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Microbial Conversion of Kitchen Waste for the Production of Bio-Organic Fertilizer and its Bio Efficacy on Crops

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ABSTRACT

Bio-organic fertilizers are produced by microorganisms through the conversion of complex organic material into simple compounds ultimately help the plants to grow by increasing the number of available nutrients. An abundant of kitchen waste is produced from the current modern society in Ethiopia and its clearance poses severe environmental and social problems in the country. In this regard, a study was conducted during the 2018-19 at phytoremediation experimental block of the Department of Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia. We isolated Kocuria rosea strain C9 from alfa alfa root nodules and were used to identify its potential for microbial conversion of kitchen waste. Azolla, cow dung, and kitchen waste were used as carrier materials. The physiochemical properties (pH, Moisture Content, Total Organic Carbon, C;N ratio, Total Nitrogen, and Temperature) were studied for attaining high microbial degradation for mass production of bio-organic fertilizer. Effectiveness of the bio-organic fertilizer were studied on the three plant species, namely Finger millet (Eleusine coracana), Field pea (Pisum sativum) and Lettuce (Lactuca sativa) in Completely Randomized Design (CRD) with thrice replicated with the following three treatments; T1 (Bio-organic fertilizer); T2 organic fertilizer (without bio-organic fertilizer as positive control) and T3 as the negative control (only the soil) The bio organic fertilizer formulated in the present study were applied on selected crops, upon screening the shoot length, plant dry weight, pods number, spikelet per plant, were measured. The majority of treatments significantly increased the yield related attributes and crops agronomic performance. The results obtained in this research indicated that this bio-organic formulation is promising and could be used as dual purpose strain, for mobilizing the soil nutrients and for achieving a safer and cleaner environment. Therefore, the current study endorses the use of recycled kitchen waste as a carrier material for the production of bio-organic fertilizer with KocurIa rosea strain C9.

Keywords: Bio-organic fertilizer, Kocuria rosea spp, finger millet, field pea, lettuce, recycled biomass.

INTRODUCTION

Environmental depravation is a considerable hazard alarming the world and the unrestrained use of chemical fertilizers bear the larger share that lead to soil health deterioration and ecological imbalance. The phenomenon like soil erosion, continuous crop cultivation, exhaustive crop residual deportation and high nutrient deficiency noticed in the country (Tadele et al., 2013). Agriculture comprises the huge fiscal sector in Ethiopia and shares 48% of the country's GDP, bring about 85% of the foreign exchange into the country and occupations about 83% of the total population of Ethiopia (Dorash et al, 2013). The cereal production and trade signify the single prevalent sub-stratum in the Ethiopian economy, which portrayed for roughly 60% of rural employment (Dorash et a, 2013). Cereals are the crucial food crops in Ethiopia and cover 82% of the total cultivable area covered by grain crops (Cereals, Pulses and Oil seeds) and backs 87% of the total grain production.

In order to meet the agricultural policies and strategies a lot has to be done by a way of finding an alternative to this chemical fertilizer which is the bio-organic-fertilizers. Bio-organic fertilizers have loomed as one of substitute for use of chemical inputs to replace the synthetic chemical fertilizers. Bio organic fertilizers utility in cropping system is preferable to chemical fertilizer, provides economic and ecological gains by improving of soil health and fertility of farm lands (Swapnil Rai and Nidhi Shukla, 2020). Field studies have established them for being low cost inputs that are effective and free from adverse implications to the environment. Bio-organic-fertilizers are endorsed through integrated plant nutrient management techniques that involved with combining in organic fertilizers, organic/green manures and bio fertilizers to boost yield, maintain soil productivity, soil health and soil microbial diversity (Rodrigue *et al*., 2004)

Bio-organic fertilizer is well-defined as an agricultural input that encompasses living microorganisms when applied to seed, plant surfaces, soil, will colonize and promote growth by escalating the supply and availability of primary nutrients to the host plant. (Vessey, 2003)

MATERIALS AND METHODS

The experiment was conducted during 2018-2019 at Addis Ababa Science and Technology University, Addis Ababa, Ethiopia located at 8° 98´ N, 38° 75´ E longitude and at an altitude of 2,355 m above the MSL. In this study, kitchen wastes were used as the chief material for composting together with cow dung and azolla. The kitchen waste was first shredded to reduce the particle size to 1-2 mm, which is the optimum value The shredded kitchen waste was mixed with cow dung and azolla to give appropriate combination of C/N ratios. All the resources were analyzed for their physiochemical properties. The composting process was carried out anaerobically in a shady area for 45 days in Addis Ababa Science and Technology University (AASTU), Addis Ababa. The study was conducted inphytoremediation block of AASTU.

Physico-chemical analysis of compost

Moisture content was estimated as weight reduction at the time of drying in hot air oven at 105 °C to a fixed weight (Lazcano et al. 2008). Total Nitrogen (TN) and Total Organic Carbon (TOC) were calculated as mentioned Pisa et al, 2020 For the evaluation of total N, samples were decomposed by concentrated H_2SO_4 and catalyst mixture in Kjeldahl flask and sequentially, N content in the digest was estimated following distillation with steam and titration protocols Total Organic Carbon was determined by was determined by combustion in a Perkin Elmer 2400 CHNS elemental analyzer. (Cajmarca et al., 2019).

Formulation of Bio-Organic Fertilizer

The carrier material (compost) was subjected to steam sterilization at 121°C for 15 minutes and dried for 12 hours at 50°C. After maintaining the growth condition, one loop full of *microbial strain* was inoculated in the Yeast Extract Mannitol Broth (YEMB) and maintained the growth condition. 40 mL of *microbial strain* were taken and mixed with 250 g of powder compost (carrier material) and the shelf life of the bio-inoculants was checked for the powder carrier by spread plate techniques. The microbial load was checked every two weeks for two months.

Evaluation of Bio-Organic Fertilizer in pot experiment

Efficacy of the Bio-organic fertilizer were tested on three plant species, namely finger millet (*Eleusine coracana*), field pea (*Pisum sativum*) and lettuce (*Lactuca sativa*) and the study was carried out under completely randomized design (CRD) with three replicates. The test was conducted on three treatments: experimental (Bio-organic fertilizer); organic fertilizer (without bio-organic fertilizer as positive control); and negative control (only soil). The experiment was carried out both in laboratory and greenhouse condition and results of the experiment were evaluated based on the agronomic and yield potentials. During the experiment, biometric observations was recorded for plant height, shoot length, pod number, seed number, dry mass and biomass weight for each experiment. All the agronomic characters were calculated on ten representative single plants. A total of ten randomly chosen, completely developed plants were taken to measure the plant height, shoot length, number of pods, dry mass and fully matured, good seeds were used for the measurement for the number of seeds, and 100 seed weight in triplicate measurements and averaged. After the agronomic traits were recorded, all the shoots were gathered, packed into the paper bags and dehydrated at 75 °C to constant weight in a forced convection air circulation oven so as to measure the shoot dry weight.

The statistical analysis

The data documented and were analyzed, put through using ANOVA. The experimental treatments were equaled against their control following Duncan's multiple range test (DMRT) (Duncan, 1955). The agronomic traits amongst treatment and control group and greenhouse data was statistically estimated by using SPSS v. 20. All the trials were replicated three times with Completely Randomized Design (CRD)

RESULTS AND DISCUSSION

Physiochemical properties of carrier martial

To raise the putrefaction of organic matter and promote the growth of microbial populations during composting, a favorable or suitable environment or condition must be provided. In favorable conditions and exceptional and optimal loading of microbial nutrients, as well as the non-subsistence of destructive toxic compounds, are indispensible to reach high microbial degradation. (Han et al., 2020)

pH

In our current study, the most of the values for the physiochemical parameters for the raw materials (Azolla, Cowdung and Kitchen waste) used in composting process were nearly in neutral pH 7.42 and 7.49 respectively and the kitchen waste was in acidic range. Table1, which in line with the results ranged from 4.3 to 8.1 that obtained by Khater, 2015. There was an overall drop in pH at the composting cycle ends. The decrease in pH observed at the cease period of the composting might be as a result of the volatilization of ammoniacal nitrogen and hydrogen ions (H^+) discharge over the nitrification process, as well as the emanation of huge quantities of carbon dioxide (Huang et al. 2004; Roca-Pérez et al. 2009). This is the resultant of the release of organic acids throughout the process of decomposition this exhibits the pH range is promising condition for beginning the composting process.

Total Organic Carbon

In the progression of the composting mechanism, the total organic carbon content of the compost declined steadily and considerably ($P \leq 0.05$) for all the treatments in this study(Fig. 3). The current investigation is also in accordance with the outcomes of Tiquia et al. (2002), who studied a total carbon deprivation that ranged from 30–54% in composting process. Similarly, by studying the works of other authors, Yadav et al. (2012) described the total organic carbon

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abatement percentage ranged between 26 to 66 % throughout the vermicomposting process. According to Sánchez, 2007 over composting process, carbon is the basis of energy for microorganisms for their growth and development. Almost the carbon is fully absorbed by the microorganisms and altered into CO_2 during the cell metabolic process.

Therefore, in this study TOC value declined this was due to the microorganisms utilized the carbon to build up their cells. The results obtained by this experiment was alike to other studies (Jusoh *et al*.2013)

Total Nitrogen

The total nitrogen of the composting techniques differed significantly ($P \leq 0.05$) with the various sampling periods, was established that the results were statistically not significant ($P > 0.05$) (Fig. 4). The total nitrogen content of the lead off raw material was low in significant in the treatments ($P \leq 0.05$) in the initial 21days of composting. But, over the subsequent sampling, there was a consistent increase of total nitrogen; the highest cost was documented at the 31^{th} day. The fall in the total nitrogen in the early 21 days might be due to the depletion of nitrogen in the form of ammonia that is evident throughout the dynamic stage of composting. The increase in total nitrogen later the 21^{st} day may be occurred by the cause of the concentration effect that ensued from deprivation of organic carbon compounds that consequently indicates to weight reduction and therefore, a comparative rise of N concentrations (Dias et al. 2010). As Paredes et al. (2000) explicated the concentration of Nitrogen generally rises all the way through composting when the loss of organic matter is higher than the removal of NH_3. The results of the present study could, therefore, validate that for the period of the first 21 days of composting, losses of N through ammonia volatilization happened at a greater rate than organic matter decomposition, while in the course of the later phases, the degree of nitrogen depletion as NH_3 might be lagging than the degree of dry matter depletion as CO_2. Additionally, the N level would have also been upraised on the grounds that the fixation of atmospheric nitrogen by the N fixing microorganisms' action which usually happens during the advanced phase of the composting process (Seal et al. 2012). In our current study, a general decline of total nitrogen was noticed while through the thermophilic phase followed by a rise which is in agreement with the co-composting experiment of pig manure with corn stalks (Guo et al. 2012)

C/N Ratio

The Carbon to Nitrogen ratio of the composting materials of all the treatments lessened steadily and significantly ($P \leq 0.05$) with the progression of the composting period (Fig. 5). The primary C/N ratio of the raw materials at 0^{th} day was 14:1 which was within the appropriate range for composting (Hussein and Mohammed 2020) During the composting method the organic matter is putrefied by the microorganisms done that the organic carbon was oxidized to CO_2 gas into the atmosphere and thus drops the C/N ratio (Jusoh et al. 2013). Our study aligned with the findings of other studies by Kumar et al. 2009; Khwairakpam and Kalamdhad, 2011. The difference primarily due to the variation in the volume of total organic carbon as would be observed from earlier research findings and the same results were given above can also be due to the deviation in C/N ratio amongst the various composting processes. Commonly, the C/N ratios in the final product were found to be appropriate since the mellowed compost material generally has a C/N ratio of fifteen or lesser (Hock et al. 2009).

As Gómez-Brandón et al. (2008) stated that C/N ratio might not be a worthy gauge of compost strength because of it can influence off beforehand the compost stabilizes. When nitrogen rich

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wastes were effective as source material for composting, the C/N ratio can be within the recommended range of constant compost. By the same findings, Zmora-Nahum et al. (2005) described a C/N ratio lesser than the determined value of fifteen during the very initial phase of composting in cattle manure, although significant stabilization progressions were taking place.

Shoot length of field pea

The shoot length which was found from treatment group and its mean values was significantly overwhelmed by bio-organic fertilizer application (at $P \leq 0.01$) (Table 2). The result revealed that shoot length was increased due to bio-organic fertilizer when applied in to crops. The highest and lowest shoot length at both group (treatment and control) was recorded. The highest mean value of shoot length over treatment was 182.33cm/plant and for both control mean value was 162.33 and 142.00cm/plant, respectively (Namvar et al., 2013). This might be recognized to the fact that bio -organic fertilizer can trigger certain enzymes which are liable for cell cycle and cell elongation that could lead to better shoot length. Likewise, erstwhile research also long-established that plant shoot was greater than with inoculant application (Ram and Katiyar, 2013)

Shoot Dry Weight of field pea

The main effect of bio-organic fertilizer application was significantly prejudiced shoot dry weight at $P \leq 0.05$ (Table 3). In broad-spectrum, regardless of the treatments applied, the data showed that shoot dry weight in all treatments groups was superior to the control.

The shoot dry weight demonstrated a rising tendency with bio organic fertilizer application. The maximum mean value of shoot dry weight over the treatment was 94.38g plant^{-1} and for control lowest mean value was 54.76g plant$^{-1)}$ and 33.15g plant^{-1}, respectively. This might be due to bio-organic fertilizer leads to improve the dry matter production of the plants (Journet et al., 2006) In accord with this study, several authors also described the positive effect of bio-organic fertilizer applications in shoot dry weight (Solomon et al., 2012; Banik and Sengupta, 2012;).

Number of Pods per plant of field pea

Number of pods per plant at treatment group was significantly affected by bio-organic fertilizer at $P \leq 0.05$. At treatment group, significantly the maximum number of pods was (26.00) but Regardless of both control group the lowest number of pods was obtained (10.50 and 10.17) respectively. Other researchers (Kayan et al., 2012, Jadeja et al., 2016) also described that number of pod increased with microbial inoculation.

Number of Seeds per plant of field pea

Number of seeds per pod showed an important response due to the prime effect of bio-organic fertilizer application at ($P \leq 0.05$) with equated to control group. The highest mean value of number of seeds per pod was (102.67) and mean value of number of seeds per pod for both control group was (43.33 and 15.50) respectively so these finding observes that Bio-organic fertilizer have potential to increase number of seed. This might be due to the fact that nutrient deficit causes prominent reduction in leaf size and photosynthetic pigments and resulted in drop seed number (Hitsuda et al., 2004). Rhizobium inoculation offers sufficient supply of N for plant and ensued for better chlorophyll synthesis and photosynthetic pigments. The result of the present study was in conformity with, Kayan et al. (2012), Sipai et al. (2016), Sharifi and Ryu(2016) have described that the role of Rhizobium inoculation increasing number of seed per pod.

Number of spikelet's in finger millet

Number of Spikelet per plant at treatment group was significantly affected by bio-organic fertilizer at $P \leq 0.05$. At treatment group, significantly the highest number of spikelet was 79.17 but regardless of both control group the number of spikelet was obtained 46.83 and 5.67, respectively. Other researchers have depicted that organic fertilizers have enhanced both the quantity and the quality of lettuce (Villagra *et al*. 2012). Lettuce quality can be altered by the bio fertilizers (Karimaei *et a*l., 2004,).

Shoot length of finger millet

The shoot length mean that was obtained from each treatments and its mean values was considerably affected by bio-organic fertilizer application ($P\leq 0.01$) (Table 3). The result showed that shoot length was increased due to bio-organic fertilizer when applied in finger millet. The highest mean value of shoot length over treatment was 78 cm plant per plant for FPT and for both the positive and negative controls (Namvar et al., 2013). This might be attributed to the fact that microbial inoculant nutrient up take of the plant and also can initiate certain enzymes that are culpable for cell cycle which could lead to augmented shoot length.(Sipai et al., 2016)

Shoot Dry Weight of finger millet

The main effect of Bio-organic fertilizer application was significantly influenced shoot dry weight of finger millet at $P \leq 0.05$ (Table 3). In this study, shoot dry weight delineated a rising tendency with bio organic fertilizer application. The highest average value of shoot dry weight was 105.64 plant^{-1} for FPT and for both positive and negative control groups 68.20g plant $^{-1}$ and 32.63 plant $^{-1}$ respectively. This might be due to bio-organic fertilizer indicated the enhancement of dry matter production of the plants (Journet et al., 2006). In accordance with this finding, several authors also conveyed the positive effect of bio-organic fertilizer applications in shoot dry weight in other crops (Solomon et al., 2012; Banik and Sengupta, 2012)

Leaf number per plant on lettuce

Leaf number of lettuce per plant at treatment group was expressively affected by bio-organic fertilizer at $P \leq 0.05$. At treatment group and positive control Leaf number was not significant which was 12.17 and 11.83, respectively but in case of negative control the lowest leaf number was 7.50 this shows bio-organic fertilizer may not increase leaf number of lettuce

Leaf length on lettuce

The leaf length that was found from treatment group and their average values was significantly affected by bio-organic fertilizer application (at $P\leq 0.01$) (Table 4). The result showed that leaf length was increased due to bio-organic fertilizer when applied in to crops. The highest and lowest shoot length at both group (treatment and control) was recorded The highest mean value of leaf length over treatment was 28cm plant^{-1} and for both control mean value was 16.83cm plant $^{-1}$ and 9.33cm plant^{-1}, respectively (Mrkovački and Milić, 2001). This might be attributed to the fact that microbial inoculant nutrient up take of the plant and also can influence certain enzymes which are liable for cell division that may perhaps repressed to increased shoot length (Cherr et al., 2006, Wilhelm et al., 2007) in their findings that microbial inoculant surge the yield and growth of various plants.

Dry mass of lettuce

Dry mass is one of the parameter in evaluating the performance of bio-organic fertilizer in harmony with their capability to fix atmospheric nitrogen *(*Journet et al., 2006). Table 4 showed that dry mass obtained from lettuce were significantly influenced by the main effect of fertilizer at P≤ 0.05. The highest mean value of dry mass was 30.50g $plant^{-1}$ for FPT and lowest mean value for both control groups was 13.50g and 6.25g $plant-^{1}$, for positive and negative control respectively. This is indicating that bio-organic fertilizer application increased with dry mass (Table 4). In agreement with this research, the application of bio-organic fertilizer has shown that bio-organic fertilizer impacts the lettuce yield and yield related traits like number of lettuce head and the biomass of the head.

CONCLUSION

Nitrogen fixation is one of the utmost vital biological processes following to photosynthesis on the global. However, due to nutrient paucity, the N_2 fixation is impaired and consequently causes low crop productivity in Sub-Saharan Africa. By guaranteeing a well-balanced supply of NPK to the crop resulted in higher seed yield through improving nodule activities and nitrogen fixation. The outcome of the present study indicated that food waste mixed with cow dung and azolla after decomposition of the waste materials using *kocuria rosea C9*, it works as an excellent raw material for the production of bio-organic fertilizer and also the result indicates that the compost that was prepared, it could be a good carrier material. The temperature, pH and C/N ratio was better indicator for maturity of the compost. The temperature, pH and C/N ratio values of the compost at day 45 were 30 ^{0}C, 8.5 and 14.1, respectively and this value indicates that the Bio-organic fertilizer was mature. The effect of the formulated bio-organic fertilizer was checked on selected crops under greenhouse condition the *kocuria rosea C9 Spp* on the carrier material. Agronomic result indicates there was a good result compared to uninoculated control.

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Table 1. Physio-chemical properties of carrier martial

Parameter	Kitchen waste	Cow dung	Azolla
pH	4.24 ± 0.06	7.42 ±0.13	7.49±0.08
TOC	22.58 ±0.56	20.82±1.46	30.60±0.83
TOM	40.66±0.03	41.33±1.50	70.53±0.50
MC	78.20±0.23	79.72±0.26	85.03±0.03
N%	0.97±0.04	0.84±0.01	22.21±0.15
C/N	23.27±0.17	18.65±0.35	0.95±0.06

Table 2. Effect of Bio-organic fertilizer on pod no, seed no, shoot length and shoot dry mass of field pea

Sample	Pod no	Seed no	Shoot length(cm)	Shoot Dry Weight (in gm.)
FPT	$26.00±8.20^{b}$	$102.67±5.43^{c}$	$162.33±25.28^{ab}$	$94.38±1.35^{c}$
FPPC	$10.50±3.99^{a}$	$43.33±16.22^{b}$	$182.50±7.58^{b}$	$54.76±1.32^{b}$
FPNC	$10.17±4.75^{a}$	$15.50±10.84^{a}$	$142.00±14.14^{a}$	$33.15±1.58^{a}$

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, FPT=field pea treatment, FPPC =field pea positive control, FPNC=Field pea negative control, LSD= Least significant difference, CV = Coefficient of variation

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Table 3. Effect of Bio-organic fertilizer on Number of Spikelet, shoot length and shoot dry mass for finger millet

Sample code	Spikelet no	Shoot length(cm)	Shoot Dry Weight (in gm.)
FMT	79.17±11.51[c]	78.00±10.70[c]	105.64±4.94[c]
FMPC	46.83±11.72[b]	50.00± 4.05[b]	68.20±1.80[b]
FMNC	5.67±1.19[a]	23.67± 3.67[a]	32.63±1.87[a]

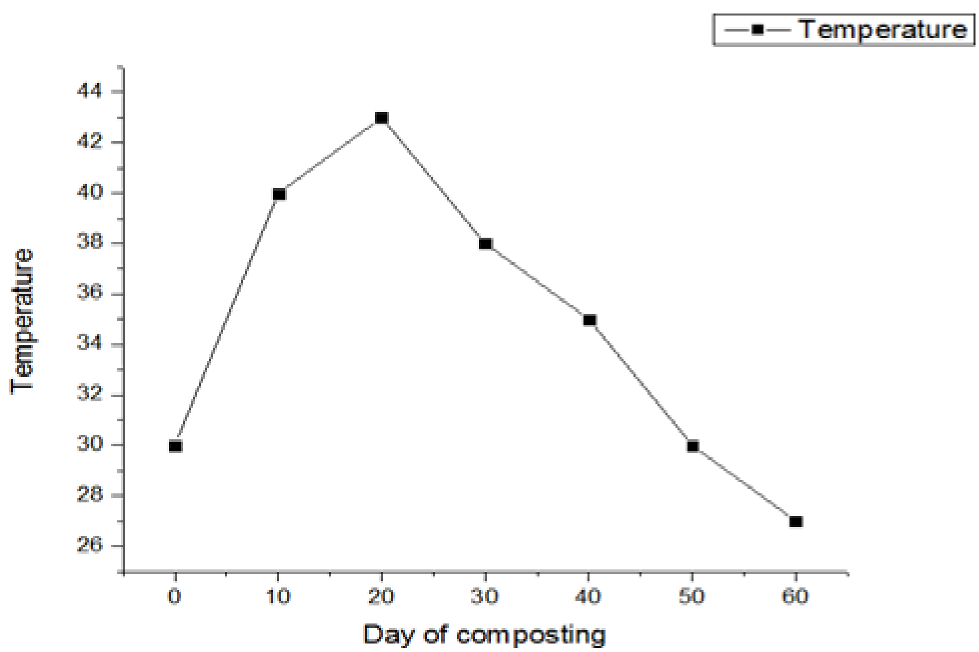
Means with the same letter are not significantly different at P>0.05 level of probability following LSD, FMT =Finger millet treatment, FMPC=Finger millet positive control, FMNC=Finger millet negative control LSD= Least significant difference, CV = Coefficient of variation

Table 4. Effect of Bio-organic fertilizer on Number of leaf, leaf length and dry mass on lettuce

Sample code	Leaf number	Leaf length	Dry mass
LTR	12.17±2.64[b]	28.00±1.55[c]	30.50±1.87[c]
LPC	11.83±2.14[b]	16.83±4.26[b]	13.50±4.72[b]
LNC	7.50 ±1.87[a]	9.33±1.54[a]	6.25±1.33[a]

Means with the same letter are not significantly different at P>0.05 level of probability following LSD,

LTR =lettuce treatment, LPC=lettuce positive control, LNC=Lettuce negative control LSD= Least significant difference, CV = Coefficient of variation



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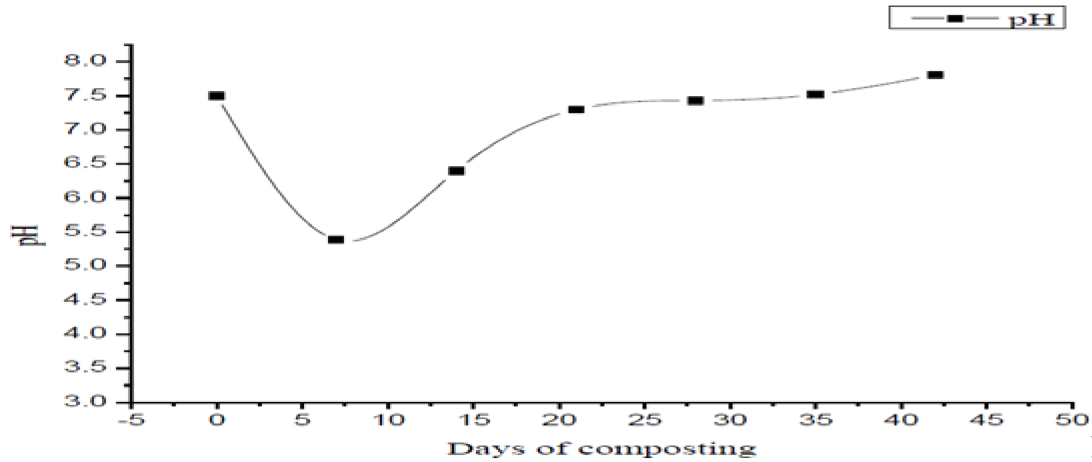

Fig. 1. Changing temperature during composting process

Fig. 2. Changes in pH during composting process

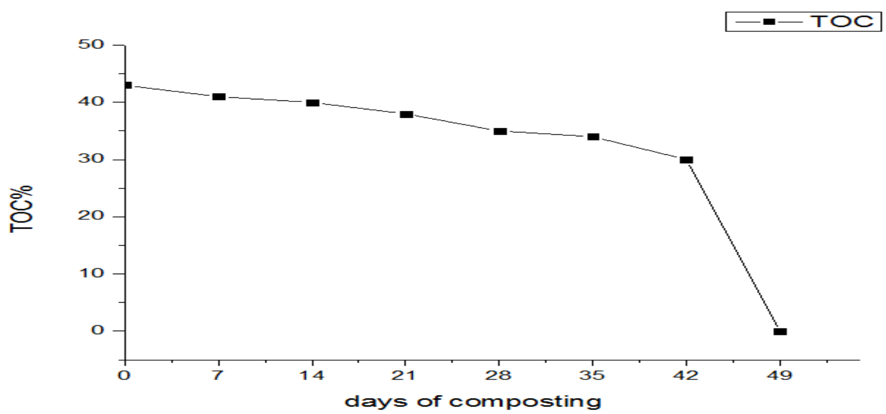

Fig. 3. Changes in total nitrogen during composting process

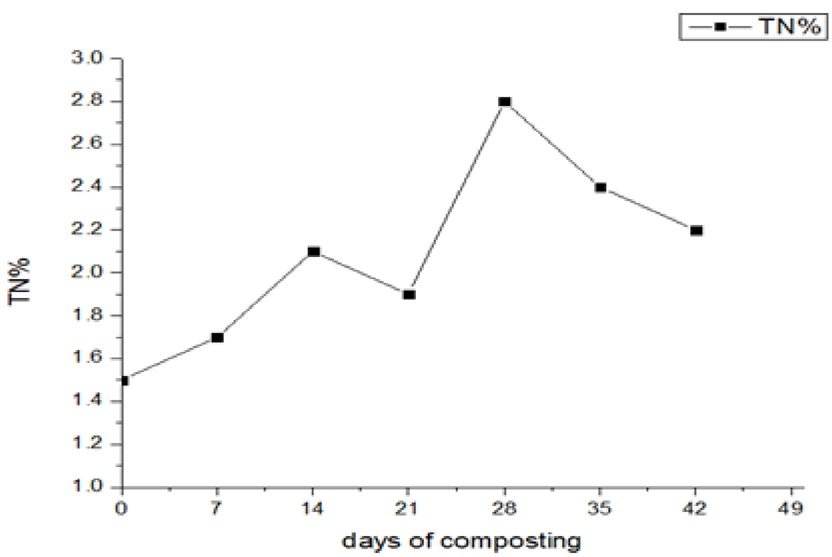

Figure 4.Changes in Total Nitrogen during composting process

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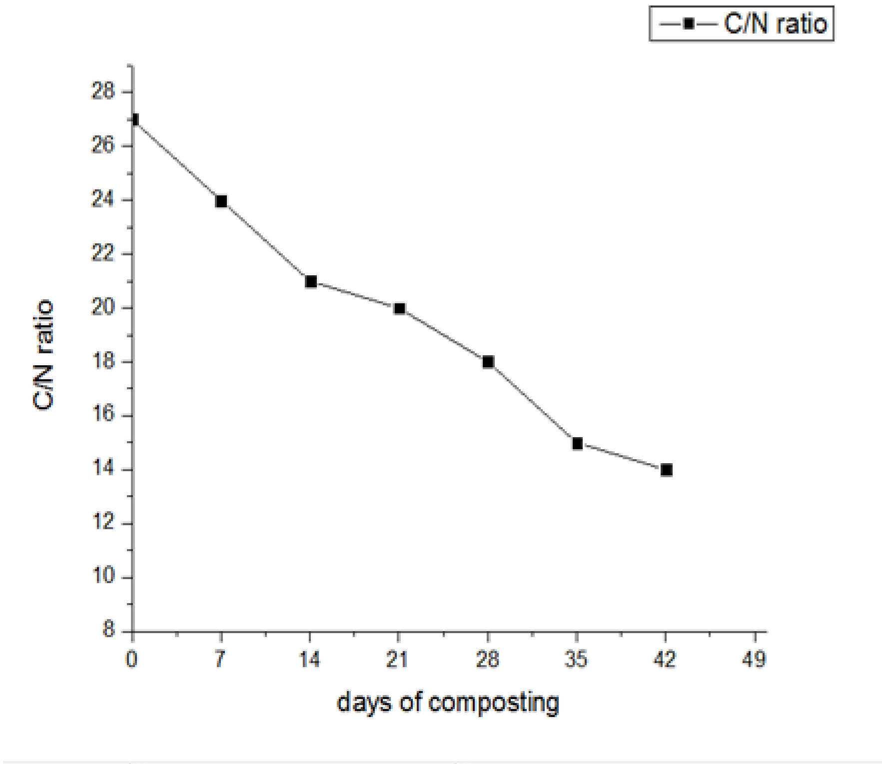

Fig. 5. Changes in C/N ratio during composting process

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