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(Original Article)

Azolla as a Source of Organic Nitrogen and its Role in Recycling Agricultural Residues and Improving the Quality of Compost

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Abstract

Composting is a process which converting the various components in organic residues into relatively stable humus-like substances that can be used as organic fertilizer or soil amendment. The aim of this study was to research the role of *Azolla pinnata* for improving the quality of compost produced from date palm trees frond residues. Treatments included two types of composts: C1) Palm tree fronds + *Azolla* + microbial inoculum, C2) Palm tree fronds + chemical activator (45kg ammonium sulfate +10 kg superphosphate /t plant residues) + microbial inoculum. For all treatments the initial C/N ratio was 37:1. The results showed that the temperature was increased to 67 and 68.34 ºC for C1 and C2, respectively and then decreased by the end of the composting period to 19.5 ºC for two treatments. The final product had brown color and natural soil odor, and C/N ratio was 16:1 and 18:1, EC was 2.60, 2.47 dSm-1, and recorded total nitrogen was 1.78, 1.70 for mixt 1 and 2 respectively. The results found that adding *Azolla* for date palm trees frond residues compost improve compost quality comparing with compost from Palm tree fronds + chemical activator.

***Keywords**: Azolla pinnata; Composting; Palm tree fronds; Quality; Microbial decomposer.*

Introduction

Azolla species is a small, free-floating fern, can survive a wide range of pH (3.5 to 10.0) and temperatures (5–45°C) (Sadeghi *et al*. 2013), has a fast growing pattern a very high growth rates can reach by asexual reproduction with a doubling time 2-5 days only (Wagner, 1997; Sadeghi *et al*., 2013). All N requirements of this fern frond can meet, by a symbiosis with cyanobacterium an N_2 fixing, *Anabaena azolla*, which lives in the dorsal lobe cavity of plant's leaves (Esteves-Ferreira *et al*. 2017). The *Azolla* biomass decomposition can release much N (75–80% of total accumulated) in paddy field for plant uptake (Roy *et al*. 2016). *Azolla* is rich in protein, vitamins (vitamin A, beta-carotene, vitamin B12), essential amino acids, growth promoters and minerals such as calcium, phosphorus, potassium, copper, iron, magnesium, etc. It contains on a dry weight basis 25-35%

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protein, 10-15% mineral content and 7-10% combination of amino acids, bioactive substances and biopolymers (Kathirvelan *et al*., 2015) *Azolla* has a high feeding value, where it is a source of nutrients (Hossiny *et al*. 2008) and very fit for many uses in agriculture and aquaculture (Pabby *et al*., 2004; Sithara and Kamalaveni 2008; Kollah *et al*., 2016). The date palm trees are available normally in the Northern Africa and Middle East. There are more than 100 million date palm trees and, which can grow for more than 100 years (Sadik, *et al*., 2012). Date palm trees produce large quantity of agricultural waste, for example, each date palm tree produces about 20 kg of dry leaves yearly (Barreveld,1993). Aswan have more than 3 million date palm trees each one produced about 35 kg could be composted instead of burning in farms and causing environment serious threat.

The soils in Egypt are very poor in their organic matter content which rarely exceeds 2 % and is most less than 1 % or even less than 0.1 % in some newly reclaimed soils. (Abdel-Motall, 2004). Composting is the natural process of decomposing organic material. Which defined as an aerobic process depends on the microorganism population, which transforming the organic ingredients of waste into stabilized humus and less complex compounds by decomposed the organic waste of animals and plants, destroying weed seeds and pathogens, nutrients stabilization, as well as controlling of diseases or toxins. Final product used as a growth substrate for ornamental and/or horticultural plants, an amendment for soils and as substrates for microbial inoculants. It therefore benefits agriculture by providing resources and natural regulating mechanisms to replace expensive inputs that may be harmful to the environment ensuring long-term agricultural sustainability (Ayilara *et al*., 2020). Carbon and nitrogen compounds transformed during the composting process and used utilize carbon as a source of energy and nitrogen to build cell structure thereby producing CO_2, H_2O, NH_3, heat, organic acids, and mature compost product at the end of the process (Marche *et al*. 2003; Hamdy 2005 and Bernal *et al*. 2009).

Therefore, this study aimed to investigate the possibility of using *Azolla* as a source of nitrogen compared to chemical sources (chemical activator) to improving compost quality produced from date palm tree frond residues.

Materials and Methods

Preparation of microbial inoculum

In this study we used a cellulose-degrading fungal inoculum containing two strains (*Trichoderma viride* and *Trichoderma harzianum*) acquired from Microbial. Department of Soil, Water and Environment. Research Institute, A.R.C. Giza. The number during inoculation of viable cells in the inoculum is 1.7 x 10^8 CFU/ml. The inoculum solution of cellulose-degrading fungi is activated in a mix of molasses (sugar cane) and water, mixing 1part of molasses to twenty parts of water. Then it’s kept in a sealed expandable container for three days to ferment. For pile inoculation, add the inoculum solution until the moisture content of the compost mixture reaches 60% (wet basis).

Preparation of compost and the composting processes

The experiment carried out at Kom Ompo Agricultural Research Station. Two piles were prepared using date palm tree frond residues. C1) Palm tree fronds + *Azolla pinnata* (500Kg dry *Azolla*/t plant residues) + microbial inoculum, C2) Palm tree fronds + chemical activator (45kg ammonium sulfate +10 kg superphosphate /t plant residues) + microbial inoculum. The chemical compositions of the date palm wastes, and *Azolla* plant used in this experiment were as given in Table (1). The date palm fronds manually chopped into small pieces and then shredded with a mechanical shredder into smaller pieces of 6-10 cm to hasten composting process. The amount of each material was calculated to attain a mixture of initial C/N ratio around 37: 1. The materials of each pile were thoroughly mixed for homogenization purpose, and the weight was about 150 kilograms for each pile. Maintained the moisture content between 50-60% during the period of composting by watering. The composts were stirred every week to ensure having a good aeration. The composting process was continued for 11 weeks, including maturation period. Every 2 days temperature was recorded at 60 cm depth. Considered the compost mature when its structure became friable and crumbly, and temperature was close to ambient temperature.

Table 1. Some characteristics of raw material used in composting process

Character	TN%	OM%	OC%	C/N Ratio	Ash	TP%	TK%
Palm tree residues	0.48	88.88	51.55	107/1	11.12	0.16	0.20
Azolla pinnata	3.00	78.20	45.35	15/1	21.8	0.7	3.5

TN: total nitrogen, OM: organic matter, OC: organic carbon, C/N: carbon/nitrogen, TP: total phosphorus, TK: total potassium

Physical and chemical analyses

- **Changes in temperature:** Every two days the temperature measured during the period of composting by using a digital thermometer. which dipped into the compost pile at depth 60 cm approximately for about five or six minutes before the reading is take.
- **Moisture content**: Compost samples were dried at 70 °C until weights constant (Page *et al*., 1982). The moisture content was calculated as a percentage.
- **Bulk Density of composts:** Bulk density was determined using the core method according to Vomocil (1965).
- **pH value:** Measured the values of pH in suspension compost, distilled water ratio 1:10 (w/v) according to (Hue and Liu, 1995).
- **Electrical Conductivity (EC):** Measuring electrical conductivity run in (1:10) compost: water extract, using EC meter ICM model 71150 according to (Richards, 1954).
- **Organic Matter (OM)**: Determined the organic matter content by burning the dried compost samples at 550 ºC to a constant weight, as recommended by Page *et al*. (1982).
- **Organic Carbon (OC):** Organic carbon was determined according to Page *et al*. (1982).
- **Total Nitrogen (TN)**: Total nitrogen was determined using the method Kjeldahl digestion reported by Jakson (1973).

- **Soluble nitrogen (ammonium and nitrate-nitrogen)**: Soluble nitrogen forms in compost i.e. NH_4^+, NO_2^-, and NO^- were determined according to the method outlined by Page *et al*. (1982).
- **Total Phosphorus (TP):** Acid solution of the digested compost samples was used for determined total phosphorus content using ascorbic acid as a reluctant (Page *et al*. 1982).
- **Total Potassium (TK)**: Digested solutions of compost samples were used for determination of total potassium content by flame photometrically (Chapman and Pratt, 1961).

Results and Discussion

Physical and chemical change during the composting process

Change in temperature

The changes in temperature during the composting process for the two piles are shown in Figure (1) The temperature curves within the piles showed three classic phases: mesophilic, thermophilic and cooling down phases. All treatments showed rises in the temperature immediately after composting begins. The temperature on day 2, rose from 38.5 and 38.9^{0}C to 56.2, 46.5 ^{0}C for composting treatments C1and C2, respectively. The temperature shows an increase especially compost C1, this rapidly rising of temperature especially in C1 during composting was due to the microorganism activity in the waste (Hafeez, *et al* 2018), which enhanced by *Azolla* addition. The temperature continued to rise until day 20, reaching its highest temperature of 67^{0}C in C1, while it reached a temperature of 68.5 ^{0}C on day 26 in C2. The temperature of composting decreased slowly and at the end of the composting processes, the temperatures of all treatments reached to a minimum level of 19.5°C. Increasing the rate of organic matter decomposition by inoculation with more active microorganisms may being the reason of the rapid increasing in temperature. (Hanajima *et al*., 2006).

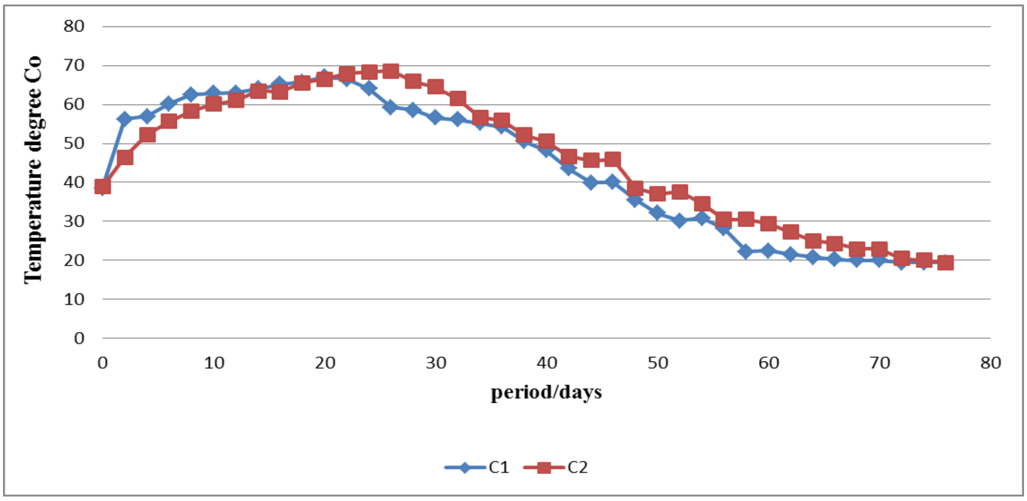

Fig.1.Change in temperature during the composting period

Change in Bulk density

Bulk density values for all treatments increased graduals as the composting proceeds. The bulk densities increased from 384 to 510 kg/m^{3}and from 310 to 465 kg/m^{3}for piles mix 1 and 2 respectively. The variations in the initial bulk density values could be attributed to the physical characteristics and weights of the organic wastes and to the additive conditioners used for making the composting mixtures at initial time before heaping. During the composting process, due to the degradation of the fibrous structure of cellulose and lignocellulosic compounds, the general particle size has changed from larger particles to smaller particles, especially the fine particle size distribution, which can indicate compost maturity (Mahmoud, 2010)

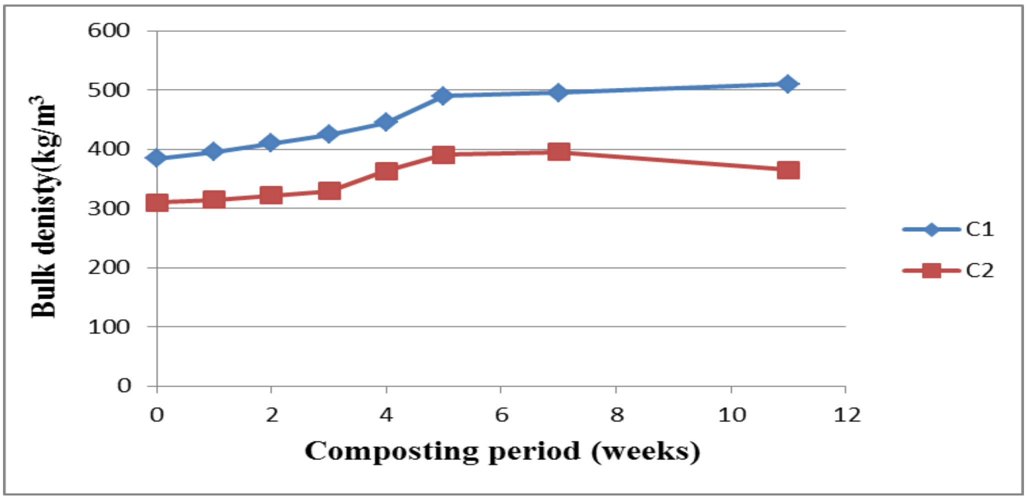

Fig.2. Change in bulk density during the composting period Change in pH

Like temperature, pH can be a good parameter for the development of bio oxidative stages and the development of microorganisms (Jimenez and Garcia, 1991). The optimum pH for composting is 5.5–8. (Zhang *et al*., 2019). The two compost heaps showed similar pH behavior with the lowest pH recorded 6.4 and 5.7 for mix1and mix 2 respectively in the initial of composting period. According to Figure (3), the data showed that pH tend to increase in C1 and C2 from 6.4 to 7.2 and from 5.7 to 6.4, in the second week. This increase in the first period may be due to the production of ammonia and mineralization of organic matters, resulting from microorganism's activities (Rasapoor *et al*., 2009; Gajalakshmia and Abbasi, 2008; El Fels *et al*., 2013)**.** This result agreed with the result of

ammonium concentration in the tables (2 and 3). At the end of the composting period pH declined to 6.8 and 6.00 for pile C1 and C2 respectively. This can be explained by the rate of ammonification and mineralization which started to slow down in end of the second week, so pH values declined gradually. In general, the value of pH remains stable with an optimum value of 6-8 in the compost (Amir *et al*., 2005; Zenjari *et al*. 2006).

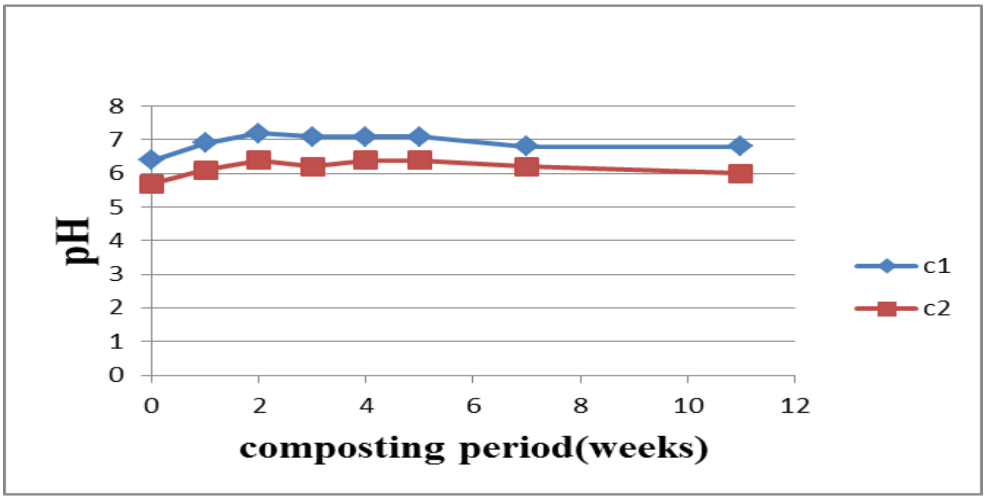

Fig.3. Change in pH during the composting period.

Electrical conductivity

The electrical conductivity (EC) in this experiment of piles C1and C2 are represented in Tables (2 and 3) and shown in Fig. (4), the EC in the initial time were 2.70 and 4.32 dsm^{-1} for C1and C2, respectively. On the first week the value of EC decreases from the beginning of the composting period in the treatment C1 and C2, then its increase and reached to the highest values 2.92, 4.46 dSm^{-1} in the third and second week in C1 and C2 respectively. The increase in the conductivity of all compost materials could be referred to the high concentration of ammonia and other nutrient ions released during the mineralization of organic matter. At the end of the composting process EC reached 2.60 and 2.47 dSm^{-1} for C1and C2. The changing in the EC value between increase and decrease could be attributed to the ions fixation and/or nutrient ions release by the changes in proliferation of the aerobic microbial populations. The relative fluctuation observed at end period of composting similar results had been reported by (Irvan *et al*., 2018).

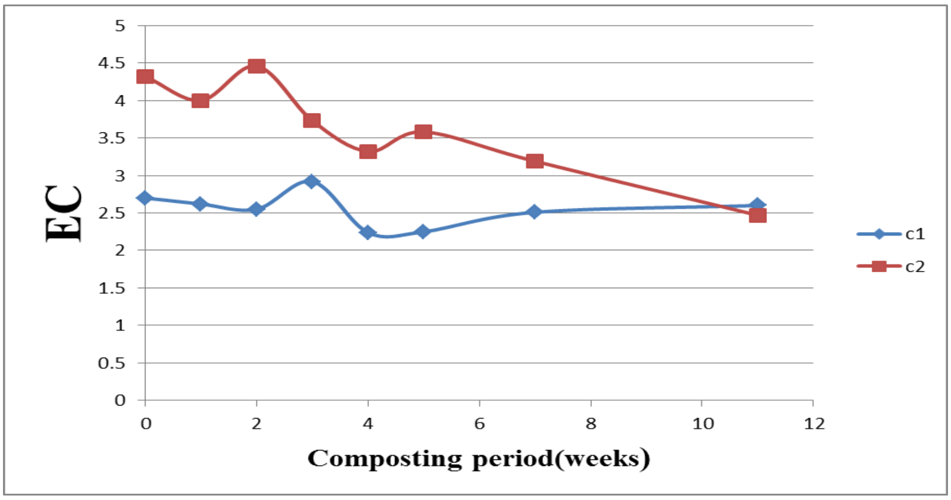

Fig.4. Change in EC during the composting period.

Table 2. Some analytical parameters of compost (C1) during the composting period*

Periods (week)	Moist Content %	Bulk Density kg/m^3	pH (1:10)	EC (1:10) dSm-1	TN %	Soluble N (mg/kg)		Organic Matter %	Ash %	OC %	C/N Ratio	TP %	TK %
						NH_4^+	NO_3						
0	61	384	6.4	2.70	1.31	25	101	83.77	16.23	48.59	37/1	0.32	0.37
1	61	395	6.9	2.62	1.47	195	112	81.45	19.55	46.66	32/1	0.35	0.39
2	60	410	7.2	2.55	1.50	230	123	80.12	19.88	46.47	31/1	0.40	0.41
3	57	425	7.1	2.92	1.55	255	165	78.95	21.05	45.79	29/1	0.45	0.47
4	58	445	7.1	2.24	1.61	143	199	73.15	26.85	42.42	26/1	0.47	0.49
5	56	490	7.1	2.25	1.66	137	226	65.31	34.69	37.88	23/1	0.49	0.57
7	54	495	6.8	2.51	1.69	110	297	60.11	39.89	34.98	21/1	0.52	0.61
11	30	510	6.8	2.60	1.78	65	300	48.21	51.79	27.96	16/1	0.54	0.62

*Each value represents the mean of 3 replicates. TN: total nitrogen, OC: organic carbon, C/N: carbon/nitrogen, TP: total phosphorus, TK: total potassium

Table 3. Some analytical parameters of compost (C2) during the composting period*

Periods (week)	Moist. Content %	Bulk Density (kg/m3)	pH (1:10)	EC (1:10) dSm-1	TN %	Soluble N (mg/kg)		Organic Matter %	Ash %	OC %	C/N Ratio	TP %	TK %
						NH_4+	NO_3^{-}						
0	53	310	5.7	4.32	1.34	711	3	85.73	14.27	49.72	37/1	0.23	0.38
1	55	315	6.1	4.00	1.45	915	65	82.53	17.47	47.86	33/1	0.30	0.40
2	58	382	6.4	4.46	1.50	950	89	80.03	19.97	46.41	31/1	0.36	0.42
3	55	429	6.2	3.73	1.60	996	103	79.73	20.27	46.24	29/1	0.37	0.49
4	60	463	6.4	3.32	1.61	450	211	76.24	23.76	44.22	27/1	0.39	0.53
5	61	491	6.4	3.58	1.65	221	251	68.92	31.08	39.97	24/1	0.42	0.55
7	55	495	6.2	3.19	1.68	112	275	63.00	37.00	36.54	20/1	0.47	0.58
11	28	465	6.0	2.47	1.70	51	293	51.66	48.34	29.96	18/1	0.50	0.61

*Each value represents the mean of 3 replicates. TN: total nitrogen, OC: organic carbon, C/N: carbon/nitrogen, TP: total phosphorus, TK: total potassium

Organic matter (OM)

In all treatments the percentage of organic matter of mature compost was decreased as a result of loss of carbon dioxide during composting (Gajalakshmia and Abbasi, 2008). Table (2) showed the ash content of mature compost increased, and organic carbon decreased. Initially, percentages of OM in all composted materials were 83.77, 85.73% for C1 and C2 respectively. Organic matter percent decreased by the time to 48.21 % in C1 and to 51.66 % in C2. The Figure (5) showed that dry matter loss percentage were high in treatments which contents *Azolla* (C1), the organic matter content decreased rapidly over time, mainly during the active period. During the curing period it slowly declines. This may be due to the fact that easily degradable OM components decompose rapidly in the initial stage, while recalcitrant components such as lignin decompose slowly and tend to decompose gradually during the curing stage (Li and Zhang, 2000; Solano *et al.,* 2001).

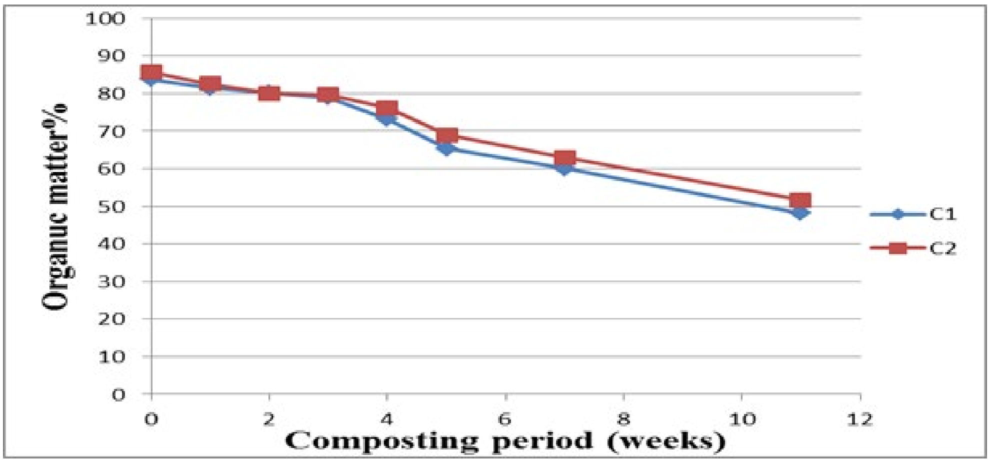

Fig 5. Change in organic matter (OM) during the composting period.

Carbon/nitrogen (C:N) ratio

C/N ratio used to characterize the organic waste decomposition, and the quality of the final compost with respect to the organic matter and nitrogen cycling. Number of studies have found that an initial C/N ratio in the range between 20:1 and 40:1 is optimal for effective composting (Tiquia, 2010). In this experiment C/N ratio decreased from 37:1 to 16:1 for C1 and decreased from 37:1 to 18:1 for C2 during the composting process (Tables 2,3 and in Fig.6). This is closely related to losing organic carbon produced by bio oxidation of organic matter and release of carbon dioxide, followed by a lower reduction in the concentration of organic acids, and an increase in the nitrogen content per unit mass of compost material (El Fels *et al.,* 2013).

C/N ratio in the range of 15 to 20 is generally considered an indicator of this compost maturity (Tumuhairwe *et al*., 2009), also it be indication to an ideal and usable compost (Dougherty, 1999).

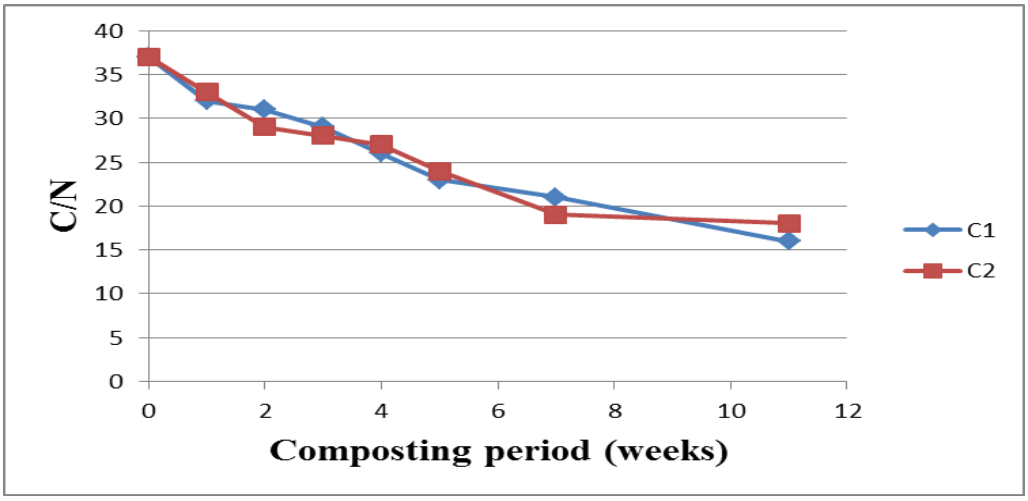

Fig.6. Change in C/N ratio during the composting period.

Total and soluble nitrogen

Nitrogen is the most important elemental nutrient in compost products, as results are shown in Tables (2 and 3). The initial value of total nitrogen in the composting period were 1.31, 1.34 for C1and C2, respectively. In all treatments the total nitrogen content increased variably (Fig.7) and was in the end of composting process 1.78 for treatment C1 and 1.70 for treatment C- 2. This may be due to the mineralization of organic matter during composting (Viel *et al.,* 1987, Zhang *et al*., 2019). In addition, at the end of the composting process the activity of nitrogen-fixing bacteria can also increase total nitrogen (Bishop and Godfrey, 1983).

The concentration changes of ammonium nitrogen NH_4 and nitrate NO_3 during aerobic composting followed the typical trends of these two forms of nitrogen (Figures 8 and 9). The initial values of ammonium nitrogen concentrations of composting treatments C1 and C2 were 25 and 711 mg/kg, respectively. High NH_4-N levels were observed during the high temperature phase of the decomposition phase, reaching maximum concentrations of 255 and 996 mg/kg after two weeks in composting treatments C1 and C2, respectively. This may be due to high ammonification rates and mineralization of organic nitrogen compounds (Mahimairaja *et al*., 1994). Thereafter, the NH_4-N content decreased as the composting process progressed and reached a lower value at the end of the composting period. The absence or reduction of NH_4-N has been found to indicate good composting and ripening processes (Riffaldi *et al*., 1986; Dougherty,1999). The final NH_4-N levels for all treatments of C1 and C2 were 65 and 51 mg/kg, respectively. This increasing and decreasing may be explained by increasing of ammonfying bacteria which reached to its maximum level after two weeks and then tended to decline towards the end of composting period (Zucconi and De Bertoldi, 1987; Dougherty,1999).

After an initial low NO_3 content, as shown in figure (9), the values increased from 101 and 3 in the beginning of composting to 300 and 293 for C1 and C2 respectively in the end of process, due to nitrification, a result that was accompanied by a significant drop in ammonia. It has been reported that nitrification rarely occurs under high temperature conditions because high temperature and excess ammonia reduce the activity and growth of nitrifying bacteria (Morisaki *et al*., 1989). After the complete decomposition reaction of organic waste, nitrifying bacteria usually become more active. This trend may be related to temperature changes and the density of nitrifying bacteria. Apparently, as temperatures drop, nitrate levels tend to rise due to the conversion of ammonia to nitrate through nitrification (Wong *et al*., 2001; Afify, 2002; Allam, 2005).

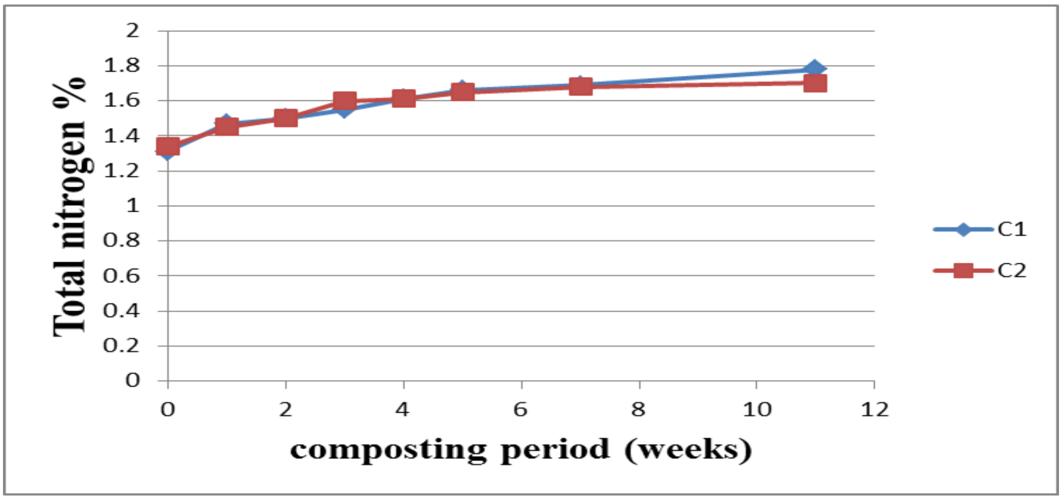

Fig. 7. Change in TN % during the composting period.

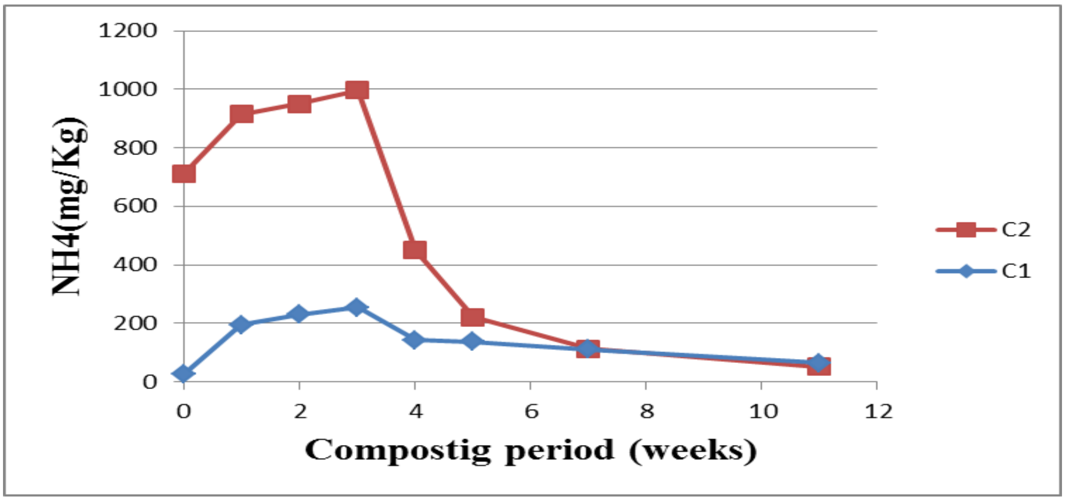

Fig. 8. Change in NH4content during the period.

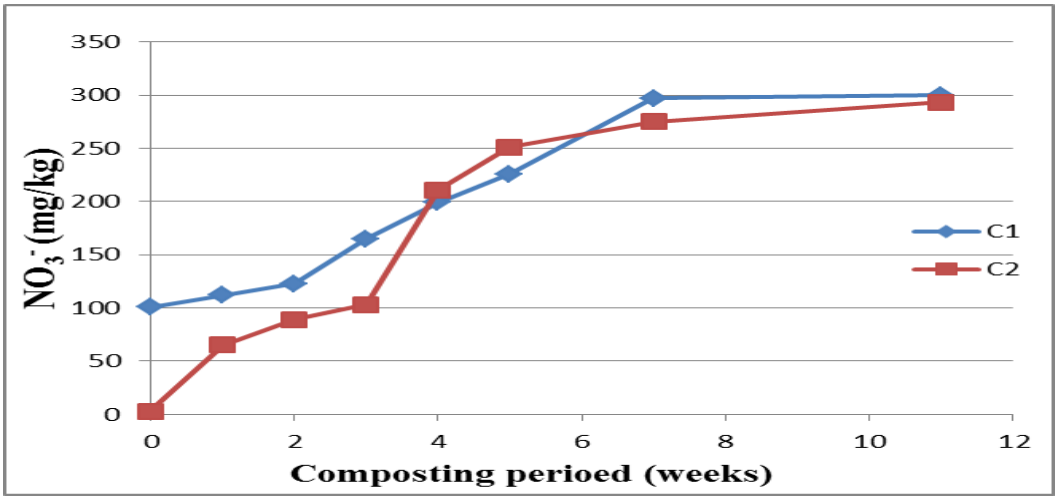

Fig. 9. Change in NO3content in the composting period.

Conclusion

The results presented here enhance the possibility of using *Azolla*, which is characterized by its rapid growth and availability in large quantities, as a good alternative and easy source of organic nitrogen compared to livestock manure or poultry waste, especially in organic agriculture with high economic returns, in addition to its clear role in accelerating the decomposition of agricultural waste and increasing the quality of agricultural waste. The final product. This can be an advantage of using *Azolla.*

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الازولا كمصــــدر للنيتروجين العضـــوى ودورها فى تدوير المخلفات الزراعية وتحســـين جوده الكمبوست

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الملخص

اجريت تجربه بمركز البحوث الزراعية محطه بحوث كوم امبو لدراســـة تأثير اســـتخدام سرخس الأزولا (*Azolla pinnata*) كمصدر للنيتروجين العضوي في تحسين جودة السماد المنتج من مخلفات النخيل. حيث تم اجراء معاملتين وهم:

1. كمبوست1 (C1) ويتكون من: مخلفات النخيل + الأزولا + اللقاح الميكروبي.

2. كمبوست2 (C2) ويتكون من: مخلفات النخيل + المنشط الكيميائي (45 كجم سلفات امونيوم + 10 كجم سوبر فوسفات /طن مخلفات) + اللقاح الميكروبي. وتم ضبط نسبة C/N الأولية لجميع المعاملات عند 37:1.

وقد اظهرت نتائج تحليل بعض الخصائص الطبيعية والكيمائية الاتي:

ارتفاع درجه الحرارة تدريجيا لتصل إلى اعلى ارتفاع لها 67 ،68.34 درجة مئوية لكل من C1، C2 على التوالي ثم انخفضـــت إلى 19.5 درجة مئوية بنهاية فترة الكمر للمعاملتين. وكذلك انخفضت نسبة C/N الى 16:1 ،18:1 لكل من C1، C2 على التوالي ووصلت درجه التوصيل الكهربائي لكل من C1و C2 في نهاية فتره الكمر الى 2.60، 2.47 ديســيســيمنز$^{-1}$ وكانت نســبة النيتروجين الكلى للمنتج النهائي 1.78، 1.70 لكل من C1، C2 على التوالي كما ظهر الســماد العضوي المنتج بلون بني داكن ورائحة التربة.

واظهـرت النتـائج إلـى أن إضـافة الأزولا لمخلفـات النخيـل لانتـاج سـماد عضـوى صـناعى يـؤدي إلـى تحسـين جـودة السـماد وصـفاته مقارنـه بالسـماد المنـتج باسـتخدام المنشـط الكيميائي مع مخلفات النخيل.