vermicompost increment in nutrient content N (22.35%), P (15.60%) and K (17.74%) and in BSS increment in nutrient content N (20.29%), P (30.00%) and K (13.04%) was observed. Addition of rock phosphate inoculated with *P. striata* led to

more availability of P, most likely due to the production of organic acids by the bacteria which might have solubilized the rock phosphate (Kumar and Singh, 2001). The enrichment of vermicompost with PSM and FNF significantly increased N, P and K contents (Padmavathiamma et al., 2008). The results of this trial clearly brought out maximum increase in the nutrient concentration of inoculated organisms on 80th day in among all enriched vermicompost treatments. Since the organisms inoculated are heterotrophs, their activities in organic manures leads to conversion of unavailable form to available form of nutrients (mineralization). The Highest available Nitrogen 369 kg/ha^{-1} observed in T11, The highest available phosphorus 25.62 kg/ha^{-1} observed in T13, The highest available potassium 405.9 kg/ha^{-1} observed in T13, and also observed available Zinc 0.68 ppm in T13. So, the enrichment of manures with beneficial micro flora contributed to significant improvement in nutrient concentration of all the organic manures. Among the inoculated organisms, Azospirillum is known to fix atmospheric N under free living condition. Phosphate solubilizing bacteria (PSB) are known to release P from bound phosphate source. Similarly enhancement of K may be due to mineralization. All these biogeochemical activities are very well established.

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