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## Chemical and Microbial Dynamics during Composting of Herbal Pharmaceutical Industrial Waste

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**Abstract:** A study was performed to analyze the dynamics of chemical, biochemical and microbial parameters during composting of herbal pharmaceutical waste. All the parameters were analyzed at three different intervals of composting (1<sup>st</sup>, 15<sup>th</sup> and 60<sup>th</sup> days). Temperature of the compost pile was initially high (46.2 °C) and on 60<sup>th</sup> day it dropped to 33.3 °C. The pH of the sample was initially acidic (2.39) and with the progress of decomposition gradually changed to neutrality (7.55). Electrical conductivity (EC) value was high (3.8 mS) during last day of composting compared to other stages. The activity of degradative enzymes namely amylase, invertase and urease were initially high (4.1, 4.79 mg of glucose/g/h and 0.19 mg of ammonia/g/h respectively) while it decreased with composting. The beneficial microbial load was initially low and very high at the last stages of decomposition. The bioassay studies using compost extracts revealed that the 60<sup>th</sup> day old sample was not phytotoxic in nature.

**Keywords:** Herbal pharmaceutical waste, Amylase, Invertase, Urease, Bioassay.

### Introduction

There are many challenges an Indian farmer has to face in agriculture with an objective of profit maximization. The most important among them are decreased production cost and increased yield. There are many ways by which increased production can be met. But only way of reducing cost is by using low cost inputs and healthy agricultural practices. One important low cost input for meeting nutrient requirement of crop is compost, which can be produced by a simple technique called composting<sup>1</sup>. Several solid waste management objectives can be achieved through composting which include; (1) controlling of pathogenic microorganisms, larvae and weeds; (2) nuisance such as odour and insect breeding can be prevented through the rapid decomposition of putrescible organic material, (3) narrowing the carbon/nitrogen ratio adequately, which implies reduced damage to crop plants through

immobilization of soil nitrogen. Further this also helps in eliminating phytotoxic substances present in organic materials. Composting period depends on the nature of matter<sup>2</sup>. Before deciding the decomposition period required for formation of mature compost the dynamics of various parameters which decides maturity of compost has to be worked out. The literatures related to dynamics of various parameters deciding maturity of herbal pharmaceutical waste compost are scanty. Hence this research work was designed and executed.

## Experimental

Composting process was carried out at Alva Pharmacy, Mijar, Moodbidri, Mangalore, Karnataka and all the chemicals and solvents used for the analyses were of analytical grade and were purchased from Himedia, Co. Pvt. Ltd., Mumbai, India.

### *Pile preparation*

Two piles were constructed of the size 2x3x5 feet (LxBxH) according to indoor method, which follows the principle of hot fermentation process<sup>3</sup>. Each pile contains around 75 to 100 kg of the fresh residues of herbal waste (Figure 1 b). Both piles were allowed to undergo decomposition and frequently (1<sup>st</sup>, 15<sup>th</sup> and 60<sup>th</sup> days) sampled and were assayed for the compost maturity parameters.



**Figure 1(a).** The pharmaceutical waste generated during preparation of Herbal medicines; **(b)** composting tank/pile designed for the study.

### *Physicochemical analysis of compost*

Temperature, pH, moisture and electrical conductivity (EC) were determined using standard AOAC procedures.

### *Chemical changes during composting*

From the compost, humic acid was extracted, fractioned, partially purified using standard AOAC procedures<sup>4</sup>. Ammonical nitrogen extracted was estimated by nesslerization method<sup>5</sup>. Total phenolic content was estimated by Folin-Ciocalteu method<sup>6</sup>.

### *Biochemical changes during composting*

The enzymatic activities of amylase, invertase and urease were estimated by literature methods<sup>7,8,5</sup>.

### *Enumeration of mesophilic beneficial microorganisms during composting*

The samples were analyzed for beneficial microorganisms mainly for phosphate-solubilizers (*Azotobacter*) and nitrogen fixers (*Rhizobium*). These were isolated and enumerated on hydroxy appetite medium<sup>9</sup> and Norris Nitrogen free medium<sup>10</sup> respectively.

### *Rice seedling bioassay*

The compost soaked rice seeds were placed in sterile petriplate lined with moist blotter paper and incubated at room temperature for two days. After two days, length of plumules and radicals of the germinated seeds were determined.

### *Statistical analysis*

All experiments were carried out in triplicates and repeated in two independent sets of experiments from two constructed tanks. Data were shown as mean  $\pm$  standard error (SE). SPSS 10.0.5 version for windows (SPSS software Inc., USA) was used. The significance of the study was assessed by one way ANOVA, followed by Post hoc comparison test. Correlations between quantitative properties were evaluated by calculating the Duncan and Dunnett's coefficient. Statistical significance value set at  $<0.05$ .

### **Results and Discussion**

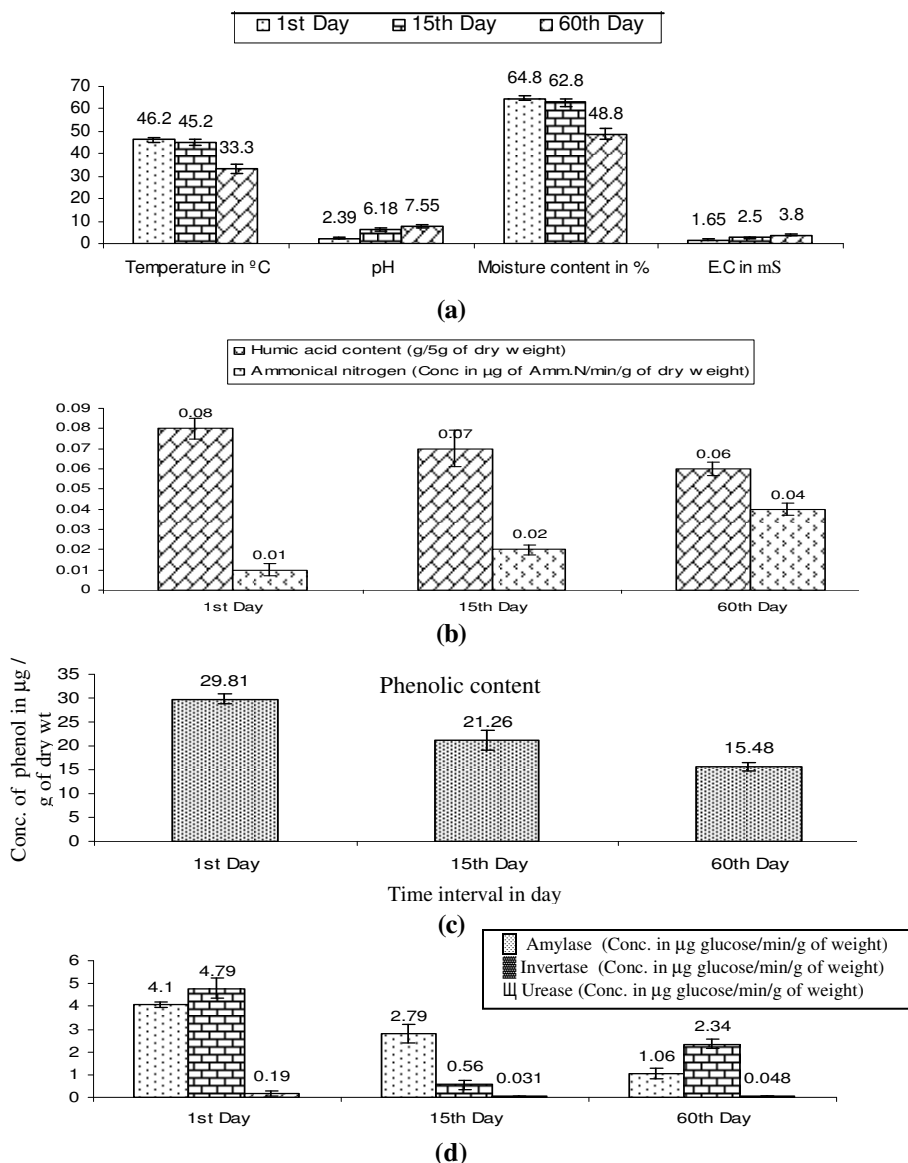
Maturity of composts critically affects their successful utilization in agriculture. In this study an attempt was made to determine the compost maturity indices of herbal pharmaceutical waste. During the decomposition of organic wastes temperature increases at initial stage and on 60<sup>th</sup> day the temperatures of the pile was observed to be optimal. The rise in temperature during composting is mainly due to the evolution of metabolic heat<sup>11</sup>. pH is an important factor that determines the quality of the compost. pH analysis of the compost showed a gradual change from acidic to neutral condition as it is evident during sludge composting. A high percentage of moisture was observed on the 1<sup>st</sup> day. As the degradation time prolonged, a fall in moisture percentage was observed. High moisture content promotes fermentative metabolism and results in production of incompletely decomposed products like organic acids<sup>12</sup>. In the light of this observation, initial low pH of sample may be is due to production of organic acid and incomplete oxidation of organic matter. During decomposition mineralization reaction takes place and one of the products of mineralization is ammonia. This product increases pH of sample. The EC value of the pile was highest on 60<sup>th</sup> day followed by 15<sup>th</sup> day and 1<sup>st</sup> day (Figure 2a). The increased moisture content during decomposition reduces EC values. This point indicates that the high water content during initial stages reduces the rate of decomposition and facilitate the release of ions like  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$  etc., resulting in decreased EC<sup>13</sup>.

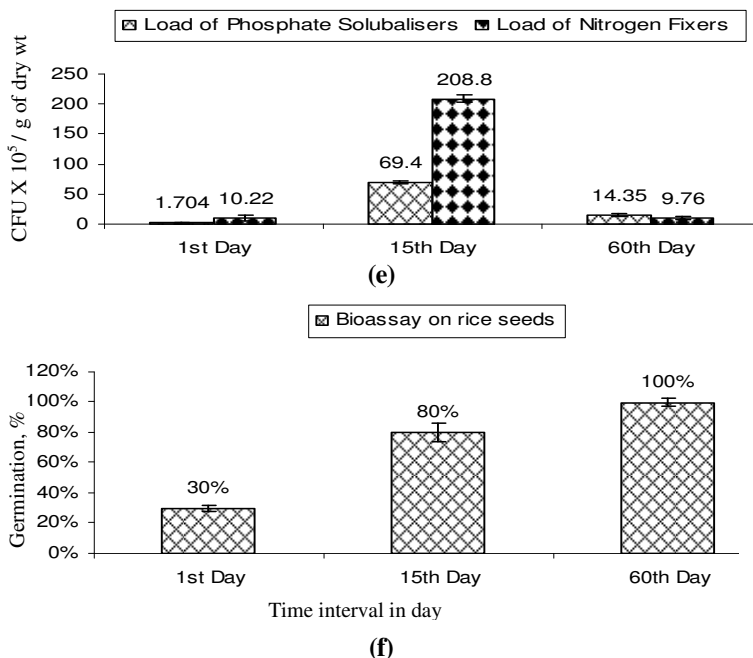
The concentration of humic acid declined as the degradation of pharmaceutical waste proceeds, this is because the humic acid during composting gets decomposed into fulvic acid and its byproducts which are inversely proportional to EC values of the compost<sup>1</sup>. The concentration of ammonical nitrogen was lowest during initial stages as the degradation precedes the concentration of ammonical nitrogen increases showing the lowest activity of urease in the degraded samples (Figure 2b). In general the concentration of phenolic compounds were highest in the initial sample followed by the sample obtained on 15<sup>th</sup> day and lowest concentration of phenolic compounds was observed during 60<sup>th</sup> day (Figure 2c).

Composting is mainly a degradation process wherein complex organic molecules are broken down to simpler components. Amylases are commonly concerned with breakdown of starch into glucose. In general activity of enzyme amylase was highest during initial stage and lowest during final stage and similarly results were observed with invertase. The activity of enzymes indicates the availability of easily degradable organic compounds such as simple sugars<sup>14</sup>. The lower activity during final stage indicates the mere completion of decomposition process. A similar kind of result was observed during decomposition of urban solid waste<sup>15</sup>. The enzyme urease catalyses the hydrolyses of urea to  $\text{CO}_2$  and ammonia with a reaction mechanism based on the formation of carbonates as intermediates. The extracted urease from soil has been found resistant to thermal denaturation<sup>16</sup>. The sample on first day contained high activity of urease but sample on 60<sup>th</sup> day showed lowest urease activity (Figure 2d).

The microbiology of composting is complex in description. When composting begins the indigenous microorganisms start utilizing the organic materials for available carbon, nitrogen and other nutrients. This stage is called as mesophilic stage<sup>17</sup>. As the activity continues the

temperature begins to increase due to the heat which is generated through microbial oxidation. At this point the mesophiles are inhibited by the high temperature and thermophilic organisms become very active (Figure 2e). From this period, stabilization with regard to its mineralization and humification occurs, and this phase is known as maturity stage<sup>18</sup>. The bioassay study was conducted to observe the influence of compost on seed germination (Figure 2f). The undecomposed organic matter contains phytotoxic substances, which inhibits seed germination<sup>19</sup>. Inhibition of seed germination by extract of initial sample (0 day old compost) observed in the present study is may be due to presence of phytotoxic chemicals. On the other hand there was no inhibition of germination by the 60<sup>th</sup> day old compost extract. This observation clearly states that the 60<sup>th</sup> day old compost is free from phytotoxic compounds and the compost is mature.





**Figure 2(a).** Physicochemical analysis of temperature ( $^{\circ}\text{C}$ ), pH, Moisture content (%) and Electrical conductivity (mS). **(b)** Humic acid and ammonical nitrogen content during composting. **(c)** Phenolic content during composting. **(d)** Biochemical indices of enzyme amylase, invertase and urease. **(e)** Microbial (*Azotobacter* and *Rhizobium* isolates) load during composting. **(f)** Bioassay on rice seeds germination during a time interval of 1<sup>st</sup>, 15<sup>th</sup> and 60<sup>th</sup> day of composting.

The compost contains some growth promoting substances or chemicals, which are structurally similar to that of growth promoting substances such as indole acetic acid (IAA)<sup>20</sup>. The enhanced germination percentage was observed in the seeds treated with 60<sup>th</sup> day compost which is due to the growth promoters present in the extract after complete decomposition.

## Conclusion

All these data would facilitate to understand the composting parameters which could help in reducing the waste generated during production of herbal pharmaceutical products and opens a new avenue for organic farming.

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