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ORIGINAL ARTICLE

Effect of Nitrogen Sources on the Composting of Date Palm (Phoenix Dactylifera) By-products Infected by Fusarium Oxysporum f.sp. Albedinis

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Abdelkader Hakkou, Khadija Chakroune, Mohammed Bouakka, Faiza Souna, Lurdes Cotxarrera, Marie Isabel Trillas.: Effect of Nitrogen Sources on the Composting of Date Palm (Phoenix Dactylifera) By-products Infected by Fusarium Oxysporum f.sp. Albedinis

ABSTRACT

The by-products of palm date (Phoenix dactylifera) contaminated by Fusarium oxysporum f.sp. albedinis (Fusarium) were composted with different rates of bovine manure, urea or ammonium nitrogen. Water was added to maintain optimum humidity at 60-70%. During the process of composting and whatever the ratio of the manure or nitrogen in the piles, the pH remained stable and relatively alkaline at values ranging from 8.2 to 8.7. By contrast, electrical conductivity decreased from 25 to 30%. The evolution of the internal temperatures in the piles depended on the volume of the manure added to the mixture, but was characterized by a mesophilic phase showing a fast increase in temperature, then a thermophilic phase, with high temperatures ranging from 50 to 70°C in the piles rich in bovine manure. However, internal temperatures remained unchanged in urea- or ammonium-containing piles. In the piles containing bovine manure, Fusarium was completely eliminated during the thermophilic phase. The rate of its destruction depended on the ratio of the manure introduced into the piles. Indeed, when the ratio increased, the elimination of Fusarium is fast. In the presence of ammonium nitrogen, proliferation of pathogenic agent increase to 62%. Experiments on rachis pieces of the palms infected by Fusarium, show that their incubation for 6h at 70°C or for 12h at 60°C destroy completely the pathogenic fungus. The C/N ratio of the date palm by-products is very high (115.4), owing to the fact that they are very rich in organic matter (90%) and very low in nitrogen (0.39%). The addition of bovine manure brought C/N ratios in the piles to values ranging from 35 to 60. During the composting process, this ratio decrease constantly from 35 to 46% of its initial value after 75 days of incubation. On the contrary, in the piles containing urea and ammonium nitrogen, C/N ratio increase by 87 and 60%, respectively.

Key words: Date palm, by-products, Fusarium oxysporum f.sp. albedinis, composting process, bovine manure, nitrogen, urea.

Introduction

In the palm plantation of Figuig, located at Eastern Morocco, in the Morocco-Algerian frontier field (latitude: 32° 07' N, longitude: 01° 14' W), the 190 000 date palms generate approximately 8000 tons by-products per year. This significant biomass, for a palm plantation of 700 hectares, causes

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environmental and phytosanitry problems [15]. Indeed, these by-products input a dangerous fungus Fusarium oxysporum fsp albedinis (Fusarium), causal agent of the vascular fusariosis (Bayoud), which is a pathogenic agent causes drastic reduction in cultivation and expansion of date palm in Morocco and Algeria [22,14]. The decomposition of this biomass, of which a part is contaminated by the agent responsible of Bayoud, constitutes a vector of propagation of the disease in all the palm plantation.

The composting of these by-products would constitute a means to attenuate the gravity of this problem while developing this organic matter. This process of aerobic fermentation leads to the formation of a compost, factor of protection, stability and fertility of the ground [16]. The use of this compost, like organic soil conditioner, will be able to cure the problems of impoverishment and of the salinisation of the oasien environment and to contribute to the fight against plant diseases. Several authors showed the importance of the compost use in the control and the reduction of the agricultural cultures diseases [11,17,28].

The needs nitrogen for composting depend on the easily biodegradable carbonaceous materials content. The date palm by-products do not possess the quality required for composting. Indeed, C/N ratio of these by products, is too high about 115 [9], whereas the ideal C/N ratio which guarantees a good starting of composting and its optimal unfolding must be located between 25 and 40 [25]. That's why it is necessary to search a nitrogen source to equilibrate this C/N ratio and to obtain sanitized and good agronomic quality compost. In this article, we will study the effect of various nitrogen sources on the process of composting of the date palm by-products contaminated by the fungus (Fusarium oxysporum f.sp albedinis). We will follow the evolution of some physicochemical and microbiological parameters significant (pH, temperatures, C/N ratio, density of the pathogenic agent) for a good decomposition of the organic matter and especially for compost sanitation by elimination of the pathogenic agent.

Materiel and methods

Preparation of the Wind-rows and Control of Composting:

The mixture of the biomass used in this experimentation is composed of 90% of by-products generated naturally from date palm and 10% of those are contaminated (generated from date palms attacked) by the vascular fusariosis. This biomass, crushed then sprinkled to reach a final water content of 60 to 70%, was mixed with various sources of nitrogen to constitute the wind-rows of experimentation according to this table:

Wind-row	F1	F2	F3	F4	F5	Az	Ur	T
By-products in m^3	1.8	1.75	1.65	1.45	1.1	2.2	2.2	2.2
Manure in m^3	0.3	0.45	0.55	0.75	1.1	0	0	0
Ammoniacal nitrogen ($(NH_4)_2HPO_4$) in G	0	0	0	0	0	922	0	0
Urea in g	0	0	0	0	0	0	320	0

Measurement of pH and Electric Conductivity:

Measurements of pH and electric conductivity were taken after each reversal during the composting process.

75 g of compost were suspended in 100 ml of sterile distilled water (pH 6.4 and electrical conductivity of 0 mS / cm). After agitation during 30 minutes and filtration, measurements of pH were taken with a pH-meter (WTW pH521) on the filtrate at ambient temperature.

Electric conductivity is measured with a conductivity-meter (WTW LF 318) on the same filtrate at ambient temperature. This measurement corresponds to the concentration of soluble biogenic salts in the substrate expressed in ms/cm.

Measurement of Internal and External Temperatures Wind-rows:

The internal and external temperatures of each wind-row were recorded daily at the semi-day using two metal probe thermometers of different lengths. The results are the averages of three successive measurements to some 15 cm (external) and 60 cm depths (internal) in both side of the wind-row.

Determination of the Fusaruim Oxysporum F.sp. Albedinis Population During Composting:

A 75g sample of each windrow was suspended in 100 ml of sterile distilled water. After agitation during one hour at ambient temperature, the suspension is filtered. Obtained filtrate is diluted successively of 10 into 10. Petri dishes containing 15 ml of the sterile KOMADA medium [20] were seeded with 1 ml of the diluted filtrate. These dishes are incubated at 27°C during 72 hours, then during two weeks under continuous lighting and at ambient temperature. The identification of the pathogenic agent is carried out on their morphological and microscopic characteristics (mycelium fine, curly, shaving) and pigmentation (pink-salmon) [13,26]. The enumeration of Fusarium is expressed in a number of colonies formed per gram of dry matter (CFU/g dries matter).

Determination of the Matter Dries:

For the determination of the dry matter, a sample of approximately 10 g of each wind-row is placed in a drying oven brought up to 105 °C during 5 hours. The sample is weighted after cooling in a desiccator. The water content is then given starting from the average of three repetitions and it is expressed as a weight percentage.

Determination of the Organic Matter, the Mineral Matters and Total Nitrogen:

The calcination method is used to determine the total organic matter (AFNOR 1999). It is calculated from the ash content after dry matter calcination at 470°C to 490°C during 6 hours. Conventionally, the percentage of carbon is equal to half of the total organic matter percentage (AFNOR, 1999). The total nitrogen content was given with the Kjeldahl method (NTK) modified (ISO 11261: 1995 (F)), AFNOR (A).

Effect of Heat Treatment on Fusarium Sheltered by the By-products:

Pieces from 1 to 2 cm of length and 0.5 cm to 1 cm of diameter of two varieties palm rachis Assiane and Bouffeggouss strongly contaminated by Fusarium. Part of these pieces humidified (content water 40 to 45%) and the other are dry ones (content water 15 to 20%) are incubated in the drying oven at various temperatures (40, 50, 60 and 70°C) during varying durations. After this heat treatment, small fragments (0.5 cm3 approximately) taken on these pieces are incubated during 8 days on PDA medium at 25°C. The percentage of inhibition of the growth is determined by measurement of the Fusarium mycelium diameter compared with the control (who does not have undergoes any treatment). The results obtained are the average of at least three repetitions.

Results and discussion

Evolution of the Ph and Electric Conductivity During Composting:

During the composting process and whatever the quantity of the manure or of nitrogen in the wind-rows, the pH remains stable and relatively basic (pH between 8.2 and 8.7) in all the wind-rows, but slightly higher than that of the control wind-row (7.26 to 7.42). Fermentation in aerobic exothermic, which takes place in the wind-rows of composting, produce the CO_2 as a volatile gas which does not influence the pH compost. However, according to several authors, the process of composting slightly modifies the pH and particularly during the first days when one attends a very light acidification allotted to the production of organic acids, followed, in thermophilic phase, of a slight alkalisation following the ammonium release by the process of proteins ammonification [23]. This difference could be explained by the perforated substrate nature.

Conversely to pH, electric conductivity falls during the composting process in all the wind-rows (figure 1). This reduction is slower in the control wind-row (17%) that in the other wind-rows (25 to 30%). The assimilation of the ions responsible of conductivity in biochemical reactions during composting and/or the scrubbing of these molecules could explain this reduction which would constitute a positive element for the agricultural compost quality.

Evolution of Internal Temperatures in the Windrows:

The presence of the manure in the wind-rows accelerates the fermentation process. The internal temperature in the wind-rows is much higher than the volume of manure added is important (figure 2 b). The evolution of the temperature can be divided in three periods: one short period (2 to 3 days) characterized by an augmentation of the temperature, followed by a thermophilous phase of 25 days when the temperature remains high between 50°C and 70°C for the wind-rows rich in manure (F2, F3, F4 and F5) and between 40°C and 50°C for the F1 wind-row and finishes by a cooling phase which lasts approximately 15 days; the temperature fall to values of 25°C to 35°C close to the ambient temperature after 42 days of composting (figure. 2 b).

The date palm by-products mixed with manure (F2, F3, F4 and F5) are rich in fermentable compounds because the temperatures of 50°C to 70°C are reached quickly. Instead of this, the wind-row F1, least rich in manure, has less high temperatures (40 with 45°C) (figure 2b). The wind-row perforated without any additive (T) reaches only a modest temperature of approximately 35°C and additions of urea (Ur) or of ammoniacal nitrogen (Az) do not show any heating effect on the process of decomposition of the date palm by-products (figure 2 a).

The temperature increases observed are the result of the organic matter oxidation by an aerobic microbial population, provided by the manure. This activity is significant at the beginning of composting process, reflected by the increase of temperature. Thermophilic actinomycetes, thermophilic fungi [10] and thermophilic bacteria [4] are likely active during this phase of composting process by accelerating the decomposition process and by ensuring the sanitation of the compost by removing pathogens carried by-products and manure. Amir [3], showed that they are the compounds easily accessible like the peripheral aliphatic chains from lipid and peptide structures which are oxidized leading to the formation of aromatic structures rich in functional groupings.

Evolution of the Pathogenic Agent in the Wind-rows During Composting:

The increase in the temperature at the beginning of the composting process and its persistence during more than 25 days in values of 55 to 70°C in the wind-rows F2, F3, F4 and F5 showed its effectiveness to eliminate completely the pathogenic agent, causes the vascular fusariosis of the date palm (Bayoud) (Figure 3).

The kinetics of destruction of this fungus is identical to the wind-rows containing manure. It is divided into two main phases (Figure 3): the first phase lasts approximately five days, during which the initial fungus population fall quickly in the five wind-rows. This decline depends on the rate of manure added to the windrow, the inhibition of the fungus is more important than the share of manure is given high. The second phase lasts approximately 12 to 15 days during which the quantity of Fusarium continues to decline but slowly until its total disappearance in all the wind-rows which contain the manure except for the F1 wind-row (the least rich in manure). The temperature of the thermophilic phase of the last period, lower than that those of the others, leads to a removal rate of the pathogen and less rapid regrowth of the pathogen from the 45th day, after the restoration of the favorable conditions in the maturation phase of compost. After the 75 th day of composting, the proliferation of Fusarium finds its initial value.

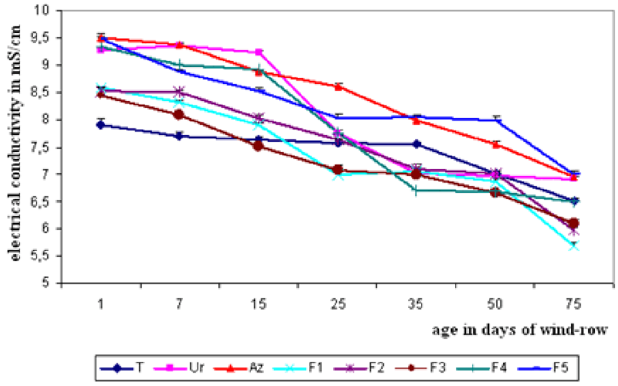
The growth of fungus starts at 7°C and stops around 38°C [8]. By studying germination of the Fusarium chlamydospores, Bounaga [7] showed that this germination is stopped at 60°C, without this temperature being lethal. The results of our experiments carried out on pieces of date palms rachis infected by Fusarium (Table1), showed that it is enough to incubate the date palm by-products infected during 6 hours at 70°C, or during 12 hours at 60°C or during 72 hours at 50°C to destroy completely the pathogenic agent placed in these by-products and this whatever the variety and moisture content of by-products. The moisture properties of conductive heat accelerates the elimination of the fungus: treatment for 6 hours at 60 ° C, the fungus byproducts wet (40 to 45% moisture) of both varieties is completely destroyed while the by-products of low humidity (20 to 25% moisture) is inhibited only 50 to 75%.The pathogenic agent seems to be more resistant in the Assiane variety by-products than in those of the Bouffaggousse variety (Table 1).

The destruction of the pathogenic agents requires an exposure of all the wind-row parts at a temperature of 55 ° C during at least three days, or preferably, five days duration under a temperature of 55°C [6]. The best results are obtained at a temperature of 60 to 70 ° C [18]. Strains of Salmonella are destroyed completely in compost at 65 ° C for one day [19]. The elimination of the pathogenic micro-organisms during the composting process can also be done by competition with other nonpathogenic micro-organisms [19,24]. Several authors showed the role of the telluric micro-organisms in the grounds resistance to the vascular fusariosis [1,2].

In the wind-rows which contain urea (Ur), the behavior of Fusarium is identical to that of the control wind-row; one observes an increase in the Fusarium population during the first week of composting then a slow reduction to return to the initial density after the 35 th day of composting (figure.3). Conversely, the presence of ammoniacal nitrogen supports the proliferation of the pathogenic agent in the wind-row (Az): during the first 5 days of composting. We note a fast increase of approximately 62% which remains stable until the end of composting. Ammonia nitrogen could constitute the preferred assimilable nitrogen source by Fusarium.

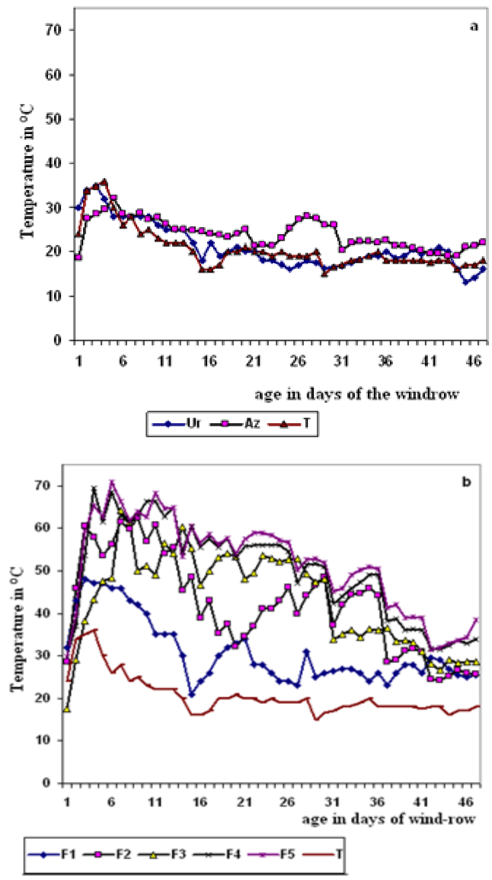
Evolution of the Organic and Mineral Matter During Composting:

The C/N ratio of the date palm by-products is very high (115.4), owing to the fact that they are very rich in organic matter (90%) and very low in nitrogen (0.39%) (Table 2). Under these conditions, the starting of the composting process did not take place in the control without additive (figure 2). The addition of the manure, much richer in nitrogen (1.5%), proved to be necessary to start the biological activity in the wind-row composting [9]. The addition of the manure reduced the C/N ratio in the F2, F3, F4 and F5 wind-rows to values ranging between 60 and 35 which started the process of composting. Although the majority of these ratios are still higher than that considered as optimal for a good starting of to the biological activity (25-35), but the composting process started well in all four windrows.



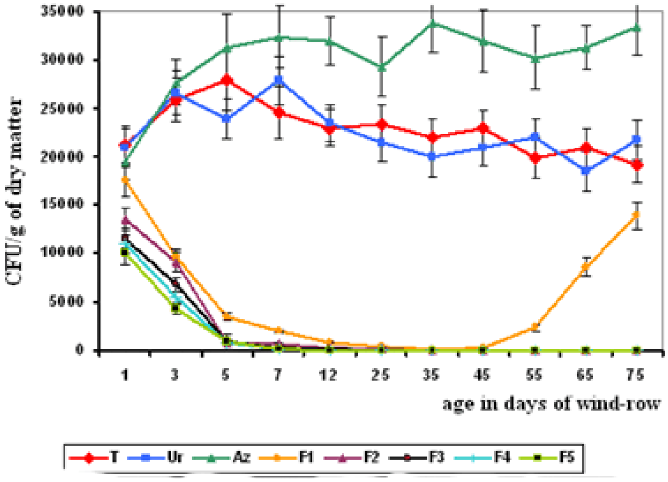
T : Control without any additive; Ur : 320 g of urea + 2.2 m3 of mixture; Az : 922 g ammoniacal nitrogen ((NH4)2HPO4) + 2.2 m3 of mixture; F1 : 0.3 m3 du fumier + 1.8 m3 of mixture; F2 : 0.45 m3 of manure + 1.75 m3 of mixture ; F3 : 0.55 m3 of manure + 1.65 m3 of mixture; F4 : 0.75 m3 of manure + 1.45 m3 of mixture ; F5 : 1.1 m3 of manure + 1.1 m3 of mixture.

Fig. 1: Evolution of electric conductivity during the process of composting.



a : in the presence of urea or ammoniacal nitrogen; b : in the presence of different amounts of cattle manure. T : Control without any additive; Ur : 320 g of urea + 2.2 m3 of mixture ; Az : 922 g of ammoniacal nitrogen ((NH4)2HPO4) + 2.2 m3 of mixture ; F1 : 0.3 m3 of manure + 1.8 m3 of mixture ; F2 : 0.45 m3 of manure + 1.75 m3 of mixture ; F3 : 0.55 m3 of manure + 1.65 m3 of mixture ; F4 : 0.75 m3 of manure + 1.45 m3 of mixture ; F5 : 1.1 m3 of manure + 1.1 m3 of mixture .

Fig. 2: Change of intern temperatures in the wind-rows during the process of composting.



T : Control without any additive; Ur : 320 g of urea + 2.2 m3 of mixture ; Az : 922 g of ammoniacal nitrogen ((NH4)2HPO4) + 2.2 m3 of mixture ; F1 : 0.3 m3 of manure + 1.8 m3 of mixture ; F2 : 0.45 m3 of manure + 1.75 m3 of mixture ; F3 : 0.55 m3 of manure + 1.65 m3 of mixture ; F4 : 0.75 m3 of manure + 1.45 m3 of mixture ; F5 : 1.1 m3 of manure + 1.1 m3 of mixture .
Values are the average of at least three measurements.

Fig. 3: Enumeration of Fusarium oxysporum f.sp albedinis expressed in CFU/g of the dry matter.

Table 1: Effect of thermic treatments on Fusarium oxysporum f.sp. albedinis placed in two date palm varieties rachis attacked by the vascular fusariosis.

		Incubation temperature in ° C				
Incubation time	variety	Humidity rate	40 ° C	50 ° C	60 ° C	70 ° C
0 hr	Assiane	15 à 20%	+++	+++	+++	+++
		40 à 45%	+++	+++	+++	+++
	Bouffaggousse	15 à 20%	+++	+++	+++	+++
		40 à 45%	+++	+++	+++	+++
6 hr	Assiane	15 à 20%	+++	+++	+-	-
		40 à 45%	+++	++	-	-
	Bouffaggousse	15 à 20%	+++	+++	+-	-
		40 à 45%	+++	+	-	
12 hr	Assiane	15 à 20%	+++	++	-	-
		40 à 45%	+++	+-	-	-
	Bouffaggousse	15 à 20%	+++	+	-	-
		40 à 45%	+++	+-	-	-
24 hr	Assiane	15 à 20%	+++	++	-	-
		40 à 45%	+++	-	-	-
	Bouffaggousse	15 à 20%	+++	+	-	-
		40 à 45%	+++	-	-	-
48 hr	Assiane	15 à 20%	+++	+-	-	-
		40 à 45%	++	-	-	-
	Bouffaggousse	15 à 20%	+++	-	-	-
		40 à 45%	++	-	-	-
72 hr	Assiane	15 à 20%	++	+-	-	-
		40 à 45%	++	-	-	-
	Bouffaggousse	15 à 20%	++	-	-	-
		40 à 45%	++	-	-	-

(+++): 0% inhibition; (++): 0-25% inhibition; (+): 25-50% inhibition; (+-): 50-75% inhibition, (-) : 100% inhibition.

Table 2: Quantitative and qualitative evolution of the mineral and organic matter during composting.

	Prelevement at 1st day of composting							
Wind-row	T	Ur	Az	F1	F2	F3	F4	F5
O. M rate	91.00 ± 7.3	88.00 ± 6.9	93.00 ± 7.6	75.00 ± 6.6	69.80 ± 6.1	63.20 ± 3.9	60.80 ± 3.9	58.30 ± 3.9
Ash rate	09.00 ± 0.6	12.00 ± 0.7	07.00 ± 0.5	25.00 ± 2.3	30.20 ± 2.5	36.80 ± 2.2	39.20 ± 2.6	41.70 ± 2.8
O.C. rate	45.00 ± 3.7	44.00 ± 3.7	46.00 ± 3.7	37.50 ± 2.3	34.90 ± 2.0	31.60 ± 1.9	30.40 ± 1.9	29.15 ± 1.7
Nitrogen rate (NTK)	0.39 ± 0.07	1.17 ± 0.6	1.15 ± 0.7	0.47 ± 0.10	0.57 ± 0.12	0.63 ± 0.11	0.73 ± 0.11	0.82 ± 0.13
C/N ratio	115.40 ± 7.8	37.60 ± 2.8	40.00 ± 2.7	79.78 ± 5.2	61.22 ± 4.2	50 .16 ± 3.8	41 .64 ± 2.9	35.55 ± 2.2

Table 2: Continu

	Prelevement at 75th day of composting							
	T	Ur	Az	F1	F2	F3	F4	F5
O. M rate	87.00 ± 6.5	79.00 ± 6.3	81.00 ± 7.3	64.70 ± 3.2	51.60 ± 3.7	47.00 ± 2.9	45.00 ± 2.9	41.70 ± 2.0
Ash rate	13.00 ± 0.7	21.00 ± 0.9	19.00 ± 0.6	35.30 ± 2.4	48.40 ± 3.1	53.00 ± 3.2	55.00 ± 3.2	58.30 ± 2.7
O.C. rate	43.50 ± 3.7	39.50 ± 2.7	40.50 ± 3.2	32.35 ± 2.0	25.80 ± 1.8	23.50 ± 1.5	22.50 ± 1.5	20.85 ± 1.6
Nitrogen rate (NTK)	0.41 ± 0.11	0.56 ± 0.12	0.63 ± 0.12	0.61 ± 0.10	0.76 ± 0.10	0.88 ± 0.13	0.88 ± 0.13	0.96 ± 0.16
C/N ratio	106.10 ± 7.8	70.53 ± 6.8	64.28 ± 5.7	53.03 ± 4.1	33.95 ± 3.1	26.70 ± 3.9	25.57 ± 3.5	21.72 ± 2.3

M.O.: organic matter; C.O. : organic carbon. Rates are expressed as % of dry matter.
T : Control without any additive; Ur : 320 g of urea + 2.2 m3 of mixture ; Az : 922 g ammoniacal nitrogen ($(NH_4)_2HPO_4$) + 2.2 m3 of mixture ; F1 : 0.3 m3 of manure + 1.8 m3 of mixture ; F2 : 0.45 m3 of manure + 1.75 m3 of mixture ; F3 : 0.55 m3 of manure + 0.65 m3 of mixture ; F4 : 0.75 m3 of manure + 1.45 m3 of mixture ; F5 : 1.1 m3 of manure + 1.1 m3 of mixture .
Values are the average of at least three measurements.

However, the C/N ratio, alone, cannot explain this phenomenon, since in the wind-rows Ur and Az whose C/N ratio are approximately 40, the process of composting did not start because, probably, its due to the insufficient microflora.

During the process of composting, the C/N ratio is steadily decreasing in the windrows containing manure and at the end of the 75 days of biotransformation. This reduction is 35 to 46%. There is no correlation between the fall of the C/N ratios and the manure quantity added to the wind-rows. On the other hand, in the wind-rows containing the urea (Ur) or ammonia (Az), the C/N ratios increased with 86 and 60% respectively (Table 2). This phenomenon could be explained by leaching of nitrate particles during repeated waterings and / or by low organic matter degradation and / or the loss of nitrogen as ammonia volatil. According to the raw material for composting, the value of the C/N ratio can go up to 13 in a finished compost [21]. This is explained by the fact that micro-organisms consume more carbon (the main component of organic molecules) than nitrogen. Veeken *et al.* [27], showed that 40% of the organic matter are degraded during 4 weeks of composting. These C/N ratios decreases observed in our wind-rows well fermented in the presence of the manure, are due mainly to the degradation of the organic matter because its rate was reduced by 26, 25, 26 and 28% respectively in the windrows F2, F3 , F4 and F5. However, the C/N ratios of the five wind-rows are still high after 75 days of composting, ranged from 53 to 22 according to the proportion of manure brought (table 2). The organic matter of these composts could still be degraded by the mesophilic flora which actively participates in the maturation of compost. According to Veeken *et al.* [27] this product still contains biodegradable organic matter, especially cellulose, could have phytosanitry capacity and to be a good ground organic soil conditioner.

Conclusion:

In contrast to chemical nitrogen sources, manure is essential to start the composting process of date palm by-products. Within the wind-rows containing the bovine manure, the temperature rise and the inhibition of the fungus depends on the amount of manure in the mixture. A contribution of 20% of bovine manure is the minimum rate which ensures a good composting of the date palm by-products crushed and, especially, which destroys completely Fusarium oxysporum. The increase in the temperature during composting destroys Fusarium and this was confirmed by the tests carried out in vitro on contaminated pieces of rachis which show well the implication of the temperature and moisture in the destruction of the pathogenic fungus.

During the composting process, the pH remains stable whereas electric conductivity undergoes a reduction whatever the composition of the wind-rows and the frequency of reversal. The evolution of the C/N ratio is a function of the raw material. This ratio undergoes a reduction in the presence of bovine manure and an increase in the windrow containing urea or ammonia.

The composting of the date palm by-products as well seems to be interesting viewpoints economic, ecological and social. This leads to the elimination of polluting organic wastes that contain pathogenic fungus Fusarium oxysporum and to the production of quality compost. Ecologically, this compost contributes to the fertility of the grounds by limiting erosion and evaporation and by saving water which is an invaluable element in these arid areas. Plant health side, the compost could contribute to the protection of the date palms against Bayoud by limiting the ground contamination due to the pathogenic fungus, although this still remains to be demonstrated and is currently the subject of research in our laboratory.

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