



## Water from Cooked Beans as Substrate for Some Heterotrophic Organisms: Case Study of Moulds

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### Authors' contributions

This work was carried out in collaboration among all authors. This work was carried out by author JCFT under the supervision of authors JPN and PT. Author SDB contributed to the exploitation of results. All authors read and approved the final manuscript.

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### ABSTRACT

The discovery of new ecological fertilizers can sustainably enhance plants nutrition. In that point of view, the present study aimed to demonstrate the high concentration of water from cooked beans in organic compounds, various mineral salts and water. For that purpose, moulds were used because of the above listed elements as their basic feeding needs. Cold water from cooked beans was collected and kept during five days at open-air; the evolution of its aspect was daily followed up. The experimental design was a randomized complete block in 10 replicates; an eleventh bucket filled with the same water was used to perform some of its characterization. The moulds are *Aspergillus L.* The water from cooked beans is a heterogeneous mixture and particularly a globular proteic suspension. At rest, it organizes itself in a superficial flaky domain and a lower liquid domain. The flaky domain is mainly organic and the liquid domain is mainly both mineral and aqueous. The density of the flaky domain was 0.964 and that of the aqueous domain was 1.011. The average speed of the growth of *Aspergillus L.* at the surface of the water from cooked beans was 3,17 cm<sup>2</sup>/H; they cover then in five days a surface of 379.74 cm<sup>2</sup>. The exponential growth of

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*Aspergillus L.* at the surface of the water from cooked beans generated a continuous decreasing of the pH; this behavior shows that the water from cooked beans seems to be an adequate substrate; it then implicitly contains all the nutrients required for their optimal development; this include water, organic matters and mineral salts among which nitrogen, potassium, phosphorus, sulfur and calcium can be named; it is then a complete liquid organic fertilizer. That water appears also as a high grade activator for soils micro flora. Peasants could thus save a lot of money by using this liquid single or in combination with other fertilizers to promote the sustainable development of agriculture in their ecosystems.

**Keywords:** Nutrition; substrate; decomposition; fertilizer; suspension; spores; globular; activator; micro flora.

## 1. INTRODUCTION

In ACP (Africa, Caribbean, Pacific) countries, the demographic explosion [1], the reduction of tillable lands, and the reducing of the fallow periods [2] represent today the most perceptible phenomena. This part of the world is peopled essentially by resource-poor farmers, with soils as their principal source of earnings [3]. Hence, to insure their survival, they cultivate even the most sloping plots [4]. Various and risky farming technics are noticeable here [4], the aim was the desire of the daily satisfaction of their needs in food. In consequence, the yields decrease years after years [5,3], attesting then the permanent depletion of soils fertility. If a minority among these peoples can easily acquire synthetic fertilizers to solve in short term this problem of soils fertility depletion [6,7,8], the majority can't. But, even in the first group of these populations, the solution for soil fertility is not completely acquired because of the remaining of queries. In fact, a fraction of them up to today lacks knowledge about the use of these chemicals, since they could be pollutants when poorly used [9]. Moreover, those chemicals are sometimes rare, and their solubility is not often guaranteed [10]. Concerning the second group of these peoples, represented by resource-poor farmers, possess synthetic fertilizers is simply a fairytale. Their survival then appears today with acuteness. That's why day after days, researchers investigate different sectors since the beginning of the twentieth century in order to provide fertilizers at low costs, easily used, efficient, and available for the poorest people worldwide. The tendency of the results obtained today are encouraging. So, in some parts of the world, many were taught in how to use rocks [11,12], plants such as *Titonia diversifolia* [13] and mixtures of organic fluids [14] among all as fertilizers. These approaches already constitute exits for that problem despite their local character; in fact, geological and floral diversity all over the world generates variations in their

application. In front of these signs of potential victories, scientists have not given up; in that way, Fopoussi et al. [14] recently showed that the mixture of human urine and water from cooked beans can pertinently enhanced the growth of plants.

The present study aims then to comfort the observations made by [14,15] concerning the potential agri-use of the water from cooked beans. In the detail, this is about demonstrating the fact that water from cooked beans is a complete organic fertilizer. In that point of view, it will be tested here as a substrate for some heterotrophic organisms, notably moulds; its position as a complete liquid organic fertilizer will be confirmed from there. The choice of moulds for this purpose is oriented by their inability to manufacture by themselves their food, and then, condemned to be often looking for external incoming of organic matters, mineral salts and water, both necessary for them to realize their vital functions [16].

Further, those external foods income represent at the same time the substances required for the improvement of soil fertility parameters as demonstrated by Fopoussi et al. [15] among other.

## 2. MATERIALS AND METHODS

### 2.1 Materials

For the present study, water from cooked beans was used as food provider for moulds according to the studies of [14,15]. Uniform buckets were also used. To guarantee a deep discussion of the results, informations about fermentation of organic matters were collected.

#### 2.1.1 Moulds

##### 2.1.1.1 Definition and classification

Moulds are nonflowering plants. They belong to the branch of Ascomycetes or to the branch of

Zygomycetes [17]. They are filamentous fungi with fluffy aspect. Their vegetative apparatus is a thallium with entangled mycelium. That thallium bears many erect conidiophores; each of those conidiophores ends at its summit by a vesicle. Their identification is easy; in fact, they form less developed flourish mycelia colonies, defining a ring-like organization [18]. Their coloration varies from an individual to another one; so, *Aspergillus candidatus* is whitish, *Aspergillus ochraceus* is reddish, *Aspergillus niger* is darkish, *Aspergillus glaucus* and *Aspergillus flavus* are greenish [19].

#### 2.1.1.2 Spreading organs and environmental parameters favoring their development

The life-cycle of the moulds comprises a vegetative stage (growth and nutrition) and quite simultaneously a reproductive stage. The vegetative stage is the consequence of the germination of the spores. During that stage, moulds produce a network of mycelium that the role is the colonization of the substrate in order to collect nutrients. During the reproductive stage, moulds release spores, which serve as their spreading and resistance organs [18]. The adequate conditions for their development includes: sufficient oxygen, temperatures ranging between 5 to 60°C, a pH from 4 to 7, an appropriate substrate, and sufficient moisture [16].

#### 2.1.1.3 Substrates

Moulds are found worldwide. In sufficient moisture conditions, they established on decaying organic matters, at the soil surface, on dust, and in air. They are essentially saprophagous [20,17].

#### 2.1.1.4 Feeding needs

Moulds are heterotrophic organisms. They collect their food from death and living things. To perform their vital functions, they need carbon (contained principally in simple sugars such as glucose, fructose, etc, or in complex sugars such as starch, cellulose, etc), besides nitrogen (contained in amino acids and peptides), mineral salts (sulphur, phosphorus, potassium, magnesium, calcium, iron, zinc, copper, manganese, boron, molybdenum) and water [17].

#### 2.1.1.5 Feeding process

Small size substances (mineral salts, simple sugars, amine acids) are directly absorbed

through the cell membrane of the mycelium. Larger molecules (starch, cellulose, proteins) are first of all split up into small size substances before being absorbed through the cell membrane of the mycelium [17].

#### 2.1.2 Water from cooked beans

Beans belong to leguminous family. It is rich in vegetal proteins and carbohydrates, with few quantity of fats [21]. Its chemical composition reveals great quantity of nitrogen, phosphorus, sulphur, potassium, iron, calcium, and a few amount of sodium [22,21]. Water from cooked beans is obtained at the end of the cooking process of beans seeds. This water is rich in chemical elements from bean seeds as demonstrated by Fopoussi et al. [14]. Its pH values measured directly after the cooling process range between 6 and 6.5 [14]. At rest, that water divides into two parts: a liquid lower part on which floats a flaky part [15]. The seeds used here is commonly called "Meringue", a subgroup of *Phaseolus vulgaris* L. [22].

#### 2.1.3 Uniform bucket

To reach the focus, eleven cylindrical plastic buckets with an internal section of 379.94 cm<sup>2</sup> were used.

#### 2.1.4 Fermentation

Fermentation is a metabolic process converting sugars into organic acids (citric and malic acids among others), gas, and alcohol; this process is the way for heterotrophic organisms to outtake a bit of energy from decaying matters necessary for their vital functions. This phenomenon requires particular physico-chemical parameters [23].

### 2.2 Methods

To reach the target of the present study, moulds were "invited" through their spores. To obtain the water from cooked beans, a variety of *Phaseolus vulgaris* L. was selected, properly washed and cooked without salt [15]. The cold water of the end of the cooking process was then collected and kept in clean cylindrical buckets with the same capacities at open-air; the experimental design was a randomized complete block in 10 replicates. The water from cooked beans was systematically checked up during five days. The parameters noticed included: the changing of the aspect of the water from cooked beans surface;

the spreading of smells; the invading of the water from cooked beans surface by a circular and whitish sail-like matter; the texture of the flaky domain and the pH. The texture of the flaky domain was evaluated wet and dried under fingers. The pH was measured with a pH-meter bearing a glass electrode. The check-up ended when the surface of the liquid was completely covered by the whitish film. The composition of the water from cooked beans was estimated by an inference approach; in fact, it has consisted to the watching of the behavior of the moulds at the surface of the water from cooked beans, followed by the establishment of the cause-effect pair between the behavior of the moulds observed and the basic knowledge about the needs of moulds; this ended by the elaboration of theories concerning the target. The surface of the water exposed was calculated using the following formula:  $S = \pi R^2$  (S is the surface of the section of the water exposed; R is the internal radius of the bucket;  $\pi$  is 3.14). The speed of the water surface invasion was calculated using the following formula:  $V = S/t$  (V is the speed of the invasion of the surface of the water; S is the surface of the water exposed; t is the time ran out from the beginning to the end of the study).

Every day, the cumulative fraction of the surface covered by the film of moulds was estimated at sight and written down. To perform some little characterization trials of the water from cooked beans, an eleventh bucket filled with that liquid was followed up during two days. The different parts of that water [15] were separated using a sieve with 1 mm mesh. 200 mL of each of those two parts were then weighted using a jeuling metter sensible at 0.1 g. The volumetric mass of those two parts was calculated using the following formula:  $\rho = m/v$  ( $\rho$  is the volumetric mass, m is the mass and v is the volume of each part of the water from cooked beans collected). This was followed by the calculation of the respective density of the different domains of the water from cooked beans, using the following formula  $D = \rho_i / 997 \text{Kg.m}^{-3}$  ( $\rho_i$  being the volumetric mass of the domain of the water from cooked beans considered;  $997 \text{Kg/m}^3$  being the volumetric mass of the pure water between 20 and  $30^\circ\text{C}$ ) according to [24]. The flaky part was dried after the weighing at open air during five days and tested between fingers. The fragments of the dried flaky domain were put in contact with the embers and their behavior followed up during about two minutes. During the study, the temperature of the water from cooked beans was measured with a liquid thermometer. At the end

of the observation, many graphics helped to orientate the different results obtained. Those results were finally described and discussed according to the previous studies made on moulds and on the fermentation processes.

### 3. RESULTS

#### 3.1 Evolution of the Moulds and the Environmental Conditions

The moulds developed at the surface of the cooked beans tested here were whitish in majority. At the beginning of the study (Do), the water from cooked beans was strong brown (Photograph 1), with a low viscosity and a light smell. Its pH was 6.4 (Table 1).

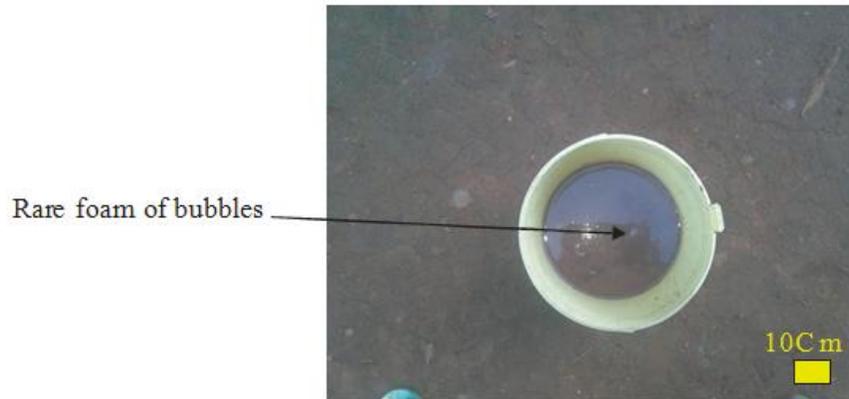


**Photograph 1. Fresh water from cooked beans with its strong brown coloration**

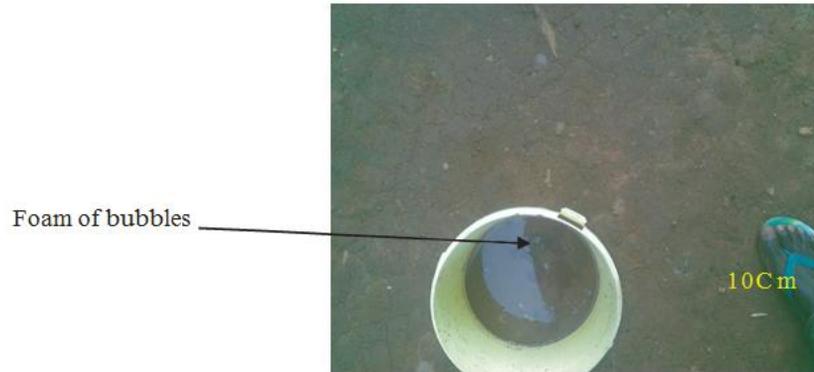
At the end of the first day (D1), the smell remained quite light and the viscosity low. Little quantities of foamy spots appeared at the surface of the water from cooked beans (Photograph 2).

They were made of coalescent millimetric bubbles. The pH of the liquid at this stage was 6.2 (Table 1).

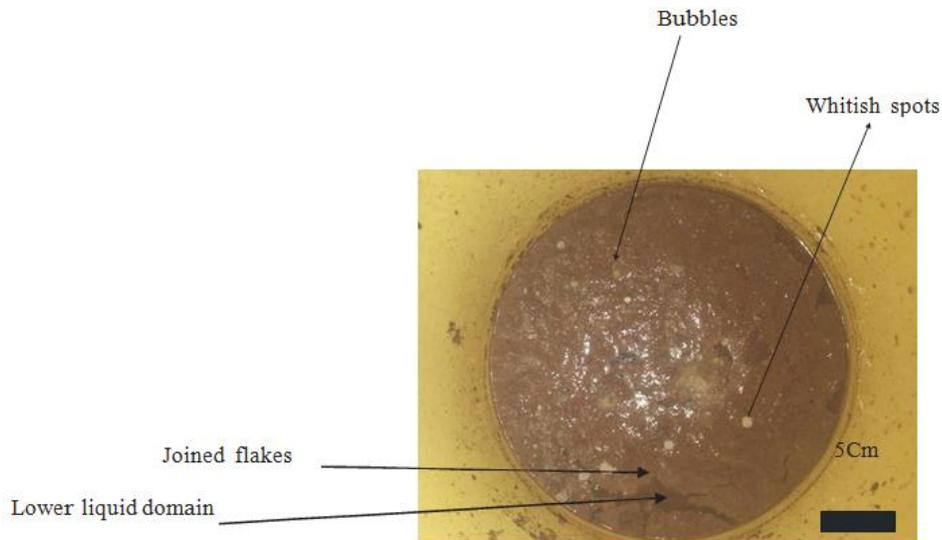
At the end of the second day (D2), the size of the bubbles constituted the foams has increased (Photograph 3). The smell produced was felt at about 1 m away from the buckets. The water presented two domains: a liquid lower domain with a light brownish coloration; on that liquid, floats the second domain, made of joined brownish flakes, covering completely the surface of the water below. At the surface of the flakes, small whitish domains were visible. They cover about 2% ( $7,5988 \text{ cm}^2$ ) of the whole surface of the flake tide. The pH of the liquid below at this stage was 5.85 (Table 1).



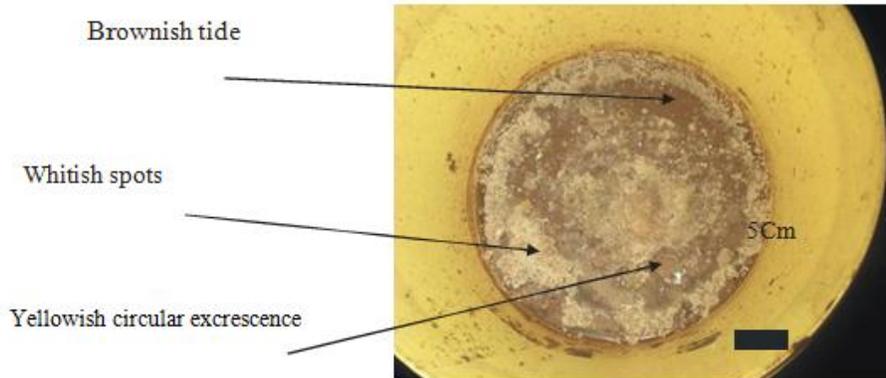
**Photograph 2. Water from cooked beans with rare foam of bubbles at its surface**



**Photograph 3. Foam of bubbles formed at the surface of the water from cooked beans**



**Photograph 4. Joined flakes at the surface of the liquid domain bearing bubbles and whitish spots**



**Photograph 5. Joined whitish spots developed at the surface of the brownish tide**

At the end of the third day (d3), bubbles were there. The smell was felt at about 3 m away from the buckets. The tide made of joined flakes has thickened; it remained brownish (Photograph 4).

The whitish domains have increased in number and size at the surface of the brownish tide. They constituted circles with millimetric to centimetric diameters; those diameters in the detail ranged between 3 mm to about 25 mm in average. They covered about 25% (94.985 cm<sup>2</sup>) of the brownish tide. The pH value of the water below at this stage was 5.2 (Table 1).

At the end of the fourth day (D4), the smell from the buckets had reduced in intensity. The whitish spots were now completely joined (Photograph 5) and form a large sail-like circle.

This sail-like circle covers about 95% (360.943 cm<sup>2</sup>) of the surface of the brownish tide. At the surface of the whitish sail-like circle, concentric local domains were recognized. Moreover, one observed yellowish circular excrescences with 8mm of diameter and 5 mm of high in average. On the wall of the buckets where the water from cooked beans was in contact with, millimetric (about 5 mm in average diameter) roundish spots of the same whitish invading matter were observed in great quantities. The pH of the liquid below the brownish tide at this stage was 4.5 (Table 1).

At the end of the fifth day (D5), the smell was concentrated at about 30 cm around the buckets; it recalling the smell from a corpse; it attracting many flies. The circular sail-like whitish matter (Photograph 6) has taken the aspect of a pan-cake covering completely the surface of the

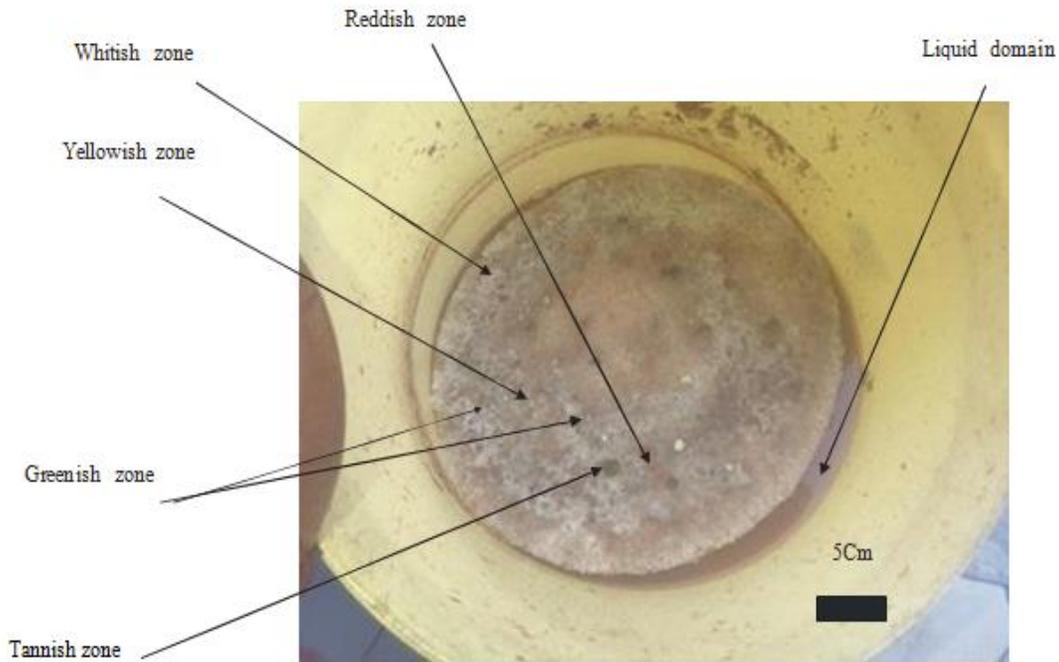
water below (100% of coverage, corresponding to the 379.74 cm<sup>2</sup> surface of the internal section of the buckets) on which it floated; a close observation of the pan-cake like matter revealed an aspect recalling that of some wet flour. It presented local variations in coloration including the greenish, the yellowish, the reddish, and the tannish, all these disseminated at the surface of the whitish pan-cake-like volume.

The brownish flakes have sunk towards the bottom of the liquid domain, leaving the place to the pan-cake-like volume. The center and the extremities of the pan-cake-like volume were locally twisted. The estimated thickness of the pan-cake-like volume was about 3 mm. The concentric local domains recognized at the end of the fourth day have increased in size. The yellowish circular excrescence have increased in size too. The millimetric roundish spots of the whitish invading matter on the sides of the buckets got dried. The pH value of the liquid below was 4.1 (Table 1).

The whole surface of the water from cooked beans was completely invaded after five days; this corresponded to 120 hours. The average invasion speed was 3.17 cm<sup>2</sup>/H.

**Di: day i**

From the beginning of the study (D0) to the end of the fifth day (D5), the pH values decreased progressively (Fig. 1). In the details, the pH turned down as follow: 0.2 unit from the beginning to the end of the first day; 0.35 from the end of the first to the end of the second day; 0.63 from the end of the second to the end of the third day; 0.7 from the end of the third to the end



**Photograph 6. The circular sail-like whitish matter bearing zones with different colorations**

**Table 1. Evolution of the pH and the surface covered by moulds during the study**

Surface covered (cm <sup>2</sup> )	pH	Days
0	6.4	D0
0	6.2	D1
7.5988	5.85	D2
94.985	5.2	D3
360.943	4.5	D4
379.74	4.1	D5

of the fourth day and 0.3 from the end of the fourth to the end of the fifth day. Globally, the difference between the pH measured on the water from cooked beans at the beginning of the experiment and that measured at the end of the experiment was 2.3 (Fig. 1 and Table 1).

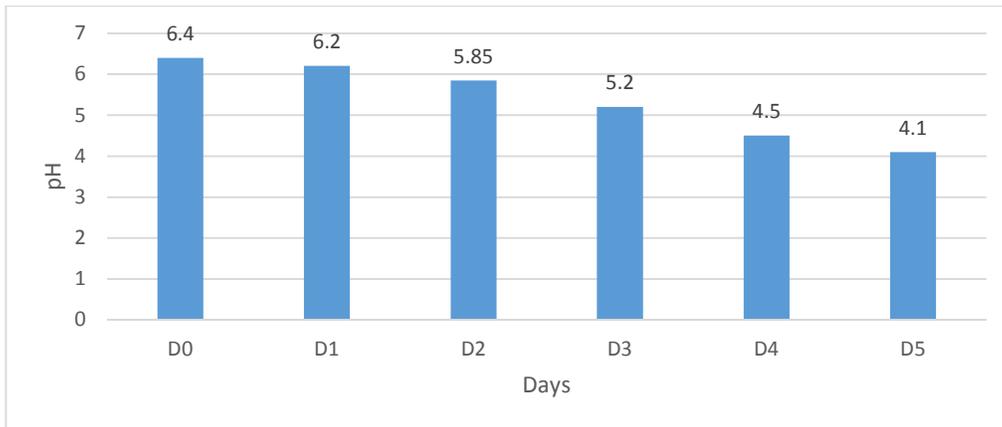
From the beginning of the study (D0) to the end of the second day (D2), the coverage of the water from cooked beans was nil to very weak (Fig. 2). From the end of the second day to the end of the third day (D3), the coverage increased. But, from the end of the third day to the end of the fourth day (D4), the coverage increases abruptly before remaining quite constant at the end of the fifth day (D5) (Fig. 2).

The correlation made between the behavior of the pH of the water from cooked beans and the

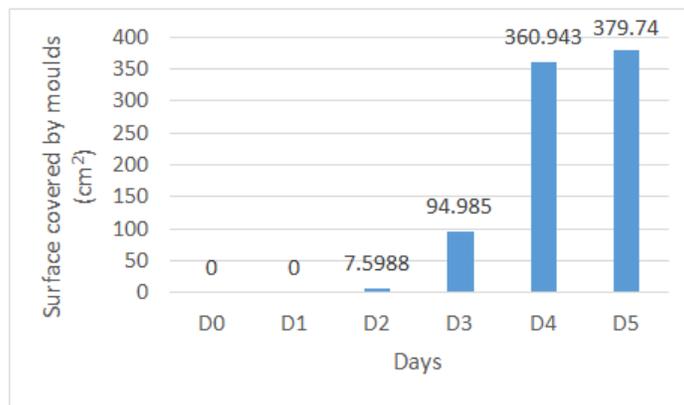
coverage of the surface of the same water by the whitish pan-cake-like volume formed in the expense of the brownish flakes floating on the water in the beginning of the study was negative (Fig. 3). The Pearson index of the correlation calculated for those two parameters was then -0.0027. According to the Fig. 4, when the coverage of the water from cooked beans increases, the pH of the same water decreases.

### 3.2 Characteristics of the Different Domains of the Water from Cooked Beans

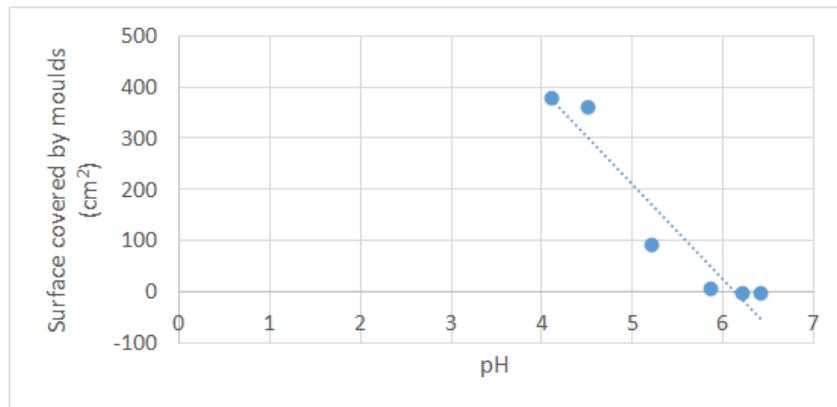
The water from cooked beans after two days divides itself into two domains. A flaky domain and a completely liquid domain.



**Fig. 1. Evolution of the pH during the period of the study**



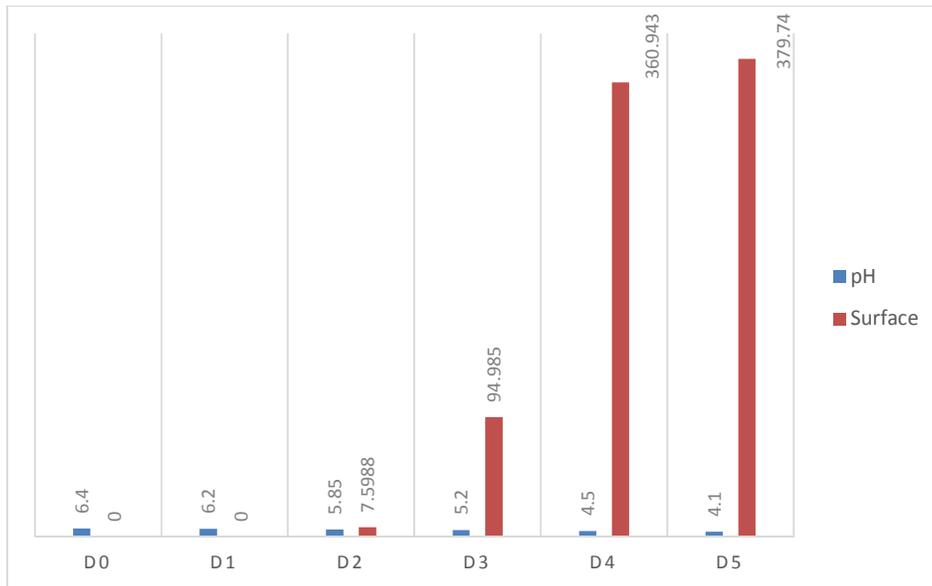
**Fig. 2. Evolution of the surface covered by the moulds during the period of the study**



**Fig. 3. Correlation between the behaviors of the surface covered by moulds and the pH of the water from cooked beans during the period of the study**

The flaky domain was creamy (Photograph 7), highly wet, sticking under the fingers. At the end of the first day of the drying, it was traversed by many disjoined circular fissures with openings ranging between 1 to 3 mm in average

(Photograph 8a); those fissures tended to subdivide the quite dried flaky domain into a dense inner core surrounded by a disc-like outer part. Many small insects with orange head were present within the fissures.



**Fig. 4. Evolution of the pH and the coverage of the water from cooked beans**

At the end of the second day of the drying, a single continual fissure was present between the inner core and the disc-like outer part; its opening reached locally 4 mm (Photograph 8b). Many centripetal fissures with opening of about 1mm appeared in the disc-like outer part, dislocating it into portions with various sizes. The insects remained present. At the end of the third and the fourth days of drying, the opening of the circular fissure reached 6mm (photograph 8c). The centripetal fissures have increased in number and size; in fact, their openings reached 2.5 mm about. The original fragments of the disc-like outer part of the quite dried flaky matter was now subdivided into smaller portions. Both in the inner core and in the fragments from the outer disc-like part, many millimetric sequent fissures were present; they subdivide the whole quite dried flaky matter into dense polygonal islets.

At the end of the fifth day of drying, the fissures were completely coalescent with openings surpassing 5mm (Photograph 9).

Completely dried, the flaky domain was spongy (Photograph 10).



**Photograph 7. The creamy aspect of the flaky domain isolated**



**Photograph 8. Evolution of the aspect of the flaky matter under drying with various sizes (Photograph 8c). The insects remained present**



**Photograph 9. General aspect of the dried flaky matter after five days of drying**

Crushed, it was flourish and silty. Many nymphs and some maggots were present at the end of the fifth day in the dried flaky matter (Photograph 12).

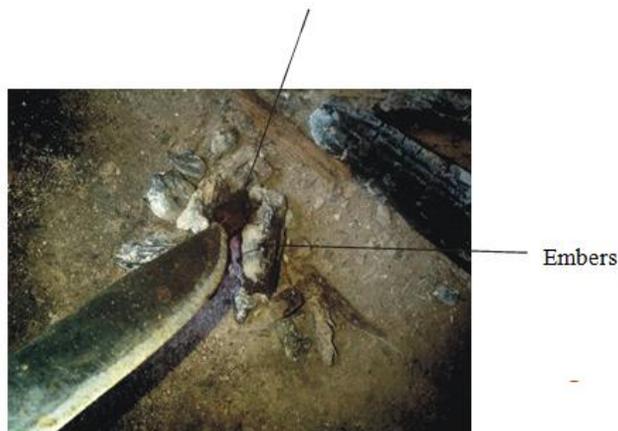
At the second day of the study, the reserved water from cooked beans presented two different domains: the liquid lower domain on which floats the flaky domain. 200 mL of the flaky domain weights 194.3 g and the same volume of the liquid domain below was weighted 201.6 g; this implied a difference of 6.3 g. The volumetric mass of the flaky domain full of bubbles calculated was  $0.9615 \text{ g/cm}^3$  and that of the completely liquid domain below was  $1.008 \text{ g/cm}^3$ . Concerning the density of each of those two domains, that of the flaky domain was 0.964 and that of the liquid part was 1.011. During the study, the temperature of the water ranged between 28 and 30°C.

In contact with embers, the dried flaky matters burns, producing a light grey smoke (Photograph 11).



**Photograph 10. The spongy aspect of the dried flaky matter**

Dried flaky matter on embers



**Photograph 11. Dried flaky matter burning on embers**



**Photograph 12. Maggots and nymphs within the dried flaky matter**

**Table 2. Some physical characteristics of the different domains of the water from cooked beans**

	Mass (g)	Volume (cm <sup>3</sup> )	Volumetric mass (g/cm <sup>3</sup> )	density
Flaky superficial domain	192.3	200	0.9615	0.964
Liquid lower domain	201.6	200	1.008	1.011

#### 4. DISCUSSION

Spores are spread worldwide with the help of water, dust, and air among others; when those microscopic organs rich on a favorable substrate, they germinate and turn into microscopic mycelia which attack their substrates to collect their food according to [18]. The strong brown coloration and the light smell of the water from cooked beans at the beginning of the study demonstrate the absence of its attack by moulds, since spores deposited would still be in their germination stage; this observations agree with the studies of Fopoussi et al. [15] and Basset and Lafont [18]. The flaky domain appeared after three days as an individualized domain floating at the surface of the liquid lower one; this could be due to the segregation acting within the water from cooked beans in response to the sequestration of gas produced and held by some components of that matter; such an assertion is justified by the presence of bubbles with different sizes at the surface of the flakes. The increasing of the size of the bubbles reveals the augmentation of the quantity of gas produced during the attack of the organic components of the water from cooked beans by the mycelia of moulds as showed previously by Basset and Lafont [18]; they are then saprophagous [17]. In fact, Magnuson and Lasure [23] demonstrated that during the process of the decomposition, many gas are produced; supplementing that observations, [25] showed

that those gas kept in died corpses make them behave as pneumatic matters capable to float on water. This floatation observed is in accordance with the difference of the respective density of the lower liquid domain and the superficial flaky domain. The signs of the decomposition highlighted here make the flaky domain an organic compound, since only organic matters can undergo decomposition [26]; such a fact logically dragged our attention towards the producing of mycelia from spores germination, requiring nutrients to feed on [18]. This is in accordance with the heaviness of the smell during the study. The capacity of the liquid domain below to carry the flaky domain is also due to its richness in mineral salt; in fact, [15] in that way demonstrated the capacity of the water from cooked beans to improve the fertility parameters of strongly acids and desaturated soils from the western Highlands of Cameroon. The presence of two domains with different characteristics within the water from cooked beans makes it a heterogeneous mixture.

Three days after the beginning of the study, flourish spots appeared at the surface of the flakes floating at the surface of the liquid domain; that aspect is common to *Aspergillus L.* according to [18]. Four days after the beginning of the study, the flourish spots got joined, forming a sort of pan-cake-like matter, alongside with the decreasing of the pH values. This recalls the

intense nutrition coupled to the development stage of these organisms during their life-cycle as demonstrated by Basset and Lafont [18]. At the surface of the whitish pan-cake like organisms, local variations in coloration are observed; they include the greenish, the yellowish, the reddish, and the tannish colorations. The studies of William et al. [19] make clearer the understanding of such phenomenon by precisating that the whitish coloration is common to *Aspergillus candidatus*, the reddish coloration is common to *Aspergillus ochraceus*, the tanned coloration is common to *Aspergillus niger* and the greenish coloration is common to *Aspergillus glaucus* and *Aspergillus flavus*. The results obtained by El-Naggar and El-Hersh [27] provide elements useful in the characterization of those organisms as heterotrophic plants with thallium as their vegetative apparatus. Restino [16] completed the characterization of those individuals by considering them as microscopic organisms; in that point of view, the speed of invasion calculated here (3,17cm<sup>2</sup>/H) can be considered as very high. In fact, Meyer et al. [17] demonstrated that in optimal conditions, those microorganisms can colonize very large surfaces in few delay; this observation corroborates the conclusions of [15,14] who present water from cooked beans as a potentially rich and complete organic fertilizer. From this observation, we can say that the basic needs in foods of those living organisms seem to be easily covered on a substrate such as the water from cooked beans where the different nutrients required for their feeding process are easily available after [18]. In consequence, mineral salts and water are directly absorbed. Concerning organic compounds (containing carbon and nitrogen in great quantity), they are digested through fermentation with the help of enzymes out of the mycelia and transformed into sugars, required for the metabolism of those heterotrophic organisms [17]. This phenomenon is followed by the releasing of organic gas that the escape at the surface of the water from cooked beans is certified by the presence of bubbles of different sizes highlighted above. Restino [16] at the end of its research confirms this assertion, insisting on the following nutrients as the main components absorbed by mycelia from their substrate to feed on: carbon, nitrogen, mineral salts, and water.

From this, we can confirm the hypothesis of [14] and [15] which put into relief the fact that water from cooked beans can be a very rich, natural,

and complete liquid organic fertilizers, concentrating easily absorbable nutrients for plants. During the study, the pH values of the water from cooked beans ranged between 4 to 6.5; these values belong to the intervals where microscopic fungi flourish [16]. The gradual decreasing of the pH during the study attested the releasing of organic acids in the water from cooked beans as demonstrated by Iqbal and Saeed [28]. In fact, Magnuson and Lasure [23], studying the fermentation process induced by moulds, reach to the conclusion that organic acids such as citric acid and malic acid among others can be produced by those organisms on liquid substrates. This observation corroborates the organic nature of the water from cooked beans. Furthermore, fermentation is known to act only on organic matters in reference to Magnuson and Lasure [23]; therefore, this shoes once more that the water from cooked beans is filled with organic matters. According to the natural richness of bean seeds with proteins, the organic compounds present here would be mostly nitrogenous (amino acids). In fact, during its reasoning,

Fopoussi [29] highlighted the fact that through the cooking process of beans, proteins are hydrolyzed to produce small size organic compounds capable to cross the envelop of the seeds through the process of diffusion. The negative correlation between the progressive coverage of the water from cooked beans surface and the pH behavior is normal. In fact, the richness of water from cooked beans in the different nutrients required for the feeding of *Aspergillus L.* makes them very active [30]. The consequence of this fact was the releasing of great quantities of organic acids as the consequence of fermentation process going on, followed by an exponential development of those microscopic organisms and the decreasing of the pH. This is in accordance with the Pearson index calculated (-0.0027). The twist of the edges of the pan-cake-like organisms made of the cohabitation of the different sub-group of *Aspergillus L.* observed here is due to the fact that the buckets at the end of the fifth day were small to content the development of those microscopic organisms, evolving on an optimal substrate. This confirms the observation of [31] who realize in Europe that in certain forests, *Aspergillus L.* can cover in optimal conditions very large surfaces, up to many kilometers.

After the days of drying, fissures appear within the flaky domain with increasing widths alongside

with the appearance of polygons with various sizes. This can be in one hand understood as the consequence of the progressive loose of water by the flaky matter. In the other hand, the polygonation observed during the drying process testifies further the richness of the flaky matter in water; that richness in water is one of the requirements for the moulds development [17]. Such behavior is commonly described within humic soils during dry season as demonstrated by Fopoussi [29]. Under the fingers, the wet flaky domain is creamy and slightly sticks. This behavior is common to proteins, particularly albumin derivatives; in that point of view, [32] qualified albumin as proteic glue. From this, the heterogeneity of the water from cooked beans mentioned above is simply the indicator of a globular proteic suspension [33], formed after the migration of proteic components from the cotyledons through the bean seeds envelop during the cooking process towards the water of the cooking process [29]. This is in accordance with the development of the albumen egg as one of the results of the double fecundation happening in angiosperms, that the role is to keep organic matters such as proteins, useful to nourish the germ during its transformation into a young plant [34]. The observations of Fopoussi [29] confirm this characterization; in fact, at the end of its fertilization trials with the water from cooked beans on strongly acid and desaturated Andosols from the Western Highlands of Cameroon, he noticed an important enrichment of soils with nitrogen within three months, which is one of the main chemical element in proteins according to Wright and Dyson [35]. Completely dried, the fragments of the flaky domain are spongy; when crushed, they are flourish and silty. The spongy aspect recalls the behavior of the egg white beat for a long time; the flourish and the silty aspects recall the behavior of egg white in powder developed by [36].

According to Wright and Dyson [35], this reveals once more the presence in great quantity of proteic derivatives in the water from cooked beans. The fragments of the dried flaky domain in contact with the embers burn, producing in consequence a light grey smoke; this is the characteristic of organic matters. In that point of view, [37] reveals that during the combustion of a matter, the organic part escapes as smoke and the mineral part remains as cinders. Small flies with orange head are present in the fissures of the dried flaky domain; these are surely vinegar flies.

Those small flies are known to feed on sugars kept in fresh or rotting matters; they are known to lay their eggs in organic matters rich in nutrients required for the nutrition of their larva to be born according to [38]. This is in accordance with the presence of many nymphs and some maggots observed in the dried flaky matter at the end of the fifth day of drying. From this, we can say that the flaky domain contains apart from proteic substances, carbohydrates. This observation contribute to strengthen the organic nature of the flaky domain. Vannier [39] demonstrated that the micro flora of soils is made of bacteria, moulds, etc.

He demonstrated also that their heterotrophic nature bring closer their feeding needs. Hence, since the water from cooked beans can enhance the multiplication of moulds, it does the same for bacteria surely. Knowing the importance of the micro flora in the pedological physiology, the water from cooked beans is not only a source of nutrients for plants, but it also positions itself as a high grade activator for soils micro flora. During the experimentation, the ambient temperature ranges between 25 and 30°C. This temperatures are common to warm and wet tropical climate [40].

## 5. CONCLUSION

The water from cooked beans is a heterogeneous mixture, and particularly a proteic globular suspension. At rest, that water divides itself into two superimposed domains: a flaky superficial domain and a liquid lower domain. The flaky domain is the organic part and the liquid domain is the water and the mineral salts provider. The density of the flaky domain is 0.964 and that of the liquid domain is 1.011. The average speed of the growth of *Aspergillus L.* at the surface of the water from cooked beans is 3,17 cm<sup>2</sup>/H; they cover in five days a surface of 379.74 cm<sup>2</sup>. The physic-chemical characteristics of the flaky domain floating on the liquid domain make it an adequate area for the development of moulds (*Aspergillus L.*). The water from cooked beans Seems then to contain all the nutrients required for an optimal development of moulds (*Aspergillus L.*), and in the same way for an optimal fertilization of soils; this include water, organic matters and mineral salts among which nitrogen, potassium, phosphorus, sulfur and calcium can be named. It is then a complete liquid organic fertilizer. That water positions itself also as a high grade activator for soils micro flora. It represents finally a way for a sustainable

improvement of agriculture in developing countries and a way for a sustainable development of soils micro flora, required for the reach of the food self-sufficiency.

## 6. RECOMMANDATION

To avoid malodorous smell during the agri-use of the present liquid, its application must be directly followed by the hilling at the foot of plants.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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