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Changes in Colour and Germination Index as Indicators for Compost Maturity

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Changes in colour and germination index as indicators of the compost maturity were monitored during the composting of *Conocarpus erectus* residues. The residues (leaves and small stems) were collected, cut into small pieces, moistend (65%) and mixed and placed in 2 bioreactors. The total volume of the bioreactor was 0.03 m^3. Airflow was supplied at a rate of 10 L/min. The results showed that the temperature increased and reached its maximum (54^{o}C) after 36 hrs and then decreased to 26^{o}C. The C/N ratio decreased from 25.22 to 18.48. A gradual darkening took place and the final compost was black in colour. The colour variables (L*, a*, b*) decreased with time and consequently, the ΔE*ab increased with time as indication of the colour change. The germination (G%) of radish seeds was 75.84, 65.50, 86.18, 99.96, 103.41 and 103.41%, the root length (RL%) was 83.33, 79.17, 116.67, 175, 191.67 and 202.08%, and consequently, the germination index (GI%) was 63.34, 52.14, 100.83, 175.37, 198.2 and 208.97% after 0, 3, 6, 9, 12 and 15 days, respectively. It can be concluded that the changes in colour and germination index could be used as indicators for the progress of composting and compost maturity.

Key words: *Conocarpus erectus* residues; Compost; Colour; Germination index.

Production of organic wastes is increasing while soils are progressively losing organic matter due to intensive cultivation and climatic conditions (Massiani and Domeizel, 1996). Recycling of organic wastes in agriculture after appropriate biological treatment can produce valuable organic matter and be of great interest in countries where soils are depleted (Hassen *et al.*, 1998). Composting is useful for waste recycling and produces a chemically stable material that can be used as a source of nutrients and for improving soil structure (Castaldi *et al*., 2005). Composting is an organic waste treatment technology having the capacity to transform organic wastes into well-stabilized product that can beneficial to agriculture (Jindo *et al.*, 2012a). Compost maturity generally relates to the agricultural value of the compost in relation to its application. The degree of compost maturity required for an agricultural application depends on the type of application, but regardless of this, there is a baseline requirement for stable compost (Cabañas-Vargas *et al*., 2005). Generally, most studies in composting have focused on physico-chemical parameters to evaluate both process evolution and compost quality (Said-Pullicino *et al.*, 2007; Albrecht *et al.*, 2008).

Colour has been described as a physical parameter to assess the compost maturity and stability (Jiménez and Garcia, 1989; Inbar *et al*.,

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1990). There is a strong possibility that the use of CIELAB colour variables to characterize the organic matter composition of the materials at different stages of composting may lead to solving the problem of assessing organic matter stabilization during composting. The CIELAB colour variable offers a new, simple, rapid and inexpensive means of evaluating compost stability and its quality prior to agricultural use (Khan *et al*., 2009b).

Phytotoxicity is one of the most important criteria for evaluating the suitability of compost for agricultural purposes and to avoid environmental risks before these composts can be recycled back to agricultural land (Tiquia *et al.*,1996; Brewer and Sullivan, 2003; Cooperband *et al*., 2003). Previous research work has demonstrated that application of immature compost onto the soil causes negative effects on seed germination, plant growth and development. These effects occur because immature compost induces high microbial activity (which reduce oxygen concentration in the soil), blocks the existing soil available nitrogen (Zucconi *et al.*, 1981a). Immature compost also introduces phytotoxic compounds such as heavy metals (Tam and Tiquia, 1994), phenolic compounds (Wong, 1985), ethylene and ammonia (Tam and Tiquia, 1994), excess accumulation of salts (Tam and Tiquia, 1994) and organic acids (Manios *et al.*, 1989), which could retard seed germination and plant growth (Selim*et al*., 2012). Acetic acid is probably the most damaging organic acid released from immature compost, but there are also other compounds that contribute to the phytotoxic effect (Ozores-Hampton, 1998). Seed germination and plant growth bioassay are the most common techniques used to evaluate compost phytotoxicity (Kapanen and Itavaara, 2001). The one of the most widely parameters accepted to evaluate compost maturity is the germination index (GI), which is a measure of phytotoxicity, has been considered as reliable indirect quantification of compost maturity (Huang *et al*., 2006). The GI is a maturity test based on seed germination and initial plant growth using a liquid extract from the compost (Zucconi *et al*., 1981b). It reflects the phytotoxicity of the compost extracts at different stages of composting (Cabañas-Vargas *et al*., 2005). Generally, several investigators reported that phytotoxic compounds are gradually eliminated during the composting process, which could explain the GI increases with the composting time.

Conocarpus erectus residues as agricultural wastes are very rarely utilized and consider as a source of environmental pollution. The utilization of these wastes in the production of compost is very important from the environmental and agricultural point of view. Therefore, the aim of this study was to evaluate the compost maturity of *Conocarpus erectus* L. trees residues in terms of changes in colour and germination index.

MATERIALS AND METHODS

Materials

Conocarpus erectus L. trees residues (leaves and small stems) were collected from the streets of Riyadh City, Saudi Arabia) and cut into small pieces.

Composting System

A static composting system was designed at the Educational Farm, Agricultural Engineering Department, College of Food and Agriculture Sciences, King Saud University, Riyadh City, Saudi Arabia. The system consisted of two bioreactors, ventilation unit and a temperature measurement unit. The bioreactor used is cylindrical (55-L) and made of thermoresistant material (galvanized iron) with a perforated plate at the bottom to distribute the air supplied from the outside. The total volume of the bioreactor is 0.03 m^3. The bioreactor was surrounded with insulator (rock wool) (2cm) to maintain minimum heat loss from the wall of the reactor. Air from a compressor was used for aeration. The airflow was supplied (12 hrs a day) at a rate of 10 L/min to the bottom of each bioreactor (0.2 L/min/kg) for 5 min period intervals. Three thermocouples (type T) were placed near the center of the bioreactor for temperature measurements. The thermocouples were connected to the data acquisition unit (Multiscan 1200) and then to computer.

Composting Method

Moisture content of the mixture was adjusted to 65% only at the start of the experiment. After adjusting the moisture content, the mixture was transferred to the bioreactors. Two bioreactors were used for composting. The reactors were filled

up to 80% of total volume. Thermocouples were connected inside the mixture to measure the temperature and the air was interred the bioreactor as mentioned above for 9 days. Afterwards, the compost from the two bioreactors was pooled and transferred to a container for maturation (6 days) and the aerobic conditions were maintained by opening a small part in the cover of container. During the composting process, changes in colour and germination index were investigated. Also, changes in temperature and C/N ratio were monitored.

Sampling

Three samples (10.0 g each) were taken at random from different locations of the bioreactor. Composite samples were transferred aseptically in closed bags to the laboratory for analyses. Sampling was done every 3 days.

Analytical Methods

Temperature

Temperature was monitored by insert three thermocouples (type T) inside the compost mixture in each bioreactor at different locations (near the center) and connected to the data acquisition unit (Multiscan 1200) and then to the computer. It was recorded every 12 hrs for 9 days during the composting process, whereas during the maturation period, it was recorded every 3 days. The ambient temperature was monitored also during the period of experiment.

C/N Ratio

The ash content (×) was determined after drying the sample at 105°C for 24 hrs and ashing at 550°C in a muffle furnace for 5 hrs (WHO, 1978; Wu *et al*., 2000; Cabañas-Vargas *et al*., 2005; Jindo *et al*., 2012a). Organic matter (OM) and organic carbon (OC) were estimated as follows: OM (%) = 100-× (%), OC (%) = OM (%)/1.8 as mentioned by several investigators (WHO, 1978; Faure and Deschamps, 1990; Abdullah and Chin, 2010). Total nitrogen (N) was determined by an automatic C/N/S elemental analyzer (Jindo *et al*., 2012a, b; Tian *et al*., 2012), while the C/N ratio was calculated using values of the organic carbon and the total nitrogen (WHO, 1978).

Colour

The changes in colour were assessed by visual observation (Khalil, 1996; Pan and Sen, 2013) and by using CIELAB colour space (Khan *et al*., 2009a,b). The changes in compost colour over the composting time by using CIELAB colour space were measured directly from the bottom of a glass Petri dish filled with wet sample or ground oven-dried sample using a Minolta Colour Reader, CR-13 (0° viewing angle and CIELAB colour space). The colour reader was initially calibrated with a clean empty Petri dish placed on a white tile. The parameters used to evaluate the changes in colour are L*, a*, b* and ”E*ab. The symbol L* is known as “lightness” and its value extends from 0 (black) to 100 (white). The symbol a* indicates the colour ranging from red through green. A high a* value means that the sample is more red and less green in colour. The symbol b* Indicates the colour ranging from yellow to blue. A high b* value means that the sample is more yellow and less blue in colour. The ”E*ab indicates the colour difference of the compost over time and it was calculated by subtracting the colour values of the initial material from the colour values of the compost on a specific day of composting and inserting these values into the following formula:

$$\Delta E*ab = \sqrt{(\Delta L*)2 + (\Delta a*)2 + (\Delta b*)2}$$

Germination Index

Germination index (GI) was determined using an adaptation of the method of Zucconi *et al*. (1981b). 10 g of sample were shaken with 100 ml of distilled water for 1 hr. The suspension was centrifuged for 15 min at 3000 rpm and the supernatant was filtered through a Whatman No.6 filter paper. Ten seeds of radish (*Raphanus sativus*) were placed on filter paper Whatman No.1 in a Petri dish of 10 cm diameter. The seeds were scattered with optimum distance between them in the dish. Two ml of the compost extract were added to each Petri dish, whereas 2 ml of distilled water were used for control. Then the dishes were covered with the lids. The test was run in triplicate. The Petri dishes were incubated at 28°C in the dark. At the end of 5 days period, germinated seeds were counted and the root lengths were measured. The relative percentage germination was calculated as follows:

$$\text{Relative percentage germination (G\%)} = \frac{\text{No. of seeds germinated in compost extract}}{\text{No. of seeds germinated in distilled water (control)}} \times 100$$

The relative percentage root length was also calculated as follows:

$$\text{Relative percentage root length (RL\%)} = \frac{\text{Mean root length of seeds germinated in compost extract}}{\text{Mean root length of seeds germinated in distilled water (control)}} \times 100$$

Finally, the germination index (GI) expressed as percentage of control was calculated using the following equation:

$$\text{Germination index (GI)} = \frac{\text{Relative percentage germination} \times \text{Relative percentage root length}}{100}$$

The control GI value is considered as 100%. The germination indices were then plotted to make a comparison between the sensitivity of seeds towards compost age.

RESULTS AND DISCUSSION

Temperature

The temperature is one of the main parameters to evaluate the composting process, since its value determines the rate at which many of biological reactions take place as well as the sanitation capacity of the process (Bustamante *et al.*, 2008). In the present study, the changes in temperature are shown in Figure 1. The temperature began to rise soon after the establishment of composting conditions and reached its maximum (54°C) after 36 hrs and then decreased gradually and reached to 26°C by the end of composting. It is noticed that the temperature remained in the range of 40-54°C (for about 80 hrs) which is suitable with the other parameters such as aeration and moisture content for microbial and enzymatic activities and therefore the increase of organic matter degradation. It is mentioned that reaching the peak temperature is very important because the peak temperature of 50-60°C causes further degradation of organic matter and destruction of all pathogens (Ko *et al.*, 2008). It is generally agreed that the temperature of the composting process should not exceed 60°C to avoid thermal inactivation of the desired microbial community necessary for the efficient degradation of organic wastes (Fogarty and Tuovinen, 1991). Temperature of the end product was very low (as the ambient temperature) and this could be attributed to the completion of compost maturity.

C/N Ratio

C/N ratio is a traditional parameter, which has been used to evaluate the compost maturity and stability as it defines the agronomic quality (Zhiyi, 2004). The initial carbon to nitrogen (C/N) ratio is one of the most important factors influencing compost quality (Michel *et al.*, 1996). In general, initial C/N ratios of 25-30 are considered ideal for composting (Kumar *et al.*, 2010). The changes in C/N ratio are shown in Figure 2. The results indicated that the C/N ratio decreased with time from 25.22 to 18.48 and this could be attributed to the suitability of moisture content, aeration and temperature for composting process. It was reported that a C/N ratio below 20 is indicative of an acceptable maturity (Poincelot, 1974), a ratio of 15 or even less being preferable (Jiménez and Garcia, 1989). Generally, the decrease in C/N ratio can be taken as a reliable index of compost maturity when combined with other parameters as mentioned by Goyal *et al.* (2005).

Colour

By using the visual observation, a gradual darkening took place during the composting process and the final compost was dark-brown or greyish-black. This gave indication of the maturity progress. The obtained result is in agreement with that reported by several investigators (Gotaas, 1956; Jiménez and Garcia, 1989; Diaz *et al*., 1993). The use of CIELAB colour space for compost characterization is a new approach in compost science. The principal advantage of using CIELAB colour space is the uniformity in its associate chromaticity diagrams (Khan *et al*., 2009a). The changes in CIELAB variables (L*, a*, b* and ”E*ab) calculated over the composting time are illustrated in Fig. 3A and B. The lightness L* value decreased from 22.47 to 20.85 and from 40.70 to 35.53, the a* value decreased from 2.13 to 1.02 and from 4.13 to 3.6 and the b* value decreased from 3.4 to 1.5 and from 13.03 to 9.88 over the composting time in case of wet and dry compost, respectively and consequently, the ”E*ab increased with time as indication of the colour change. The obtained results are in agreement with those found by Khan *et al*. (2009a, b), except a* value decreased with time. In general, composting materials gradually turn black due to the evolution of humic substances from the decomposition of organic matter (Fukai and Matsumoto, 2000; Wu and Ma, 2002). Therefore, as expected, the value of L* gradually decreased over time. It has been reported that the yellow colour (b* value) decreases gradually and turns more blue with increasing composting time. The changes in colour of compost are not only due to simple changes in physical properties like

moisture but also due to changes in the content of carbon dioxide or volatile organic acids (Brinton and Droffner, 1994). In the present study, change in the measured CIELAB variables of the *Conocarpus erectus* compost reached near the plateau after 12 days of composting; indicating that compost was nearly stabilized after day 12. It was mentioned that the major colour changes, especially the L* and b* values, occurred during the immature stage, and the CIELAB variables were more stable during the mature stage. The directly measured colour variables (especially b*) are very closely related with the decrease of easily degradable organic matter indicated by OC, C/N, though less obvious for a* and L* during the composting process (Khan *et al*., 2009b). Generally, CIELAB colour variables have the potential to be used for characterizing compost quality for agricultural use as mentioned by Khan *et al*. (2009b).

Germination Index

Compost maturity is an important factor affecting the successful application of compost for agricultural purpose (Inbar*et al*., 1990; Wu and Ma, 2002) and their general marketability (Butler *et al*., 2001). The seed germination test is a widely accepted protocol for evaluating the compost phytotoxicity as well as the compost stability (Zucconi*et al*., 1981b; Tiquia *et al*., 1996). Fuentes *et al*. (2004) observed that seed germination has been regarded as a less sensitive method than root length when used as a bioassay for the evaluation of phytotoxicity. The seed germination bioassay could be relatively low sensitive to many toxic substances, because many chemicals may not be absorbed by seeds and the embryonic plant draws its nutritional requirements internally from seed stored materials and is effectively isolated from the environment (Kapustka, 1997; Araujo and Monteiro, 2005). The roots are responsible for absorption and accumulation of metals so the root lengths were more affected by the concentration of the compost (Oncel *et al*., 2000; Araujo and Monteiro, 2005). The response of radish

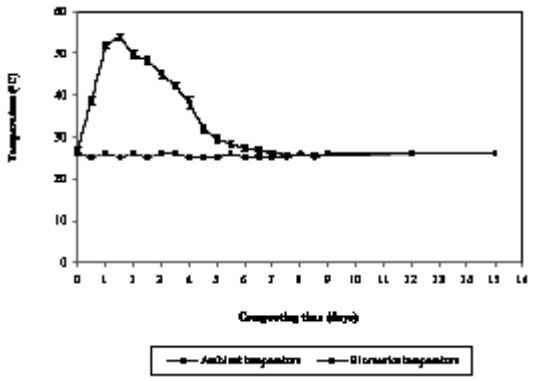

Fig.1. Changes in temperature (°C) during the composting of *Conocarpus erectus* residues. Values are means of 6 replicates ± standard deviations.

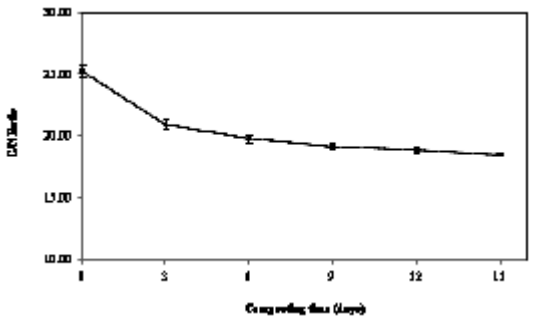

Fig. 2. Changes C/N ratio during the composting of *Conocarpus erectus* residues.Values are means of 3 replicates ± standard deviations**.**

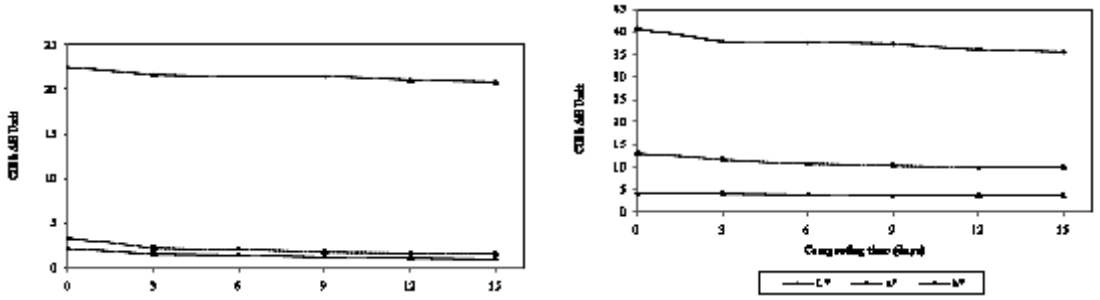

Fig. 3A. Changes in colour CIELAB variables (L*, a* and b*) during composting of *Conocarpus erectus* residues (wet compost (A), dry compost (B)). Values are means ± standard deviations (error bars are not shown since they are smaller than the symbols).

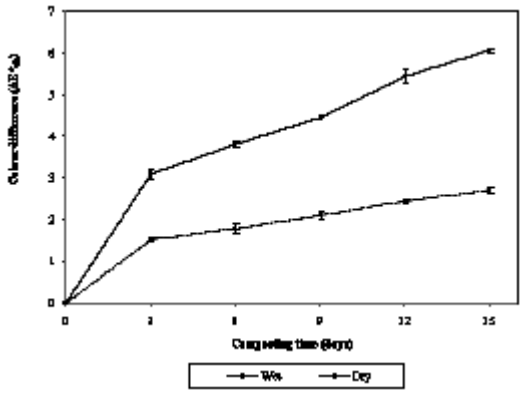

Fig. 3B. Changes in colour CIELAB variable (ΔE*ab) during composting of *Conocarpus erectus* residues. Values are means ± standard deviations.

(*Raphanus sativus*) to the toxicity of the compost water extract of *Conocarpus erectus* residues during the composting period in term of the relative seed germination and relative root length percentages was illustrated in Figure 4a and b. The relative percentage germination (G%) as % of control was 75.84, 65.50, 86.18, 99.96, 103.41 and 103.41%, whereas the relative percentage root length (RL%) as % of control was 83.33, 79.17, 116.67, 175, 191.67 and 202.08% after 0, 3, 6, 9, 12 and 15 days, respectively. From the beginning of the composting up to 6 days, the germination rate of seeds decreased compared to the control (distilled water). The percentage of seeds that germinated increased significantly at the later stages of composting. The initial reduction in germination may be attributed to the release of a high concentration of ammonia and to the presence of low-molecular-weight organic acids as mentioned by Wong (1985). Also, the root length (RL%) decreased compared to the control up to 3 days and increased significantly after that. Generally, the obtained results of the germination tests indicate the compost maturity and the lack of any compounds that would be toxic to the plants or interfere with seed germination as mentioned by Koledzi *et al.* (2011).

The germination index (GI), which combines measures of relative seed germination (G%) and relative root length (RL%), has been used to evaluate the toxicity of compost (Tam and Tiquia, 1994; Tiquia *et al.*, 1996; Wong *et al.*, 2001). The GI is a sensitive indicator of maturity and phytotoxicity (Tiquia *et al.*, 1996). The GI of 50% has been used

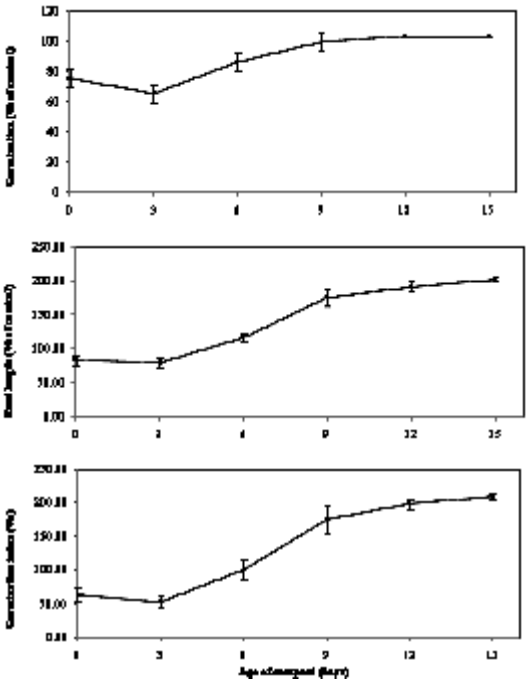

Fig. 4. Changes in germination (A), root length (B) and germination index (C) of radish (*Raphanus sativus*) using compost extract of *Conocarpus erectus* residues. Values are means ± standard deviation. G (%) or RL (%) in the control was always 100 ± 0.0.

as an indicator of phytotoxin-free composts (Zucconi *et al.*, 1981a; Madan *et al.*, 2012). A GI value of 80% indicated the disappearance of phytotoxins in composts (Zucconi *et al.*, 1981b), a GI of more than 80% indicates phytotoxic-free and mature compost (Tiquia and Tam, 1998). Also, the compost is considered mature when the GI is higher than 60% as mentioned by Zucconi and de Bertoldi (1987). It was stated that the values of GI for final composts were higher than 60%, which means that composts did not exhibit phytotoxic characteristics (Cunha-Queda *et al.*, 2007). In the present study, GI% of radish (*Raphanus sativus*) using the compost water extract of *Conocarpus erectus* residues during the composting period is shown in Fig. 4c. It was 63.34, 52.14, 100.83, 175.37, 198.2 and 208.97% after 0, 3, 6, 9, 12 and 15 days, respectively. At the beginning of composting, the GI of radish was low compared to the control, probably due to the phytotoxic effects of ammonia and low molecular weight organic acids as mentioned by Zucconi*et al.* (1981b) and Wong *et al.* (2001), but as composting preceded, the GI value

increased. However, after 6 days, the GI value increased to greater than 100% and reached to 208.97% at the end of composting; revealing that the phytotoxicity was eliminated after composting. The elimination of phytotoxicity has also been widely used as a measure of compost maturity (Wu and Ma, 2001; Butler *et al*., 2001; Meunchang *et al*., 2005). Generally, the high observed GI value for the produced compost (208.97%) indicates that it is stable and mature.

CONCLUSION

It can be concluded from the obtained results that the changes in colour and germination index during the composting process are useful indicators for assessing organic matter characterization and the progress of composting and compost maturity. The decreases in temperature, C/N ratio and CIELAB colour variables at the end of composting are good indicators for the compost maturity. There is a good relationship between the decrease in these parameters and the increase in germination index. Therefore, the changes in colour and germination index could be used as indicators for the progress of composting and compost maturity when combined with other parameters such as temperature and C/N ratio.

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