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Cultivation of Canola (*Brassica napus* L.) Using Composted Agro-industrial Waste

Hanaa E. Ahmed[1,2], Mohamed E. M. Ali[3], Nabila S. Ammar[*3], Hanan S. Ibrahim[3]
[1]*Department of Botany and Microbiology, Faculty of Science, University of Cairo, Giza 12613, Egypt.*
[2]*Department of Biology, Faculty of Science, University of Jeddah,21589, Saudi Arabia.*
[3]*Department of Water Pollution Research, National Research Center, Dokki,12622, Egypt.*

COMPOSTING is an effective procedure for recycling of agricultural and industrial waste and converting into valuable material. This work focuses on conversion of solid waste of tobacco manufacturing to compost which is useful for agricultural application. As well the compost from tobacco waste was characterized using different techniques and employed as soil amendment for improving its properties. The produced compost showed variation in characteristics; pH, total phosphorus, total organic content, total nitrogen and C/N ratio with time of composting regarding to original waste. Noticeably, total organic was decreased by 35 % and C/N ratio was improved by decreasing from 26.6 to 13.48 after 75 days of composting process. Also, the Potential Toxic Elements (PTEs) in the composted material had been measured as it is a key to use of mature compost for land application. A mixture of sand and tobacco waste compost has a 46.61% of water holding capacity, while it was 25 % for sand alone. Canola seeds grow well in a mixture of sand and tobacco waste compost in referring to some physiological and morphological parameters. Therefore, tobacco compost may be used as a soil amendment for cultivation land. Conclusively, the waste was converted to wealth, and produced compost valorized added value of waste.

Keywords: Tobacco waste, Compost, Canola, *Brassica napus* L., Soil amendment, Antioxidant enzymes

Introduction

Since the industrial revolution, worldwide produce annually large volumes of agro-industrial waste, which cause a serious disposal problem. Therefore, development of appropriate technologies for the reprocessing and reuse of these waste will minimizing their adverse environmental effects [1-3]. A tobacco (Nicotiana tabacum) plant is used for cigarettes production. Where, cigarettes consumption has been rising more or less steadily since the 1970s [4]. For more than 200 years, tobacco farming has been virtually nonexistent in Egypt because of a ban on tobacco cultivation [5]. However, the Egyptian cigarette market is dominated by the Eastern Tobacco Company (ETC), which maintains a nearly 80% market share [4]. ETC has long been the dominant firm in the Egyptian cigarette market and the largest cigarette manufacturer in the Middle East.

Many tons of tobacco waste is generated from various stages of cigarette production process which require its safe disposal. The majority of tobacco plant waste is burned, although it has a high cost of operation to minimize air pollution problems [6]. Also, the presence of high organic content in tobacco waste considers a potential for using it as a soil amendment [7]. Therefore, there was an urgent need to find eco-friendly and

*Corresponding author e-mail: nabilamammar@gmail.com
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economic technology for safe utilization of solid waste of tobacco manufacturing.

Accordingly, the most proper way for utilization of tobacco waste is composting technique, which is considered to be an alternative method for organic waste treatment.

Composting of tobacco waste would be useful for agricultural purposes. The effectiveness of compost use in agriculture depends mostly on the quality of the compost, which is closely related to its stability and maturity. The quality of compost is mainly determined by measuring some physico-chemical properties (temperature, C/N ratio, pH, cation exchange capacity, total organic carbon, NH4, phenols, humic-like substances).

Several studies have been confirmed the biodegradation of nicotine during the process of composting for tobacco waste and it is the available paramount alternative for preventing of environmental pollution [8-14]. Furthermore, different studies were focused on the positive yield responses after addition of the matured tobacco compost waste, which will increase the soil organic matter content as well as to enhance the soil structure and cation exchange capacity. Additionally, improve soil porosity, density, water holding capacity and provides soil with array of nutrients and suitable organic matter [15-18].

Canola (Brassica napus L.) is an important crop for edible oil production, and recent as a biodiesel. Canola is currently the third most important crop after soybean and maize for biodiesel production [19]. In spite of this important it is not widely cultivated in Egypt. Herein, the current work targets the valorizing value of solid wastes of tobacco manufacturing and achieves the waste to wealth perception. Therefore, the objectives of this study is to convert tobacco manufacturing waste to compost and employed as a soil amendment for cultivating canola plants. Moreover, some of antioxidants as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX) of plant extract will be investigated.

Materials and Methods

A suitable amount of tobacco waste was collected from the Eastern Tobacco Company (ETC) for cigarette production, Giza, Egypt. This tobacco waste consisted of by-product of a cigarette manufacturing process such as: tobacco sticks, tobacco dust and rejected cigarettes. An effective microorganism (EM1) of a mixed culture of beneficial microorganisms (primarily photosynthetic and lactic acid bacteria with a minimum of one million colony forming units/ml, and yeast was purchased from the Agricultural Research Center, Egypt.

Experimental design for compost production

Tobacco waste compost (TWC) was performed during the period of 75 days from October to the middle of December. In Egypt, this period of the year is characterized by mean monthly maximum and minimum temperatures of around 28 and 10 °C.

Two kilograms of dry tobacco waste were decomposed in polyethylene plastic container with demissions of 0.35 x 0.50 x 0.30 m. An applicable amount of (EM1) was added to increase the microbial activity and enhance the composting process.

Preliminary experiments were conducted for mixing different ratios of tobacco waste and EM1 in order to optimize the C:N ratio and adjust the moisture content which are necessary for starting composting process. Moisture content of TWC was adjusted to be between 40% and 65% with tap water all over the composting process. The aeration was performed manually by manual flipping of tobacco compost in each container every 7 days at first month, and then manual flipping was performed every 10 days [20].

Analysis of Compost Samples

Compost samples were taken from five symmetrical locations during performing of TWC with time intervals of five days until the end of the composting process (75 days). The data which have SD < 0.1 % were not presented.

Physicochemical Analyses of TWC

The ambient temperature and TWC temperature were recorded directly every 2 days using a digital thermometer probe at a depth of 15 cm at different positions inside the container. The pH was measured in the supernatant suspension of 1:10 compost: deionized water mixture using a standard pH-meter (Jenway, model 3510, United Kingdom). The total nitrogen content of the 10 g of compost sample was determined using the regular- Kjeldahl method [21]. Organic carbon in each sample was measured using TOC analyzer (Analytik Jena Multi N/C 2100).

Total phosphorus (TP) concentration was determined every two weeks using (ammonium vanadate-molybdate method) and measured colorimetric using a spectrophotometer (HACH, model 2800, Germany) at 470nm. Samples moisture content was determined gravimetrically by drying a suitable sample weight at 105 °C over night till constant weight.

Heavy Metals Analysis

The total concentrations of certain heavy metals (Cu, Cd, Pb, As, Zn, Cr, Fe, Hg and Ni) in the applied tobacco wastes, as well as the final matured tobacco composted forms were determined according to Rice et al [22]. All heavy metals analyses were performed on an Atomic Absorption Spectrometer (SpectrAA 220, Varian, Australia). For each series of measurement absorption calibration curve was constructed, composed of a blank and three or more standards. External reference standards from Merck, Germany, and quality control sample from US EPA were used to confirm the instrument metal concentration reading.

Cultivation of Canola plant in the presence of tobacco waste compost

Seeds of canola were sterilized by immersing in sodium hypochloride (2 %) (v/v) for 20 min, rinsed with sterilized distilled water, and sown in plastic pots with area of 0.12 m × 0.12 m, containing 500 g sterilized sand (A) and with mixture of sand and compost (B), (1:1 w/w). The pots were irrigated with tap water and kept in a growth chamber for 16/8 h light/dark photoperiod, light intensity (5000 lux) and temperature of 25 ºC. Water holding capacity was measure for both soil (A) and (B). After 30 days, the dry weight and the photosynthetic pigments of the harvested plants were determined. Total activity of some antioxidant enzymes (CAT, POX and APX) were also measured in canola leaves extract.

Determination of enzymatic antioxidant activities in canola leaves

Crude Canola leaf extracts were prepared from 30 days old plants according to EL-Sath [23] with slight modifications. A known weight of fresh leaves was homogenized to a fine powder in a pre-chilled mortar by aid of acid washed sand with a known volume of 50 mMTris-HCL, pH 7.0 containing 20% (v/v) glycerol, 1mM EDTA, 5 mM $MgCl_2$, and 1mM dithiothreitol (DTT). The extracts were centrifuged at 12000 g for 15 min at 4oC. Extracts were kept at 20 ºC until determination of enzymatic activates. Catalase (CAT, EC 1.11.1.6) activity was measured at 25 °C according to Gong, et al., (2001) [24]. CAT activities were estimated by measuring the decrease in absorbance of H_2O_2 at 240 nm. One unit of CAT activity was defined as the amount of enzyme required to oxidize 1 µmol of H_2O_2 per minute (extinction coefficient 22.4 mM^{-1} cm^{-1}).

Peroxidase (POX, EC 1.11.1.7) activity was measured at 25 ºC according to Nakano and Asada [25], using guaiacol as substrate. POX was estimated by recording the increase in absorbance at 470 nm every 30 sec. One unit of POX activity was defined as the amount of enzyme required to oxidize 1 µmol of guaiacol per minute (extinction coefficient, 0.25 µmol cm^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by monitoring the change in the optical density at 290 nm according to Nakano and Asada [25]. One unit of APX was defined as the amount of enzyme that oxidizes 1 µmol ascorbic acid per min (extinction coefficient, 0.189 mM^{-1} cm^{-1}).

The photosynthetic pigments (chlorophyll a, b and carotenoids) were determined by the spectrophotometric method recommended by Fadeel [26]. The pigment contents were calculated as mg/g fresh weight of leaves.

Results and Discussion

Analyses of produced compost

Temperature profile

Temperature profile for TWC was shown in Fig. 1. There is gradual increase in temperature with composting time till 25 days, after that it was decreased. The heat content is related to microbial activity. The highest temperature (50°C) was observed after 23 days of starting composting process. This increasing in composting temperature levels owing to the heat energy released from the biochemical reactions of the microorganisms in the compost [6]. Thereafter the temperature tended to decrease gradually due to a consumption of the starting substrates and a decrease in microbial activity [27]. Tiquia et al [28] recommended that the variation in compost pile's temperature is a good indicator of compost maturity. Compost temperature was increased with time from 32 to 50 °C, where, the biodegradation rates are maximized those between 45 and 55°C.

pH profile of compost

The pH of tobacco waste composting increased from an initial pH of 5.3 to pH of 8.4 after 31 days (Fig. 2). This increasing in pH values during the composting process may be explained by the production of ammonium as a result of the mineralization of tobacco proteins [29]. Additionally, it may be due to the microbial decomposition of the acids accompanied by release of alkali and alkali earth metals previously bound in organic matter [30]. Gradual increasing in pH values were observed till steady values of 9.3 (Fig. 2). This Gradual increase in pH values was recommended during the composting process the acidity contributed by the small molecule organic acids and CO2, produced from carbohydrates under the function of microbiology, could not be completely neutralized by alkalinity from the dissolved ammonia nitrogen [31]. Therefore, gradual increase of pH is a good sign for succeeding the composting process.

Moisture content of TWC

The moisture content of the composting is an important environmental variable as it provides a convenient medium for the mobilization of dissolved nutrients required for microorganisms metabolic and physiological activities [32]. The efficient moisture content percentage was adjusted to be between 40 and 65 % for composting. While, the moisture levels less than 40 % cause the microorganisms to slow their activities Below 15%, microbial activity cease altogether (dormant or die) [33]. Figure 3 represents the moisture content percentage for the compost. Initially, the moisture content of TWC was approximately 61.63% then; it decreased finally to 37.3%. The gradual decreasing in moisture content is owing to the heat generated by biological metabolism and the high water evaporating rates at the thermophilic phase of the composting process [34].Therefore, the addition of water was uniformity till the composting process was completely continued.

Total carbon content of TWC

Organic matter plays a survival role in increasing water holding capacity, nutrient availability and improving soil structure. Total

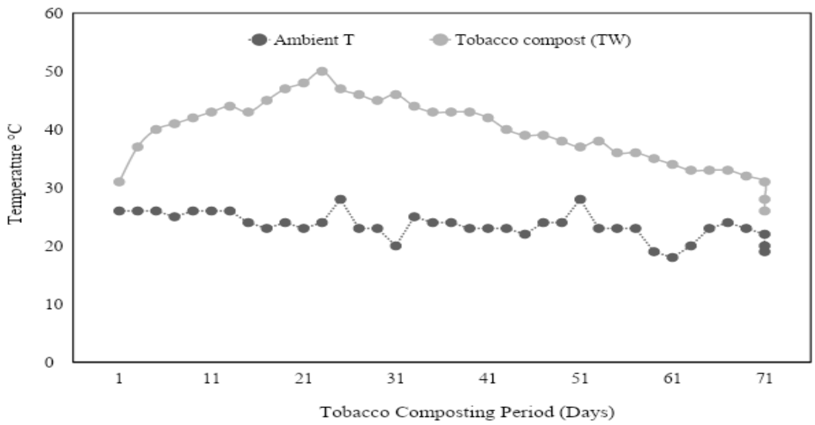

Fig. 1. Temperature profiles during composting of tobacco waste

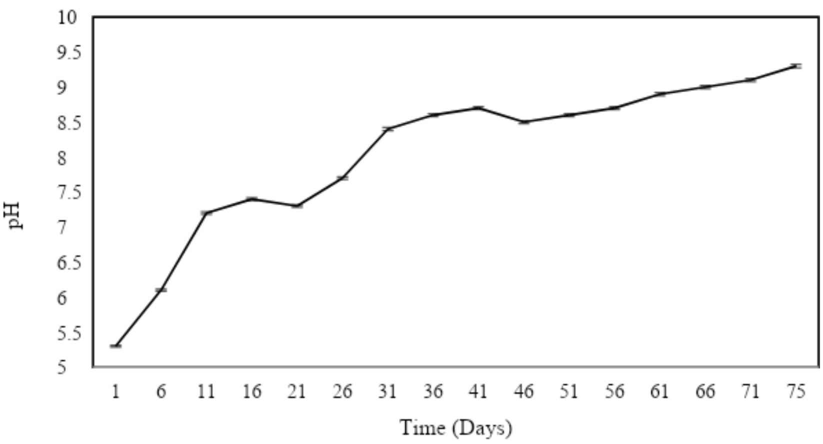

Fig. 2. pH profile of TWC during composting process

organic carbon is the carbon fraction of organic matter. Total organic carbon reduction for TWC after composting process was presented in Table1. Total carbon content decreased by 35 % of the initial during the composting process. This decreasing in total organic carbon content for composting process was related to the microbial population action [35]. A part of the carbon in the decomposing residues evolved as CO_2, and another part was assimilated by the microbial biomass [36].

Total nitrogen content of TWC

The total nitrogen content of TWC is represented in Table 1. The total nitrogen content of TWC was increased by 27 % of the initial value. This increasing in total nitrogen percentage is due to loss of dry mass in terms of carbon dioxide as well as the water loss by evaporation of organic matter [37, 38].

Change in the Carbon/Nitrogen Ratio (C/N ratio) of TWC

C/N ratio is an essential parameter that determines the extent of composting and degree of compost maturity irrespective to the materials used for composting and so it should be presented in the convenient balance in order to compose efficiently [39]. C/N ratio should be between 20-25 parts for C/N; a departure from this ratio leads to slow down the composting [40].

The obtained results showed decreasing in C/N ratio from 26.6 to 13.48 after 75 days of composting (Table 1) with decreasing total organic carbon and increasing nitrogen content. The high C/N ratio in tobacco solid waste suppresses composting process leading increasing maturing time of compost. This was agreement with previously reported [41], they mentioned that, the carbon is released as CO_2 while nitrogen concentration fixed in the composting system. The calculated C/N ratio of compost in this study was below 20. This value was in agreement with previously reported [42].

Total Phosphorous of TWC

Phosphorous is considered to be one of the essential nutrients for plant growth.

Figure 4 shows increasing in total phosphorous concentrations of TWC along composting process. The total phosphorus content gradually elevated during composting process, as well as the water

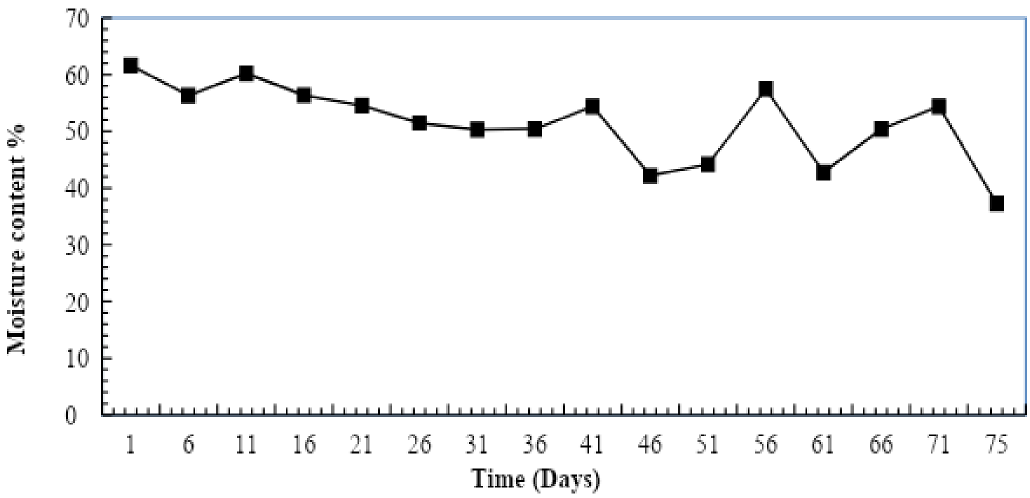

Fig. 3. Moisture content of TWC during composting process

TABLE 1 Changes of carbon, nitrogen and C/N ratio of TWC

Parameter	TC %	TN %	TC/TN
Tobacco waste	47.9±0.45	1.8±0.04	26.6±0.07
Composted Tobacco waste (TWC)	31.01±0.29	2.3±0.05	13.48±0.04

solubility of phosphorus decreases due to the humification [32]. Therefore, the phosphorus solubility during the decomposition was subjected to further immobilization [43].

Metals content of TWC

Detection for Potential Toxic Elements (PTEs) or heavy metals concentration in the composted material is a key to use it for land application. This is because of their indirect risk to human health. Therefore, the level of heavy metals concentration in the composted material are considered as a restriction factor in the characterization and classification for the quality of composted material [44]. Composting process can increase or decrease heavy metals present in the organic waste. Decreasing the heavy metals concentration could be attributed to the loss of the metal through leaching. However, increasing in heavy metals level is due to releasing of carbon dioxide and water as well as mineralization processes [45].

Table 2 shows the decreasing in tobacco metal concentration (Cu, Cd, Pb, As, Zn, Cr, Fe, Hg and Ni) after composting, which mainly related to metal loss through leaching, or releasing from decomposed organic matter and the changing in cationic and anionic state of the medium [46]. It was observed that metals concentration of TWC was lower than the limited concentration; the limit was compared by European composted materials quality of PTEs content for composted bio-waste materials supposed to be used as organic fertilizers [46; 47].

Application of TWC as soil amendment

Soil water holding capacity

Water holding capacity was determined for different mixture of sand soil and TWC (1:0.25, 0.5:1, 1:1, w:w) regarding to soil. The results showed that the water holding capacity was raised from 25% to 46.61% with introducing different amounts of compost to soil. The addition of the compost increased soil aggregation as well as increasing water holding capacity. The results of compost could improve the soil properties; water holding capacity and nitrogen content. Also, it was found that the application increased bulk density, field capacity, available water content, and structure stability index of soil when compared with the control. The effect of compost on soil physical and chemical properties and the plant yield was investigated and. It was previously stated water holding capacity values of soil were significantly affected by all the amendments. The capacity values of soil were changed around 16.6 – 21.9 %. After application of 50 t/ha TWC in the soil samples, the available water amount increased by 10.73% [48].

Dry weight, photosynthetic pigments and some antioxidant enzymes of Canola leaves cultivated in presence of TWC

The mixture of soil with compost was used for cultivating canola, comparing with that cultivated in sand only. The germinated canola plants (photo 1), were left to grow for 30 days. Then, the dry weight photosynthetic pigments and some antioxidant enzymes canola leaves that cultivated in sand soil (A) or in mixture of sand soil and TWC (B) were measured.

Table 3 includes the data for the dry weight, photosynthetic pigments and some antioxidant

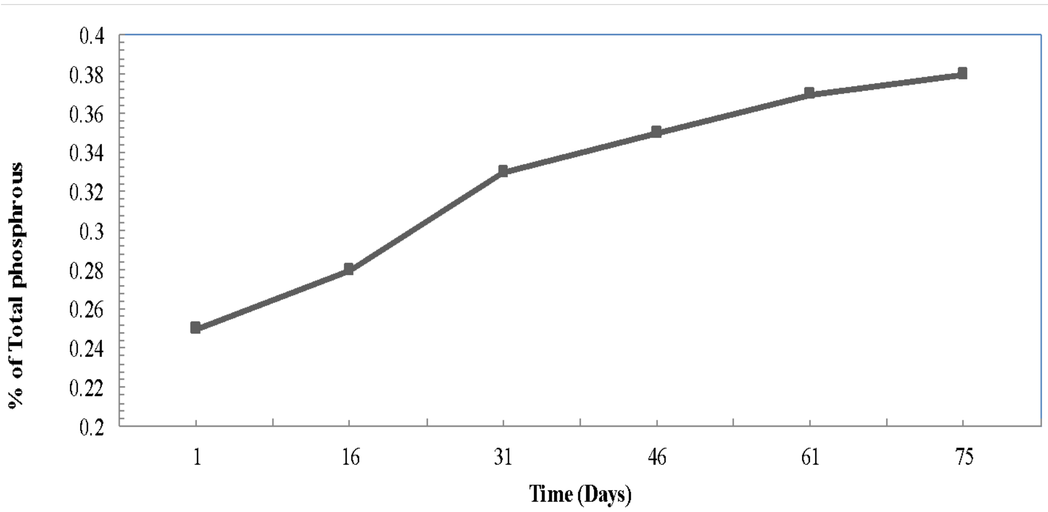

Fig. 4. Total phosphorous changes during composting of TWC

TABLE 2. Metals concentration for tobacco waste before and after composting

Parameters	Before composting	After composting	European composted materials quality		
			Mean	Max.	Min.
	mg/ kg (dry solids)				
Copper	11.2± 0.11	9.8± 0.1	143	600	25
Cadmium	0.39± 0.007	< 0.05	1.4	3	0.7
Lead	2.2± 0.02	1.8± 0.019	121	280	45
Arsenic	0.9± 0.009	< 0.05	23	50	5
Zinc	94.6± 0.5	34± 0.18	416	1500	75
Chromium	0.95±0.04	< 0.05	93	250	50
Iron	976± 1.2	375± 0.3	----	----	---
Mercury	< 0.05	< 0.05	1.0	3	0.2
Nickel	0.18±0.004	< 0.05	47	100	10

(A)

(B)

Photo 1. Germinated canola plant cultivate in sand (A) or sand and compost (B)

enzymes (CAT, POX and APX) in 30 day old canola leaves cultivate in sand

(A) sand and TWC mixture (B). It is worth to say that the plants cultivated in the presence of TWC had a greater dry weight, and more photosynthetic pigment chla, b except the carotenoids. Also, CAT, POX and APX of canola leaves increased by 100, 38, 69 %, respectively within the plants cultivated in mixture of sand soil and TWC. The increments of these different parameters indicated that the TWC improved the growth of canola plants. As the TWC implied N, C, P and list of beneficial elements, all of them are enriching the planting conditions. Additionally, TWC has an effect on soil microbial properties and plant biomass yield [18]. By the greatly increased microbial populations, some of plant growth regulators such as indole-acetic acid, gibberellins and cytokinins and humic acid simulate the plant growth [48; 49). Therefore, our results substantiated by Malhi, et al [50] who mentioned that some organic amendments can be used to improve crop yields. The soil properties as well plant growth were improved by addition of TWC to sandy soil.

TABLE 3. Dry weight, photosynthetic pigments and some antioxidant enzymes in 30 day old canola leaves cultivate in sand (A), sand and TWC mixture (B).

Parameter	Control (A)	Test (B)	% of change from A to B
Dry weight (g/plant)	0.05	0.072	44
Photosynthetic pigments			
Chl a (mg/g f wt)	496.15	540.80	8.99
Chl b (mg/g f wt)	307.08	449.74	46.46
Carotenoids (mg/g f wt)	136.18	104.94	(-)22.94
Antioxidant enzymes			
CAT (μM / g/ min)	0.005	0.01	100
POX (OD/g/min)	28.01	38.82	38.59
APX(μM /g/min)	0.56	0.95	69.64

Conclusion

The composting is considered sustainable management for tobacco solid waste, and implementing the produced compost for soil improving. It is the available paramount alternative for prevention of environmental pollution. The produced compost showed variation in characteristics with time of composting comparing to original waste. Where, after maturation of composting there was decrease in total organic carbon content by 35 % of the initial during the composting process because of microbial population action. The final C/N ratio for composted tobacco waste is < 20 which indicative for its acceptable maturity. Also heavy metals concentration of TWC was lower than limit concentration of PTEs. It is worth to say that the plants cultivated in the presence of TWC had a greater dry weight, and more photosynthetic pigments (chla, b). Additionally, the antioxidant enzymes such as CAT, POX and APX of canola leaves increased by 100, 38 and 69 %, respectively when compared to that planted in sand soil only. Conclusively, TWC improved the soil properties as well plant growth. The compost production is a green beneficial tool for sustainable management of agro-waste.

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زراعة الكانولا (*Brassica napus* L.) بالأسمدة المحوله من مخلفات الصناعات الزراعية

هناء البدوى أحمد[١،٢]، محمد عيد محمد على[٣]، نبيلة صالح عمار[٣]، حنان سيد إبراهيم[٣]
[١]قسم النبات والميكروبيولوجى - كلية العلوم - جامعة القاهرة - الجيزة ١٢٦١٣ - مصر.
[٢]قسم الأحياء - كلية العلوم - جامعة جدة - ٢١٥٨٩ - المملكة العربية السعودية.
[٣]قسم بحوث تلوث المياه - شعبة بحوث البيئة - المركز القومي للبحوث - الجيزة - ١٢٦٢٢ - مصر.

يعد سماد الكمبوست الناتج من مخلفات التبغ احدى الطرق الفعالة لإعادة تدوير المخلفات الزراعية الناتجه من مخلفات الصناعات الزراعية و التى يمكن إستخدامها للأغراض الزراعية لذلك كان الهدف الرئيسى من هذا البحث هو دراسة الخصائص الأساسية لكمبوست مخلفات التبغ ومن ثم إستخدامه كوسط زراعى. لقد أثبتت الدراسة إنخفاض في إجمالي محتوى الكربون العضوي بسبب زيادة نشاط الكائنات الحية. وكان هناك زيادة في الخصائص الفيزيائية والكيمائية لكبوست التبغ و منها (الأس الهيدروجيني، الفوسفور الكلي، والنتروجين الكلي ونسب الكربون/ النتروجين). كما تم قياس عناصر السمية المحتملة (PTEs) لكمبوست التبغ حيث تعد احد الاساسات التى بناء عليها يتم استخدام الكمبوست الناضج فى التطبيقات الزراعية. كما أثبتت الدراسة أن سعة الاحتفاظ بالماء لخليط التربة الرملية وكمبوست التبغ وصلت إلى ٤٦,٦١ ٪ في حين كان ٢٥ ٪ فقط بالتربة الرملية. وكان نمو بذور الكانولا بشكل جيد في خليط التربة الرملية وكمبوست التبغ بالإشارة إلى بعض المعايير الفسيولوجية والمورفولوجية. لذلك ، يمكن استخدام كمبوست التبغ كمخصب للتربة الرملية.