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ROLE OF MICRO ORGANISMS IN TEMPLE WASTES MANAGEMENT ASSISTED BY *EUDRILLUS EUGENIA*

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ABSTRACT The increasing solid waste generation rates and their high collection cost were some of the major problems in India. This paper deals with the effective management of Temple wastes. The temple waste consists of coconut, leaves, flowers, fruits etc which are of biodegradable in nature.It also includes milk products, grains and water, which could facilitate the growth of microorganisms. Such biodegradable temple wastes are released as such into the water bodies (or) dumped haphazardly which causes environmental pollution. Recycling of such temple waste is performed through vermitechnology employing *Eisenia fetida*. Three types of waste were prepared. They were as follows: T_A (control), T_B (Temple waste+ Cow dung), T_C (Temple waste+ Cowdung+ Microbial inoculum) in different proportions in the 1 kg capacity Reactor vessels. The physic chemical parameters like pH, Electrical Conductivity, Total Kjeldahl nitrogen, Total Organic Carbon, Total Organic Matter and Carbon-Nitrogen ratio and phosphorus were tested in the vermicompost. Bacterial strains isolated from vermicompost and identified and are used as an inoculum to check whether they could enhance the rate of degradation. The impact of the prepared vermicompost on chick pea seed germination was also studied. These studies showed a significant growth in terms of length and height of the plant with T_C vermicompost when compared to T_A and T_B vermicompost. Thus, it is concluded that the vermicomposting supplemented with the bacterial cultures is comparatively efficient technology for the effective management of the temple waste in an eco friendly manner.

Keywords: **Vermicompost, Temple waste, *Eisenia fetida*, Chick pea.**

INTRODUCTION

Solid waste includes items that are discarded by the public, and is now a growing problem at global, regional and local levels. In Indian cities, the average solid waste generation is nearly about 1.6 million tonnes per day, (http://panchabuta.com/2012/10/02/india-green-it-spendings-to-reach-70bn-by-2015-gartner/). The problem is more acute in developing nations than in the developed nations (Sudhanshu kaushik and Bishambhar Datt Joshi, 2001). So, we are in need of an useful and an effective technology for the waste management. Vermicomposting offers rapid recovery of valuable resources from biodegradable waste by producing humic like substance called vermicompost. (Pramanik et al., 2007). The waste material is decomposed by the earthworm and their gut associated microbes. These earthworms also have great capacity in assimilating heavy metals present in solid waste as pollutants (Azizi et al., 2013) .Many latest studies are available regarding the potential of earthworms for vermicomposting of solid waste (Appelholf et al., 1998; Yan et al., 2013). During vermicomposting the soil micro flora such as bacteria, fungi, yeast and actinomycetes plays a very important role directly or indirectly. In addition, the composted materials have a clear impact on soil biological properties, such as increase in microbial biomass, soil enzymes, and soil structure (Hossein et al., 2014;Ros et al., 2006) .Several examples in the literature show that compost and vermicompost are able to enhance the growth of a wide range of plant species by the supply of nutrients (Grigatti et al,, 2007). Vermicomposting not only provides nutritional elements but also shows insecticidal activity and also they were proved to contains plant growth stimulating hormones (Tomati et al., 1983). So this technology is considered as one of the best method for managing the waste generation . Solid wastes of varied composition are seen in and around cities and also at sacred places like temples. Comparing other types of solid waste, large amount

of waste are left over by pilgrims (Gurav and pathade,2011). This causes several issues such as environmental pollution and also serves as source of inoculums for many diseases (Blackman, 1995). Astemple waste contains nutritive rich material it may act as a best raw material for the vermicomposting. In the present study, attempts were made for the conversion and utilization of temple waste in an eco friendly manner

MATERIALS AND METHODS

Collection of the wastes

Cow dung was collected from dairy yard at the Agriculture field near the Bharathidasan University campus, Trichy. Tamilnadu, India. Temple wastes were collected from Malaikottai temple, Trichy Tamilnadu, India and the Epigeic (or) surface dwelling earthworms ***Eudrillus eugeniae*** was obtained from Tamilnadu Agricultural and Research University, Madurai, Tamilnadu, India.

Preparation of experimental media

Three type of experimental media were prepared as follows. T_A contains only cow dung and temple waste which is considered as control, T_B contains cow dung, temple waste and earthworms and, T_C contains cow dung, temple waste, earthworms and microbial inoculum. These feed mixtures were subjected for Vermicomposting in reactor vessels with the following dimensions (diameter- 40 cm, depth -9cm). The mixtures were turned over manually for 15 days in for acclimatization process. Further, 30 non-ciliated earthworms were introduced into each reactor vessels. Also 50 ml of microbial Inoculum was added to the T_C media. The moisture content was maintained at 60-80% throughout the study period by periodically sprinkling of adequate quantities of water.

Physico chemical parameter Analysis

Physicochemical analysis was performed by collecting the sample once in a week. The physico chemical parameters like Total Kjeldahl Nitrogen is analysed (Singh and pradhan, 1987) method Total Organic Carbon (Walkely and Black 1934), Total Organic Matter and Carbon-Nitrogen ratio and pH, Electrical Conductivity, Phosphorus were analyzed (Tandon ,2009)

Enumeration and isolation of microorganism

The bacterial population of the vermicompost was estimated by serial dilution technique (Travors and cook, 1992). Identification of the bacterial strains was performed by conducting Biochemical tests like gram staining, indole production test, methyl red test, voges proskauer test, triple sugar iron test, catalase test, oxidase test, citrate production test, urease test.(Benson ,1990).

Seed germination

The chick pea seeds were selected for the seed germination studies. Five seeds were selected and surface sterilized with 0.1% mercuric chloride for 1 to 2 min followed by repeated rinsing with distilled water. The percentage of germination was calculated using the following formula.

Germination % = Number of seeds germinated / total number of seeds X100

Statistical Analysis

One-way analysis of variance (ANOVA) was done to determine any significant difference among the parameters analyzed, at 0.05% level of significance. (SAS Procedures Guide, 1990)

RESULTS AND DISCUSSION

The physico-chemical parameters were analysed from the prepared vermicompost and it is figuratively represented in Figures 1-7.

pH

The pH range of 6.0-8.5 is found to be suitable for the soil in order to ensure compatibility with most plants (Hogg et al., 2002). As evident from **Fig.1**, there were changes in the pH throughout the vermicomposting process. The pH was in the range between 6 to 8 in all the 3 different types. The pH shift has been reported due to mineralization of nitrogen and phosphorus into nitrites/ nitrates and orthophosphate (Ndegwa et al., 2000)

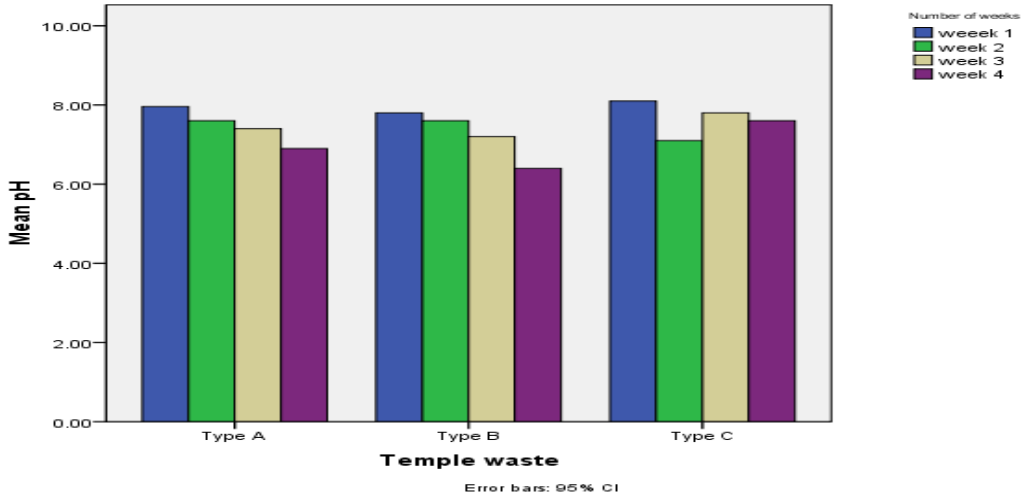

Figure 1. Comparison Chart Of Ph Different Types Of Vermicompost

Electrical Conductivity

At the initial stage, the EC values were in the range of 12.1±0.002, 11.3±0.003, 10.12±0.003 in 3 different types. It has increased significantly in final vermicompost and was in the range of 19.15±0.06, 19.1±0.035, 17.7±0.12. (**Fig. 2**.) The increase in EC may be due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium, etc (Kaviraj and Sharma, 2003)

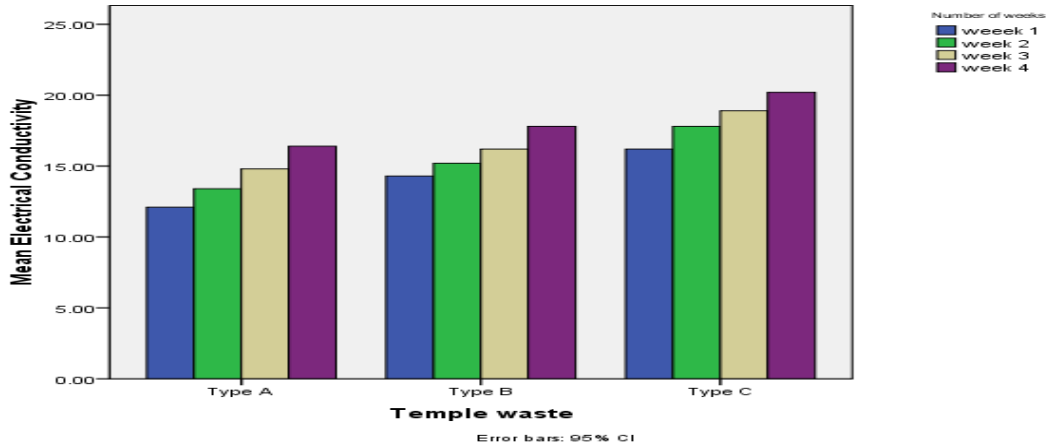

Figure 2. Comparison Chart Of Electrical Conductivity Different Types Of Vermicompost

Total organic Carbon

Total organic carbon (TOC) in all 3 different ratios of temple waste has reduced when compared to their initial levels. (Kaushik and Garg, 2003) reported 20-45% loss of TOC in the form of CO_2 from different industrial sludge's during vermicomposting. In temple waste the TOC reduction was in range of 21.04±0.01 to 16.66±0.06 in T_A, 21.5±0.06 to 17.2±0.06 in $T_B$and 21.4±0.06

to 14.4±0.06 in T_C(**Fig.3**). It is well known that the earth worms modify the substrate condition which consequently promotes the carbon losses from the substrate through microbial respiration in the form of CO_2 and even through mineralization of organic matter and hence leading to the reduction in the TOC content has been noticed.

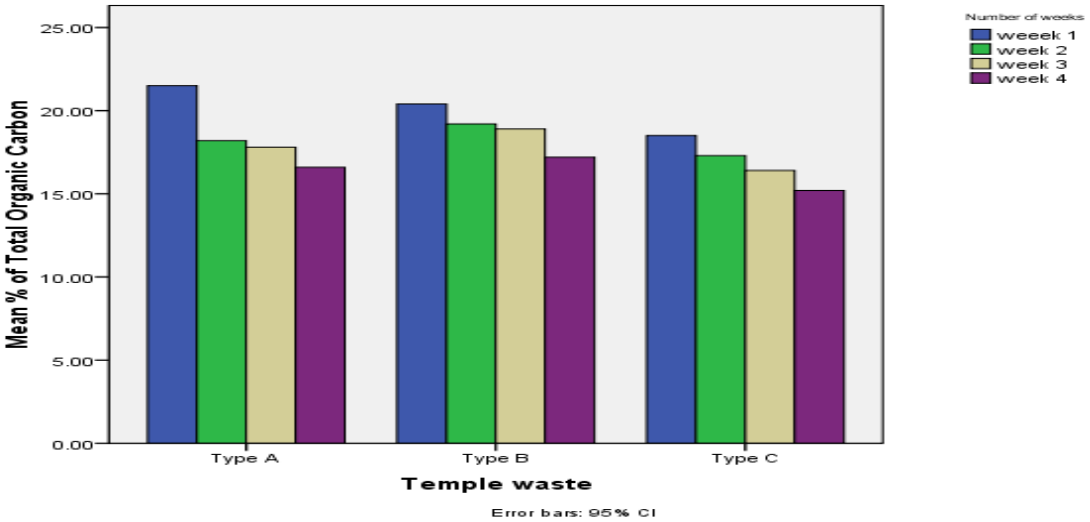

Figure 3. Comparison Chart Of Toc Of Different Types Of Vermicompost

Total Kjeldahl Nitrogen

The Total Kjeldahl nitrogen content has increased by 57.6%, 65% and 73.9% in all the three different types of treatment (**Fig 4**). Losses of organic carbon might be responsible for nitrogen addition in the form of mucus nitrogenous excretory substances, growth stimulatory hormones and enzymes from the gut of the earth worms (Viel et al., 1987)

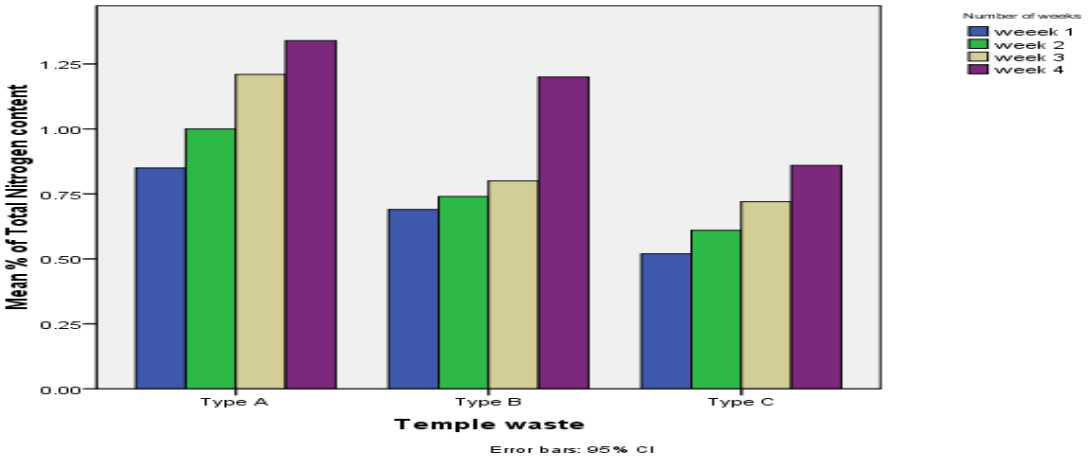

Figure 4. Comparison Chart Of Tkn Of Different Types Of Vermicompost

C: N ratio

C: N ratio is one of the most widely used indicators to measure the vermicompost maturation, which is reported to decrease sharply during vermicomposting process (Suther, 2008). During the temple waste degradation process, the reduction in C: N was in the range of 49.2% inT_A, 53.04% in T_B and 61.3% in T_C (**Fig 5**). According to (Sensei,1989)the decline in C: N ratio <40% indicates an advance degree of organic matter stabilization and reflect a satisfactory degree of organic wastes degradation

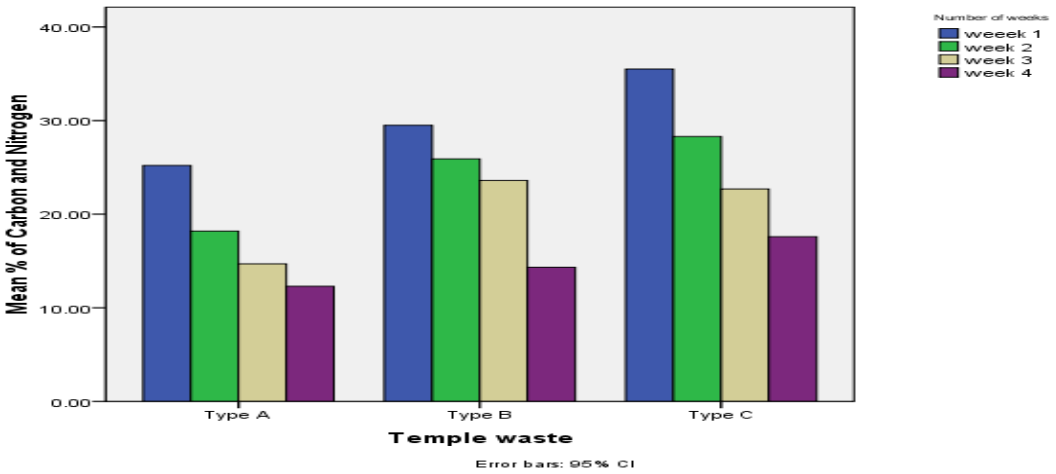

Figure 5. Comparison Chart Of C:N Ratios Of Different Types Of Vermicompost

Phosphorus

Total phosphorus (TP) was greater in final vermicompost than in the initial feed mixture. In this study **Fig 6** shows the increase of 34%, 35%, 37% in TP in all three types. This indicates that the degradation activity is performed by the phosphate solublizing microbes present in earthworm gut. (Satchell and Martin, 1984).

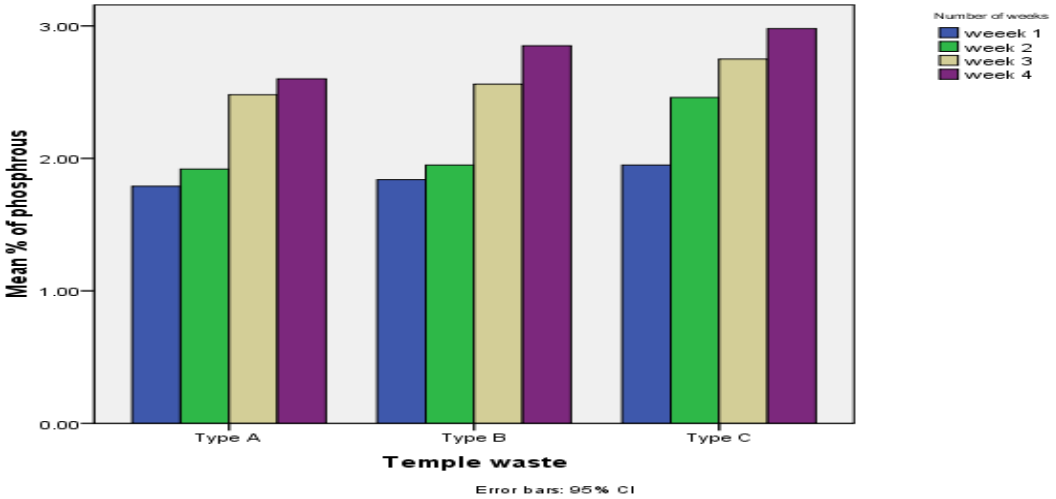

Figure 6. Comparison Chart Of Phosphrous Different Types Of Vermicompost

Total Organic Matter

Many types of organic wastes were found to be readily decomposed by soil microbes. The main product of decomposition of organic matter is carbon dioxide and water (Kaushik and Garg, 2003). Thus the decomposition of organic matter reduces the amount of TOM but leaves the compost enriched with nitrogen. The reductions in TOM were in the range of 39%, 40%, and 59% (**Fig 7**) with respect to all the three different types of temple waste.

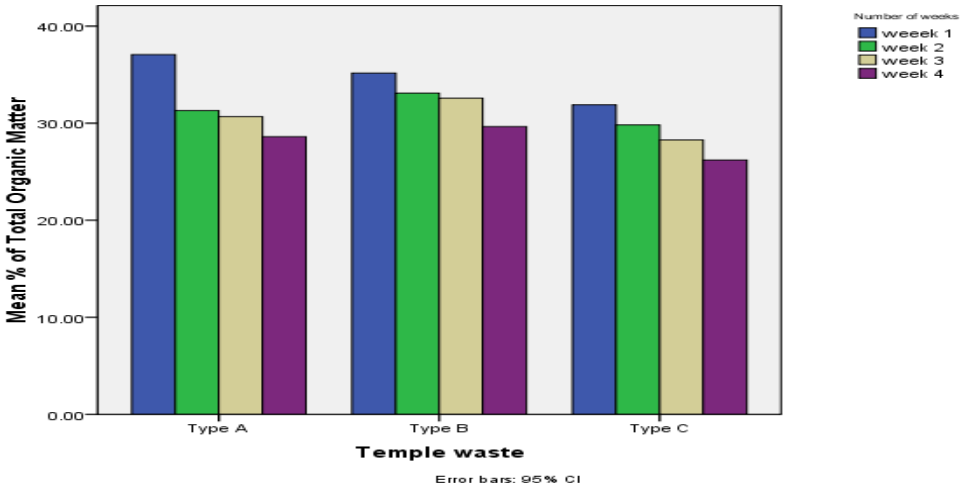

Figure 7. Comparison Chart Of Tom Of Different Types Of Vermicompost

Statistical analysis

Table 1 represent the statistical data for the physico chemical parameters. The results were statistically analyzed at 0.05 levels using one way analysis of variance (ANOVA) using SPSS version 16.0. In the results since the p value is <0.05, the variance between the three different types is significant. Therefore it is concluded that the samples mean of $T_{A,}$ T_B and T_C are different from each other significantly.

Table 1. Statistical representation of Physicochemical Analysis of Vermicompost using One way ANOVA

S.No	Parameters	F-Value	P-value	Df	Interpetation
1.	TOC	2.209	0.166	2,9	Not Significant
2.	TKN	4.384	0.097	2,9	Significant
3.	C:N	1.677	0.24	2,9	Not significant
4.	TOM	2.224	0.164	2,9	Not Significant
5.	pH	1.856	0.211	2,9	Not significant
6.	EC	5.962	0.12	2,9	Significant
7.	Phosphorous	0.605	0.567	2,9	Not significant
8.	Moisture	1.856	0.211	2,9	Not significant

The significance of variance, ie., p-value, given under the head sig. is 0.000. Since the significant value is less than 0.05 (p<0.05), the variance between the mean value are not significantly different. This reflects the stabilization of the compost. But in cause of TKN and EC the varicance is significantly different.

Isolation and identification of micro organisms

During Vermicomposting, microorganisms are considered as the primary decomposers. These microorganisms include bacteria, fungi, actinomycetes and protozoa. They break up waste debris and facilitate the decomposition (Haritha et al., 2009). In our study with respect to **Table 2** the microbial activity was found to be higher in the initial stages THTC (Too High To Count) due to enrichment of nutrient, and subsequently reduced during the final stages due to the degradation of the waste.

Table 2. Total Bacterial count of Vermicomposts

S.No	Compost	Total Heterotrophic bacterial population(CFU/ml)	
		Before	After
1.	T_A (control)	THTC	84±0.5X 10^{-4}
2.	T_B(temple waste+Cowdung)	THTC	106±0.5 X 10^{-4}
3.	T_C (temple waste+Cowdung + microbial inculation)	THTC	158±0.5 X 10^{-4}

This will affect the complete degradation of the waste. To overcome this we identified the microbes present in the vermicompost and re inoculate it manually into the vermin bin to enhance the degradation process. **Table 3** shows the Biochemical Characterization of the isolated microorganism. The bacterial genus identified from vermicompost is *Shigella, Proteus, Salmonella* and *Citrobacter*. The isolated organisms were grown as a consortium in 50ml nutrient broth and then the culture was inoculated into the T_C reactor vessel for enhancement purpose. Plates 1 and 2 represent the waste before and after the vermicomposting of T_C waste.

Table 3. Identification of heterotrophic bacteria

S.No	Isolated colonies	Shape	Gram staining	Motility	Indole	MR	VP	TSI	Citrate	Oxidase	Catalase	Urease	Name of the genus
1.	S1	Rods	Gram negative	Non Motile	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	*Shigella*
2.	S2	Rods	Gram negative	Motile	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	*Proteus*
3.	S3	Cocci	Gram positive	Non Motile	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	*Micrococcus*
4.	S4	Rods	Gram negative	Motile	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	*Salmonella*
5.	S5	Cocci	Gram positive	Non Motile	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	*Micrococcus*
6.	S6	Rods	Gram negative	Motile	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*Citrobacter*

Initial
Temple waste

Final
Vermicompost

Plates 1 & 2 -Preparation of vermicompost during experiment

Seed germination

Seedling stage is the most sensitive stage in the life cycle of plant and hence it is susceptible to physical and chemical adversities (Copper et al., 1998). In the present germination studies, chick pea seeds were treated for 7 days with the three different types of vermicompost. The morphological growth parameters such as germination percentage and seedling length were taken into consideration. **Fig 10** represent the effect of vermicompost on seed germination. With reference to **Fig 9**, the highest germination percentage was observed in T_C (92%) compared to T_A (58%). which may be due to activities of microbes. Also the growth and length pattern of Shoot got increased in the plate where vermicompost is used.

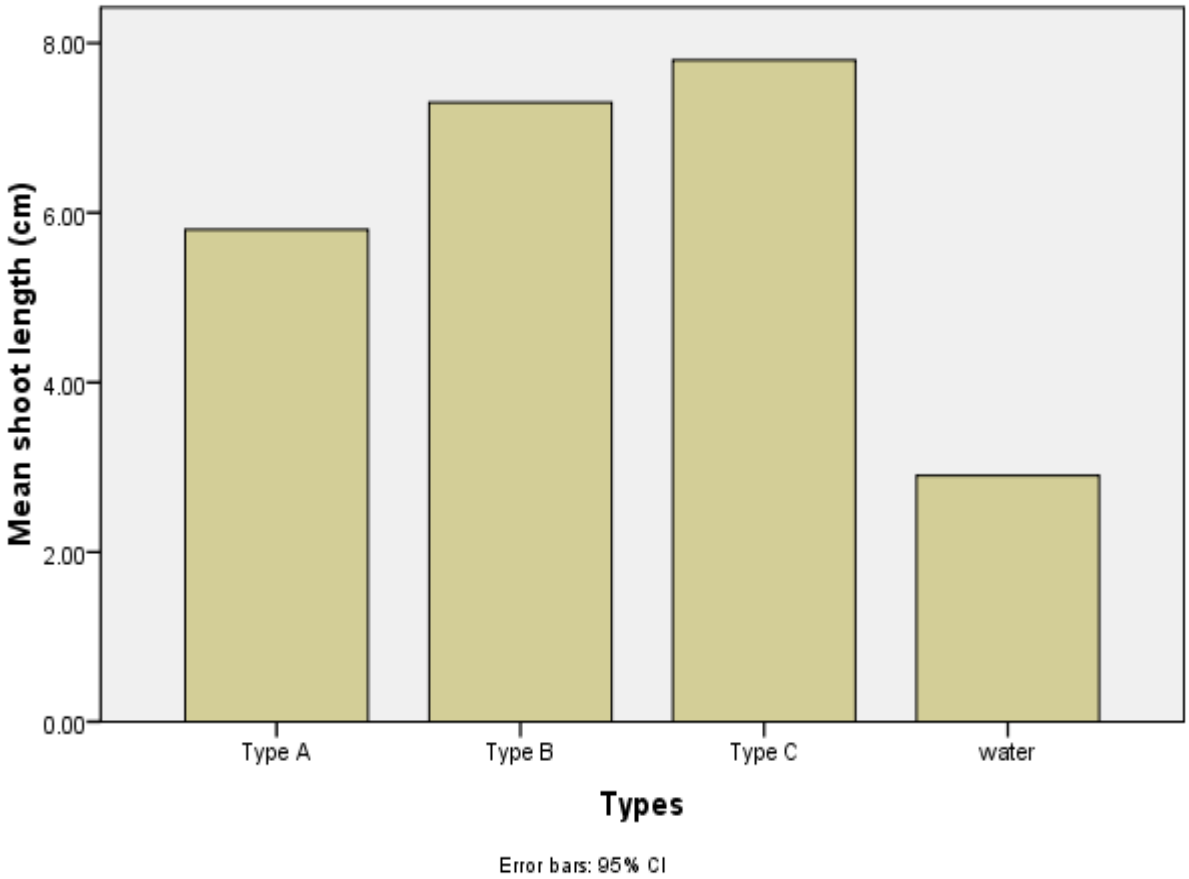

Figure 8. Effect of application of different types of vermicompost in shoot length of chick pea

As evident from **Fig 8** the hieghtest Shoot length was recorded in the T_C (7.8cm). The marked difference in the shoot length of chick pea may be due to the presence of growth promoting hormones which could be present in the vermicompost prepared by using temple waste along with cow dung and microbial inoculum. Similarly, Arancon et al., (2004) found that maximum benefit from vermicompost is obtained when it constitutes between 10 to 40% of the growing medium Neilson, (1965) and Tomati, (1988) have also reported that the vermicompost contains growth promoting hormone 'auxins', 'cytokinins' and flowering hormone 'gibberellins' secreted by earthworms. Similarly good growth of three flowering plants such as *Mirabilis jalapa, Calendula officinalis, Clitoria ternatea* were reported by Bhat and Limaye, (2012) when vermicompost was applied to the soil.

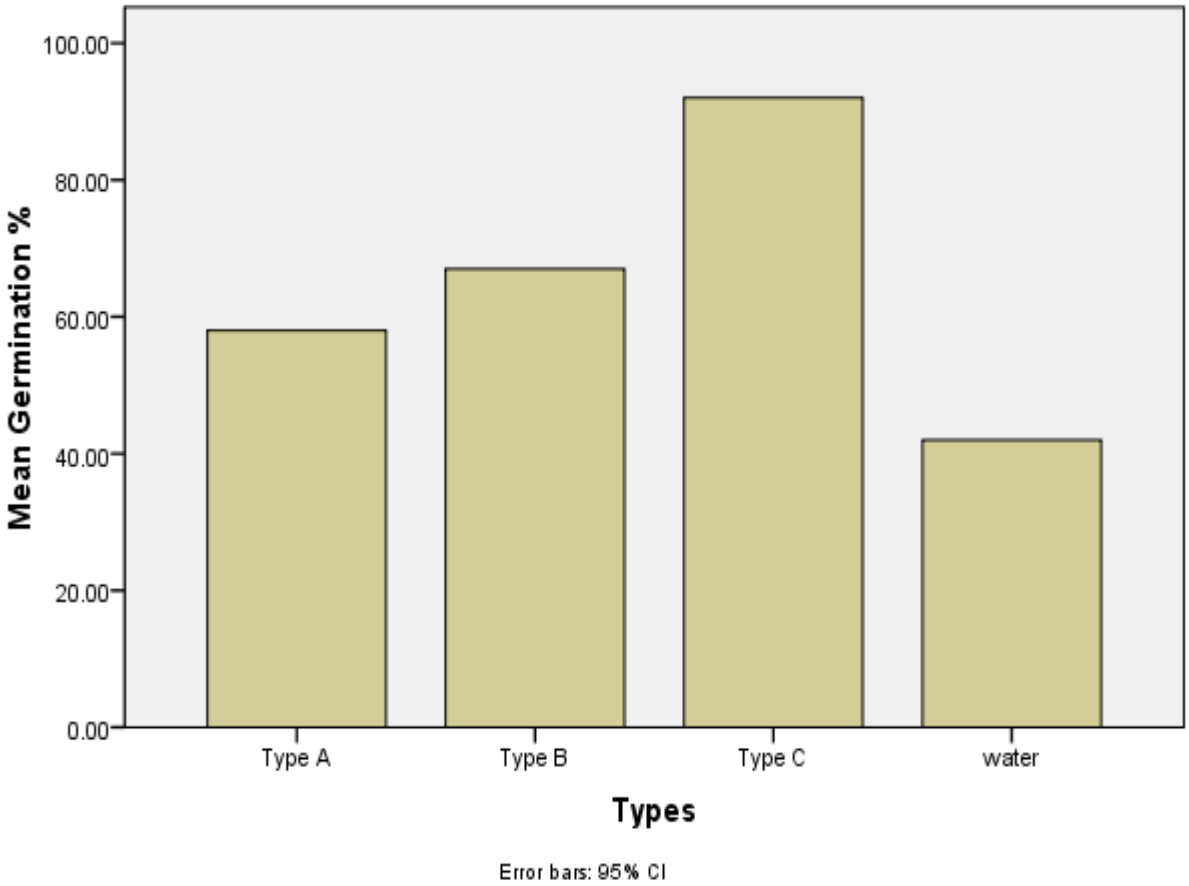

Figure 9. Effect of application of different types of vermicompost in percentage of seed germination of chick pea

CONCLUSION

In this study, the temple waste along with cow dung and microbial inoculums works as an excellent raw material for vermicomposting using *Eudrillus eugeniae*. This study concludes that the degradation was enhanced only after the addition of the microbial inoculum. This work proves that the major role of degradation was played by the microorganisms present in the vermicompost. Also the vermicompost prepared from temple waste act as a good fertilizer for the plantation, which were confirmed by seed germination studies. Hence this ecofriendly method of temple waste management could be extended for the temples to minimize the solid waste pollution. This method greatly help us to potentially convert the temple waste into value added product in a shorter time effectively.

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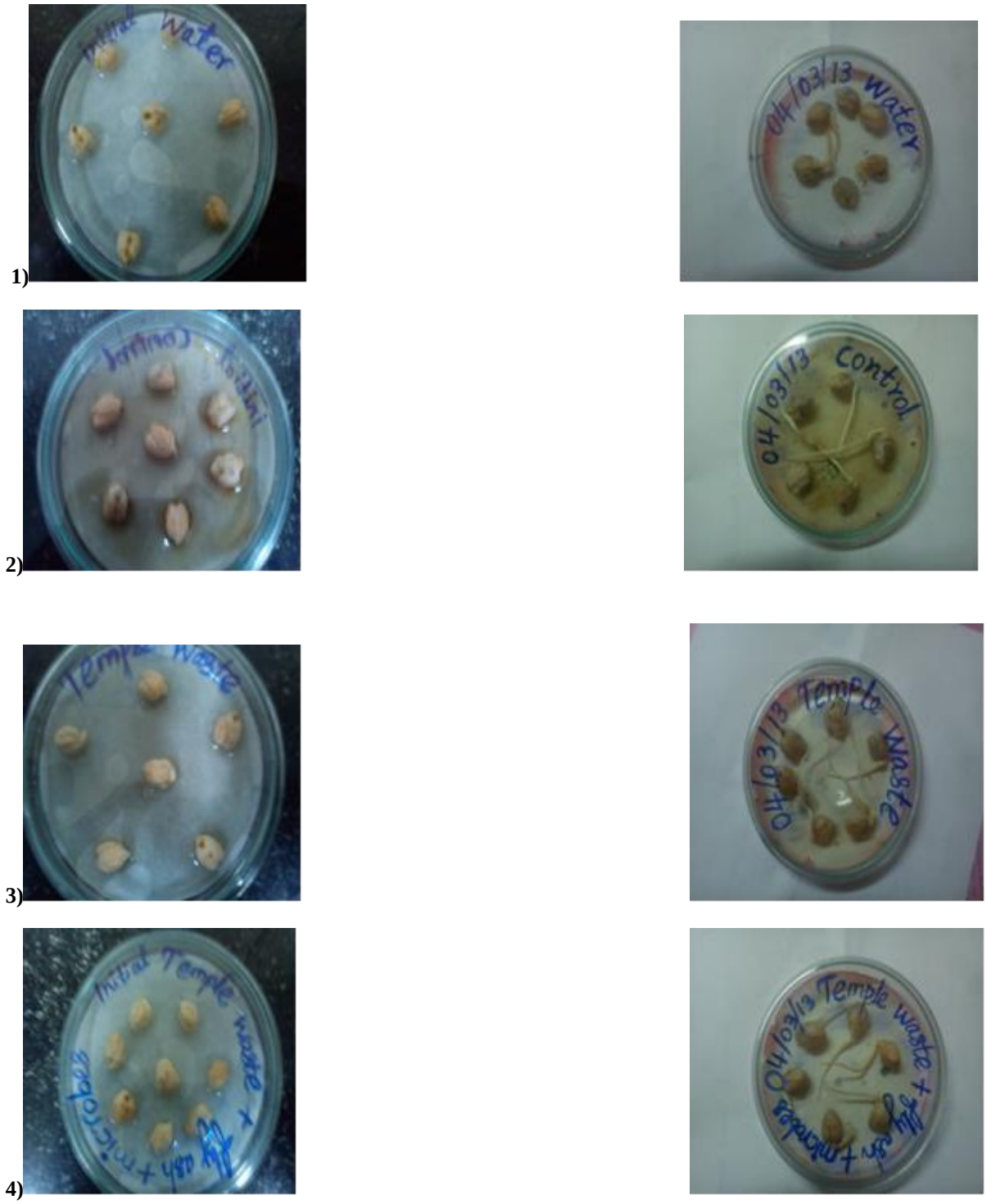

Figure 10. Effect of different types of vermicompost on seed germination and seedling length of chick pea

Plate 1-Water; Plate 2-Control; Plate 3: Temple Waste; Plate 4: Temple waste +Inoculum

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