

EVALUATING THE ROLE OF HEAT STERILIZATION IN SUPPRESSING COMPOST BIOTA FOR ENHANCED NUTRIENT USE EFFICIENCY AND DISEASE MITIGATION

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Abstract

Compost sterilization was mainly carried out in protected crop system. Concerns about widespread use of chemicals/pesticides and their effect on human health and environment have prompted the use of heat as an alternative method of sterilization. Continuous cropping of monocultures or different host crops needs to be sterilized after each cropping. The current study was designed to analyze the effect of sterilization on nutritional components and compost biota. The present study revealed that various temperatures have different effect on compost biota. Among ten tested temperature and time levels, T10 (110⁰C/60min) was found best for controlling microbial biota with less than 30 bacterial colonies and no fungal colony per petri plate, while T2 (70⁰C/30min) was observed to be least effective in controlling soil biota. Analysis of variance for pre and post-compost showed significant not differences in pH and electrical conductivity. However, pH and EC slightly increased after sterilization. Significant variations were observed in organic matter contents of pre (25.86 %) and post sterilization (18.3%) at 110 ⁰C for 60 minutes. Lime content was slightly increased with sterilization as compared to the unsterilized compost sample. Analysis of nitrogen, phosphorous and potassium levels showed significant variations in pre and post sterilization samples, while non-significant variations at different temperatures. Maximum concentration of nitrogen was recorded at 90⁰C/30 minutes (1 mg kg⁻¹) while the lowest (0.43 mg kg⁻¹) recorded at 110 ⁰C/60 minutes. Similarly, the highest levels of phosphorous and potassium was recorded at 90⁰C/45 min (3.1 mg kg⁻¹) and 90⁰C/60 min (271.3 mg kg⁻¹).

Keywords: Soil microbes; Organic matter; Nutrients; Time duration; Temperatures

Introduction

The sterilization process kills and suppresses the microorganism's activities such as bacteria, fungi, unicellular, eukaryotic, spores and viruses. There are various sterilization procedures including heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, radiation and so many procedures have been used (Rutala and Weber, 2001). Sterilization processes are used for many purposes that have more importance in agriculture research. Sterilization of soil and composts are used to killed weed seeds, pathogens, to make microbes free that may transmit diseases (Rutala, 1997).

Compost is the decomposing of organic solids or materials converted into simple and inorganic compounds through a process called composting. Composting is an aerobic (oxygen presence) process in which organic solid wastes are decomposed into inorganic or available forms of plant nutrients by diverse varieties of microorganisms (Masters, 1997). The compostable material includes residential and agricultural waste such as plants leaves, fruits and vegetables, eggshells, tea bags, coffee grounds and filter paper, shredded newspapers, cardboards, houseplants, hay and straw, sawdust, wood chips, ashes, cotton and wool Rags, hairs and fur, etc. The application of compost is used to refine the soil health and fertility level and also improve organic farming and reduce chemical fertilizers use (Nierenberg and Amelia, 2020). Natural compost contains plant nutrients and soil microbes. A large number of microbes are useful while a few of them are pathogens diseases in plants (Tjamos, 1989).

A group of scientists from western countries evaluated the suppression of causing disease pathogens in compost through sterilization (Termorshuizen *et al.*, 2006). The activity of compost microorganisms can be controlled or inhabited by several procedures such as pasteurization or sterilization (Cotxarrera *et al.*, 2002; Reuveni *et al.*, 2002). Compost microorganism has a direct or indirect role in improving soil and plant health. The compost biota can be controlled by physical, chemical and biological techniques (Whipps, 1997). Chemical control of compost microbial population in compost is harmful to soil and plant health while physical and biological procedures are well suited for the crop as well as for the environment. In biological control, the useful microbes show resistance to disease causing pathogens by producing antibiotic lytic, or enzymatic compounds while in physical control sterilization, radiation, pressure, light are used for suppressing soil biota (Lorito *et al.*, 1994).

Method and Material

The current study was conducted at Soil and Microbiology Laboratory, Agriculture Research Institute, Tarnab Peshawar. The pre and post sterilization compost samples were obtained for analysis of various physio-chemical properties of compost soil according to standard methods/procedures. The compost samples for the experiment were collected from Qarshe industries, Hattar Haripur, KP.

Sample preparation

Sterilization was carried out applying the protocol of Jain *et al.*, (2020) with slight modification. Briefly, 1 kg sample was wrapped in aluminum foil 60cm x 30cm in a hot air oven (Memmert). The applied time and temperature for each treatment in Table 1.

Microbe Collection

Isolation as well as microbial population counting, the procedure of (Eisenhauer *et al.*, 2018) was used. Simply, 1g dry compost samples were mixed in 9 ml distilled water. The samples were shaken for 15 to 20 minutes and poured on the Petri plates containing 20 ml media. Two different medias were used for microbial growth, one was potato distros for fungi and the 2nd was nutrient agar for bacteria growth. The microbial populations were counted after incubating the samples at 28⁰C for 3 to 5 days (Roohi *et al.*, 2014).

Chemical analysis of Compost

Chemical composition of pre and post sterilized compost samples was analyzed for the vital constituents i.e. The EC and pH of compost sample was determined from compost extracts. In extract the ratio of compost and water for both EC and pH was (1:5) mean 1g compost and 5ml water. The compost extract EC was determined by the method of Ryan *et al.* (2001), using EC meter, while the pH was determined by Mclean, (1982) method.

The organic matter (OM) was determined by (Walkley and Black, 1934) method. One-gram soil/compost sample was taken in conical flask and 10 ml of 1 N K₂Cr₂O₇ solution was added along with concentrated 20 mL Sulfuric Acid and titrated with 0.5 N FeSO₄ solution.

Calcium carbonate was (CaCO₃) determination by (Dhyan Singh *et al.*, 1999) mothed. 1g soil was transferred into 250 mL flask and mixed with 10 mL of 1M HCl. The suspension was heated for five minutes followed by filtering through Whatman No. 42 after cooling. Add 50 ml DI water. The filtrate was titrated against standardized 1N NaOH by adding phenolphthalein as an indicator till the pink color appeared as end point.

Nitrogen content in compost sample was determined by the Kjeldahl method (Bremer and Mulavaney, 1982). Phosphorus in compost samples was digested with perchloric acid and nitric acid and their P concentration was determined calorimetrically (Kuo, 1996). The potassium concentration in compost samples was determined by flame photometry according to (Ryan *et al.*, 2013).

Table 1: By various temperature and time intervals were used for suppressing microbial activity.

Sample No.	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Temperature ($^{\circ}\text{C}$)	control	70	70	70	90	90	90	110	110	110
Time (min)		30	45	60	30	45	60	30	45	60

Results and Discussion

Compost biota

The purpose of compost sterilization was to provide ideal conditions for plant growth by destroying all types of microorganisms in growing media. Statistical analysis of compost biota was presented in Table 2. The present study revealed that sterilization temperature and duration had a significant effect on the microbial population. Among treatments T10 (110°C /60min), proved to be the best sterilization procedure for suppressing the microbial biota with less than 0.005 cfu bacterial colonies and 0.001 cfu fungal colonies per Petri plate reported. However, T9 (110°C /60min), proved to best condition after T10, while T2 was observed to be least effective in controlling soil biota (Table 2). Our observations are in line with the findings of Eagles, (1967), discussing that an increase in temperature directly reduces the number of soil biota. The entire microbes of compost were killed by applying heat sterilization.

Table 2: Suppressing of compost microbial population at various temperature and time intervals.

Treatments	Temp	Time	Bacteria population cfu g^{-1} compost (10-4)	Fungi population cfu g^{-1} compost (10-4)
T1	0	0	6.5	5.4
T2	70	30	3.4	3.1
T3	70	45	3.1	2.5
T4	70	60	2.7	1.6
T5	90	30	2.9	0.25
T6	90	45	2.1	0.11
T7	90	60	1.1	0.021
T8	110	30	0.1	0.004
T9	110	45	0.01	0.002
T10	110	60	0.005	0.001
Mean			2.19	1.44
Standard Deviation			2.02	1.90
Standard Error			0.64	0.63

pH and Electrical Conductivity

Sterilized compost pH and EC were mentioned in Table 3. The statistical analysis shows that sterilized and unsterilized compost pH was non-significantly. The sterilized compost becomes slightly acidic with pH (min 7.4) as compared to control (mix 8.3). It might be due to the degradation of various volatile organic compounds by heating. Similarly, the electrical conductivity significantly increased after sterilization as compared to Control from 0.12 to 1.15 dSm^{-1} . However, among the various sterilization procedures, the compost sterilized with 90⁰C temperature for 60 min and 110⁰C for 30 min, showed the higher EC values (1.15 dSm^{-1}). At a soil temperature range of 25⁰C-39⁰C the soil pH increased as a result of organic acid denaturation which increases at high temperatures (Menzies *et al.*, 2003).

Organic Matter and Lime content (%)

Organic matter (OM) contents were significantly reduced by sterilization. Data relating to organic matter and lime were listed in Table 3. The highest OM contents (25.86 %) were found in the pre-sterilized sample (control) while the lowest was found in T₁₀ (18.39 %). The rate of organic matter decomposition increased with increasing temperature (Burke *et al.*, 2003; Davidson & Janssens, 2006). Significant effect of temperature was observed in sterilized (16%) and unsterilized (5%) samples but the rest of the treatments had no significant effect on lime contents at varying temperatures and time duration. According to Onwuka and Mang, (2018) increase in temperature do not affect soil lime content.

Nitrogen content (mg kg^{-1})

Nitrogen is an important element for plant growth and regulation. Analysis of nitrogen content of compost was listed in Table 3. The concentration of nitrogen was significantly decreased by increasing the temperature and time duration during the sterilization process. The maximum concentration of nitrogen was recorded in T₅ (1 mg kg^{-1}) while the lowest was observed in T₁₀ (0.43 mg kg^{-1}). The temperature had a great role in nitrogen fixation, volatilization, mineralization, immobilization and denitrification. Initially, when the sterilization temperature reached 70⁰C some nitrogenous processes stop such as fixation, mineralization and denitrification and a few of them such as volatilization, immobilization processes start slowly. With further increase in temperature, the amount of nitrogen becomes less available up to a certain level. Increase in temperature up to 80⁰C or 90⁰C the nitrogen content become stable and a very small amount is available as most of the nitrogen evaporates in the form of Ammonia (Bunemann, 2015).

Phosphorus Content (mg kg^{-1})

Analysis of phosphorus content is shown in Table 3. Our results showed that sterilization had a significant effect on phosphorus concentration. The higher concentration of phosphorus was noted in T₇ (3.10 mg kg⁻¹) and followed by T₆ (3.03 mg kg⁻¹). Phosphorus availability decreased in the soil while increased in manure with temperature. In sterilization, initially, the phosphorus concentration increases in the presence of moisture and temperature due to increased P-mineralization processes (Zimmerman and Ahn, 2010). The water-soluble phosphorus concentration increased with increasing soil temperature from 45 to 100°C due to the increase in the movement of phosphorus in the soil, controlled by diffusion (Onwuka and Mang, 2018).

Potassium concentration (mg kg⁻¹)

Potassium concentration in compost was presented in Table 3. Our results revealed that the sterilization process had a significant effect on potassium concentration. Increasing temperature and time duration significantly enhanced the potassium content in comparison to control treatment. The maximum concentration of potassium (271.3 mg kg⁻¹) was recorded in T₇ where compost samples were heated at 90°C for 60 minutes, followed by T₆ 268.3 mg kg⁻¹ potassium content observed when the samples were heated at 90°C for 45 minutes. Our results are in close agreement with the findings of Williams & Wilkins, (1982) who also reported that sterilizing the soil and compost at 90°C results in maximum availability of calcium, magnesium and potassium.

Table No.3 Physio-Chemical analysis of compost sample after sterilization.

Treatments	pH	EC	Lime	OM	N	P ₂ O ₅	K ₂ O
T1	8.3	0.12	4.97	25.87	0.9	0.5	180
T2	7.60	1.14	11.49	22.03	0.84	2.26	242.67
T3	7.57	1.12	14.50	22.20	0.60	2.83	264.00
T4	7.43	1.10	15.40	18.44	0.59	2.90	267.00
T5	7.80	1.16	11.93	22.15	1	2.42	246.67
T6	7.67	1.14	14.73	23.00	0.81	3.03	268.33
T7	7.63	1.11	15.63	18.49	0.77	3.10	271.33
T8	7.70	1.15	11.69	21.97	0.77	2.33	239.33
T9	7.67	1.13	14.57	21.75	0.56	2.90	261.67
T10	7.53	1.11	15.53	18.39	0.43	2.93	267.00
Mean	7.69	1.03	13.04	21.43	0.73	2.52	250.80
S.D	0.24	0.32	3.27	2.38	0.17	0.77	27.43
S.E	0.07	0.10	1.04	0.75	0.06	0.24	8.68

Conclusion:

In conclusion, the interactive effect of heating and time duration strongly suppressed the microbial biota. A combination of 110°C temperature and 60 min time favored the optimal microbial

sterilization and nutrient availability. In short, the current protocol plays a key role in the sterilization of compost microbial biota suppressing and to ensure the optimum level of desired nutrient availability incased such K^+ , P_2O_5 .

Authors' Contributions

Aziz Ullah conceived and designed the experiments. Aziz Ullah and Zakirullah Jan performed the experiments. Hammad and Badshahe Rome analyzed the data. Sheheryar Hafeez and Tausif Ahmad contributed reagents, materials, and analysis tools. Aziz Ullah & Zubair Shah wrote the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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