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RESEARCH ARTICLE

SEED PRODUCTION TRIALS OF *VOLVARIELLA VOLVACEA* ON VARIOUS SUBSTRATES IN DALOA (CENTRAL WEST - CÔTE D'IVOIRE)

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Abstract

The objective of this study was to improve the seed production of *Volvariellavolvacea*in Daloa(central-west; Côte d'Ivoire). Tissue fragments were cultured on PDA (Potato Dextrose Agar)medium for the production of mycelium. Once the mycelium was obtained, it was inoculated into jars containing pre-cooked cereals (maize, sorghum) and legumes and sterilized (at 121°C). When the jars are completely covered with mycelium, the different parameters are measured. The results obtained showed that the mycelium of *Volvariellavolvacea*can propagate on PDA medium, legumes and also cereals. The seed production of *Volvariellavolvacea*is susceptible to contamination. Results after 10 days of incubation showed that the mycelium grows best on the following substrates: sorghum (14.1 cm), soybean (13.4) and bean (12.9 cm). Corn showed low values (6.3 cm) for mycelial growth.

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Introduction:-

Fungi are an integral part of most natural ecosystems and contribute to the redistribution of food resources used by all organisms in the environment (Barros et al., 2007; Reis et al., 2012). Many fungal species are of interest in human nutrition and health. More than 2000 species are edible and nearly 700 species have interesting pharmaceutical properties (Barros et al., 2007; Reis et al., 2012). Nutritionally, edible forest mushrooms are rich in protein, fiber, and low in fat. They contain important vitamins and trace elements (Ndoye et al., 2007).

In sub-Saharan Africa, work on wild mushrooms has been conducted and has identified approximately 400 species with use value (Muluwa et al., 2013). In Côte d'Ivoire, ecological, taxonomic, ethnomycological, and socioeconomic studies on edible mushrooms have resulted in a list of 67 species (Kouassi, 2012). The most consumed genera are Amanita, Auricularia, Chantarellus, Lactarus, Termitomyces, Psathyrella, and Volvariella (Degreef et al., 2016). The Volvariellavolvacea mushroom represents an important source of protein, its nutritional value is comparable to that of eggs (Oei, 2003). This mushroom is widely consumed in the world, particularly in sub-Saharan African countries and by a large part of the Ivorian population (Boa, 2006). Mushroom cultivation is not widely practiced and this aspect of mycology remains under-explored in Côte d'Ivoire (Soko et al., 2019). Their appearance occurs during the rainy season for a few weeks per year (Bram Van and Janna, 2007).

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Given the importance and year round availability of *Volvariellavolvacea*, the production of its seed is a necessity in Côte d'Ivoire. The objective of this work is to use cereals and legumes as supports for the improvement of *Volvariellavolvacea*seed production in Daloa.

Material and Methods:-

Material:-

The material used is of two types: biological material and technical material.

Biological materials

The biological material used, is derived from tissue fragments of fresh *Volvariellavolvacea*mushrooms, potato tubers (Figure 1), two cereals: maize, sorghum and two legumes: soybean, bean (Figure 2).

Figure 1:- Some biological material ; A : Carpophore de *Volvariellavolvacea* ; B : Potato tubers.

Figure 2:- Some mushroom seed production media ;A) Corn seeds ; B) Soybean seeds ; C) Bean seeds ; D) Sorghum seeds.

Technical material

The technical material used, is the usual and indispensable equipment of a microbiology laboratory (Table I).

Table I:- Some materials and their uses.

Materials	Uses
Fume hood	Workunderasepticconditions
Autoclave	Sterilizingsubstrates
Agar-agar	Solidify the environment
Benzenespout	Handle under good aseptic conditions
Glass bottles	Storing grains and seeds
Fragment of mushroom tissue	Producemycelium
Alcohol at 70°, bleach, cotton.	Disinfect the equipment

Scalpel	Remove the tissue fragment
Balance	Weigh the products
Petridishes	Contain the culture medium
Beaker Syringe	Prepare medium Casting the medium
Sucrose	Enrich the PDA medium with glucose
Graduatedruler	Measuring the length of the mycelium

Methods:-

Preparation of the PDA (Potato Dextose Agar) culture medium

For the preparation of 0.5 L of PDA medium, 100 g of diced potatoes, 10 g of agar powder, 10 g of sucrose and 0.5 L of water were used. The preparation of the medium is done in several steps.

Step one :

The potatoes are washed and weighed, then cut into small pieces. Then to these pieces of potatoes, is added half a liter of water. The mixture is boiled at 90°C for 15 to 20 minutes. The broth obtained is removed and completed with water until half a liter of solution is obtained.

Step two :

Sugar and agar are added slowly to the resulting solution while stirring until the agar has melted. Finally, the final product is sterilized in an autoclave at 121°C under a pressure of 1bar for 1h. Once the medium is sterilized, add half a capsule of amoxicillin and half a capsule of norflloxacin and homogenize.

Step Three :

Using a syringe, a quantity of the culture medium is withdrawn, taking care to close thelid of the jar immediately. Around the flame, the petri dish is opened and the medium is poured to a quarter of its volume. Finally, the boxes are left to cool.

Culture of a tissue fragment

The culture started by washing a young carpophore and sterilizing the scalpel. Using the scalpel, a fragment of tissue (about 2 x 2 mm) of the harvested fungus was taken from decayingpalms at the experimental site. The tissue is then gently placed on the PDA medium and the petri dish is immediately closed. Finally, all plates are inoculated and placed in an oven at 37°C for 5 days.

Preparation of the white of semi

The culture consists in transferring the mycelium from petri dishes to flasks containingthe substrate. First, 500g of each of the cereals and legumes (soybean, corn, bean and sorghum) are rinsed in water, this operation is repeated 2 to 3 times, until the rinse water becomes clear. After rinsing, cover with water, are added to the mixture, 5g (about 1% of the weight of plant support) of calcium carbonate ($CaCO_3$). Cover the containers well and let the cereals and legumes soak for 12 hours.

The seeds and grains are then boiled for 10 min before draining with a strainer. The jarsfilled to 2/3 of the substrates, are placed in the autoclave to sterilize them for 1h30 min. Once cooled, the jars are removed from the autoclave. The flame scalpel is sterilized, then cooled next to the flame for 20-30s. The agar is cut into 4 pieces and a piece of agar is used to inoculate each jar. Between each agar sample, the flame scalpel is sterilized. Thus, at the end of the experiment, the jars containing the inoculated grains and seeds are placed in the oven for ten (10) days at a temperature of about 37°C. Parameters at the level of the PDA culture medium and at the level of colonization of cereals and legumes (spawn) were evaluated.For crop contamination rates, the following relationship was used :

$$T = \frac{\text{Number of contaminated boxes}}{\text{Number of boxes}} \times 100$$

Regarding the length of mycelium cover, the values were taken every day for 5 days.As for the speed of recovery of the medium was calculated according to the following expression :

$$v = \frac{\text{Length of mycelium cover}}{\text{Overlap time}}$$

Results and Discussion:-

Results:-

Culture on PDA medium

Lengths of mycelial propagation

The culture of the tissue fragment of Volvariella volvacea on PDA medium after five (5) days showed good development. The evolution of mycelium was observed on the forty-two(42) Petri dishes. The length of mycelium propagation is 8.71 cm. At the end of the five days of culture, the mycelium covered the entire 9 cm dish. The average length is 1.45 cm/day (Table II).

Table II:- Mycelial propagation lengths on PDA.

Time	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Lengths(cm)	0	1.36	3.17	5.86	7.69	8.71

Fungal contamination rate

After incubation, some petri dishes showed mycelial colonies of various colors. The appearance of yellow, blue, green or grey mycelia on the surface of the culture medium corresponded to contaminations. Fungal contaminations concerned 12 plates out of 42 plates. The uncontaminated plates showed a whitish and cottony mycelium. These results gave a contamination rate of 28.43 % (Figure 3). Figure 4 shows a contaminated Petri dish and an uncontaminated dish.

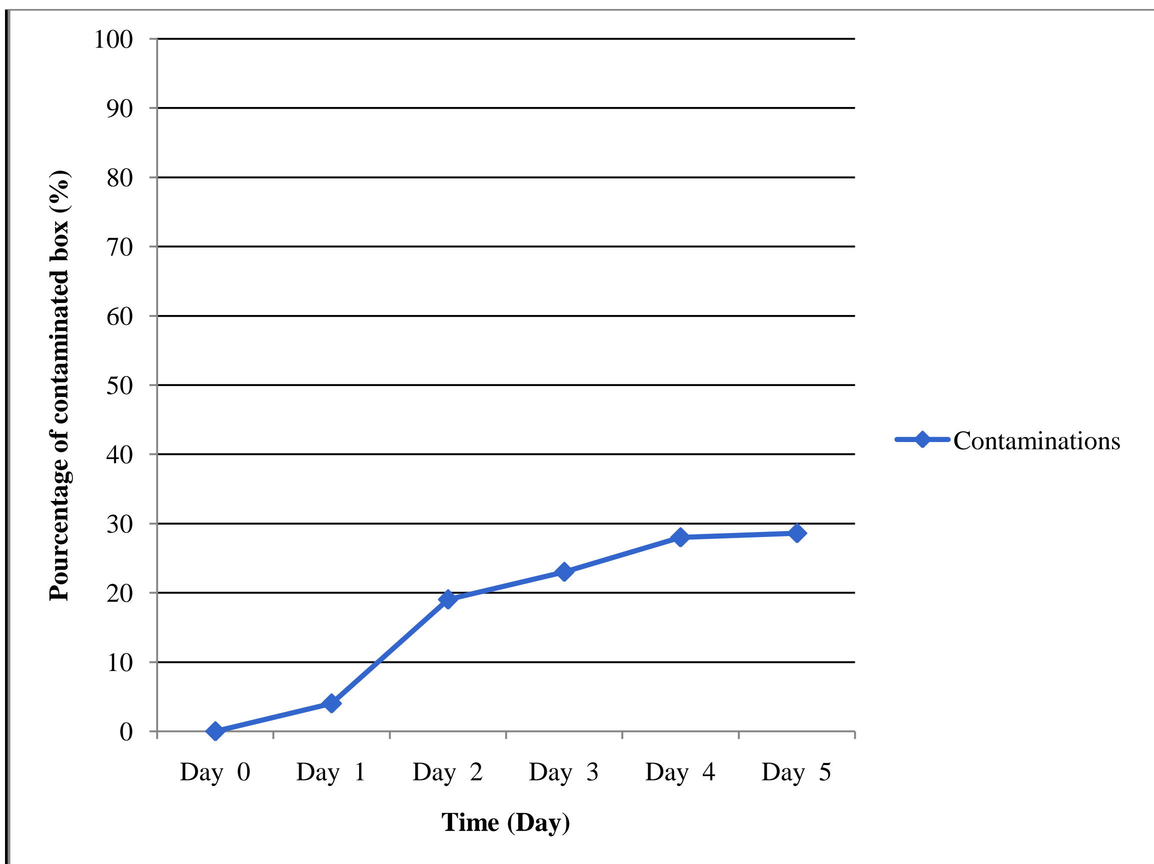

Figure 3:- Pourcentage of contaminated box.

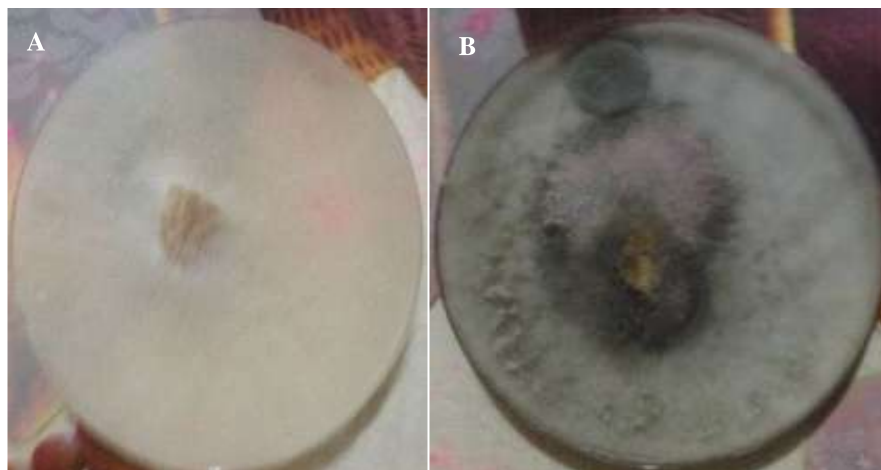

Figure 4:- Boxes containing mycelial cultures ; A : Non-contaminated petri dish ; B : Contaminated petri dish.

Production of semi white

Length of substrate invasion

The mother culture was prepared on different cellulosic substrates: corn grains, sorghum grains, bean seeds and soybeans. The results obtained after 10 days of incubation at 37°C, presented the behavior of the mycelium on the different substrates. The length of invasion of the mycelium on the different substrates was measured every two days during the incubationperiod. The results of this experiment, are recorded in Table III. The mycelium of *Volvariellavolvacea*spread differently in ten (10) days of incubation on the four (4) types of plant substrates used namely corn, bean, soybean and sorghum. Sorghum (14.1 cm), soybean (13.4 cm); and bean (12.9 cm) showed good mycelium growth. Maize (6.3 cm) showed poor mycelial growth (Table III). These results showed that the mycelium grows progressively on the different propagation media. The highest lengths were observed on sorghum, soybean and bean. However, the lowest lengths were recorded on maize.

Table III:- Mycelial growth on different media.

Days **Length (cm**	**Day 0**	**Day 2**	**Day 4**	**Day 6**	**Day 8**	**Day 10**
Soybeans	0	1.6	4.9	12.6	13.3	13.4
Corn	0	1.9	2.2	2.4	3.9	6.3
Bean	0	2.4	4.6	12.1	12.6	12.9
Sorghum	0	0.9	4.9	11.4	12.9	14.1

The speed of invasion of the mycelium on the different grains and seeds

The velocity curve shows a progressive evolution for sorghum, soybean and bean (Figure 5). It reached its peak on day 10ème. Corn showed a good evolution during the first two days. From day 2ème to day 6ème growth was steady. After the 6ème day, a weak recovery of growth was observed. The average growth rate was 2.23 cm/day for soybean, 1.05 cm/day for corn, 2.15cm/day for bean and 2.35 cm/day for sorghum. The spread was fastest on sorghum; then soybean, then bean and finally corn (Figure 5).

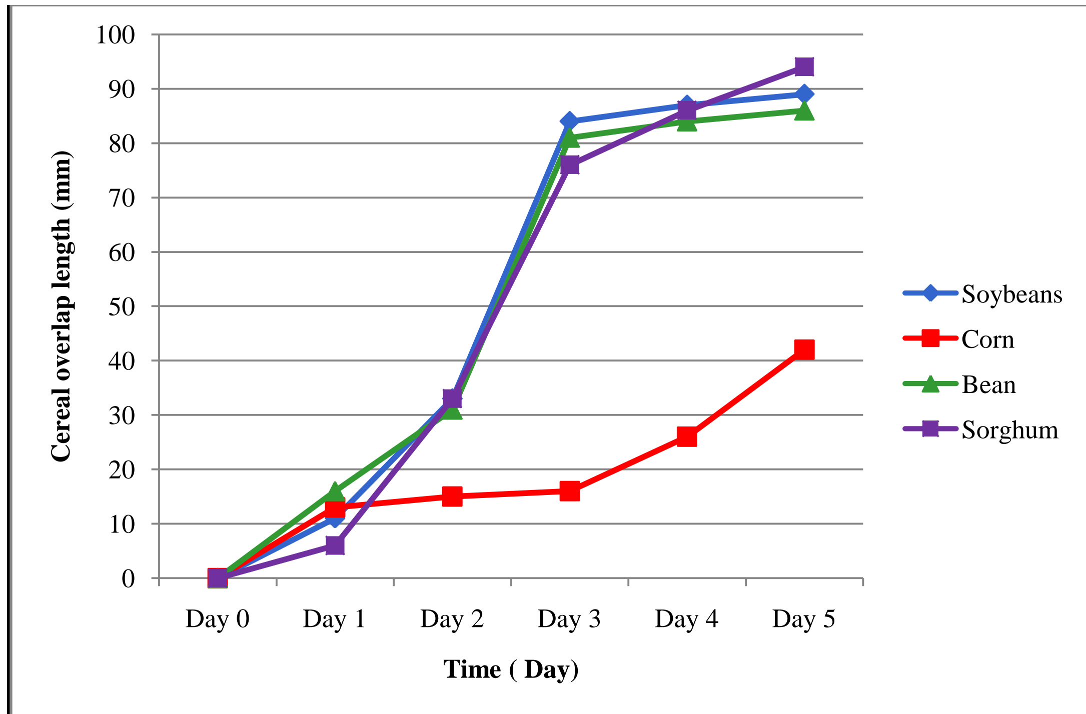

Figure 5:- Growth rate curves for powdery mildew on soybeans, corn, beans and sorghum.

Contamination rates of the crop

The mycelium obtained on grains and seeds is longitudinally linear, then aerial and disorganized. The color is white to grayish-white at first and light gray in the end, often with discolored areas. However, contamination was observed in some jars including 1/3 corn, 1/3 sorghum and 1/3 beans. Only the soybean jars were not contaminated. In the bean jars, the PDA medium was rapidly degraded. Based on the results, sorghum was found to be the best substrate of the four (4) culture media used for the production of spawn of Volvariella volvacea. Soybean and bean can also be used although they are susceptible to contamination. However, corn was found to be a substrate with low spawn production of *Volvariella volvacea*(Figure 6).

Figure 6:- Jars containing seeds ;A) Semi-blank on sorghum, B) Semi-blank on bean, C) Semi- blank on soybean and D) Semi- blank on corn.

Dicussion:-

On the PDA medium, the rate of mycelial development was progressive. This mediumused favors a good growth of the fungal mycelium. Indeed, the PDA medium is a standard medium designed for the cultivation of a wide

spectrum of fungal species. This medium has been described by several authors, its composition is simple and rich in carbohydrates (Ellis, 1979).

The success rate of the mycelium culture is globally high showing that the experiments were performed under adequate environmental conditions. These environmental conditions are: a temperature between 27°C and 30°C and a relative humidity in the range of 80-90%. Benamar-Mansour et al, (2016) indicated that temperature is a determining factor in the production of good quality mycelium. Therefore, a good temperature would ensure a high success rate for seed production.

The relatively short seed incubation time results in a high mycelium growth rate of thisspecies. This observation corroborates with the work of (Dibalukaet al., 2010) who indicated that the faster growth of mycelium in cultivated species reduces their incubation time. After three to four days, the mycelium will have covered the tissue which will branch out onto the agar.

Seeds or any other type of substrate contain a large number of contaminants. Preparation for 45 minutes in an automatic sterilizer at 121°C on average, is usually sufficient to eliminate all harmful organisms in the growing medium (Sobeiralski, 2011). It takes a specific amount of time for the steam to heat all parts of the substrate. The rate of pest removal, as well as the duration of sterilization, is dependent on the amount of seed in the bottles, the intensity of the heat, and the type of sterilizer used (Ninkwango, 2014).

The jars containing the inoculated seeds were placed in the oven for ten (10) days at a temperature of about 37°C and in the dark. The mycelium obtained appeared as a whitish mat covering the seeds forming the culture media.

The incubation allows the invasion of the culture medium by the inoculated mycelium.The incubation period is the time taken by the mycelium to colonize all the medium contained in the bottles. This period depends on the composition of the substrate, the rate of inoculation, the aggressiveness of the mycelium, the temperature (15 to 25°C), as well as the relative humidity of the room. Incubation takes place in a dark room, commonly referred to as a dark room, because darkness promotes rapid colonization of the substrate (Guillaume et al., 2014). It is necessary to wait one to two additional weeks for the mycelium to be sufficiently mature and vigorous, for a good colonization during the next inoculations. It is best to store the spawn in the dark around 5-7°C (Ninkwango, 2016).

Results showed that mycelial growth was high in bottles containing sorghum, soybean and bean. Sorghum has a dominant resistance during the sterilization period, the seeds do not break, resulting in a lumbar growth of mycelium for some seeds, it can go up to 15 days. For maize, the low efficiency recorded is due to the rigidity of the cellulosic rind of the kernel envelope and not to the ability of the mycelium to penetrate and feed on the protein stock (Goose, 2003). The appearance of yellow, blue, green or gray mycelia in other parts of the surface seen is evidence of fungal contamination on the medium.

The mycelial culture is whitish and extends outside the tissue fragment. The appearance of blackish, yellowish, greenish layers of mycelium indicated the presence of fungal contamination. Creamy, shiny growth is often a sign of bacterial contamination (Aurelie, 2008; Wayne, 2001). Many fungi are parasites of many cultivated mushrooms, most attack the mycelium. A major fungal attack results in total crop loss (Tanaschuk, 2000).

Experiments must be conducted in a sterile environment. Operations during which sterilized containers are opened, must be conducted under aseptic conditions (Ninkwango, 2014). The ambient air contains an infinite number of particles and microorganisms that can easily infect the sterilized medium. It is not possible to completely sterilize an environment inwhich experiments are conducted, but the degree of contamination must be kept below a certain threshold. An average of 5 % contamination is reasonable for tropical countries, considering the infectious environment and the lack of material and financial resources (Proust, 2017). The preparation of culture media and experiments must be done with appropriate equipment, (test tubes and transparent glass jars), and then classified in incubation rooms. Several sources of contamination are reported namely: the manipulator, the substrate (more than 50% of contamination sources), the inoculation, living beings transporting microorganisms, air, respiration and perspiration of the manipulator and additives of the substrate (Ninkwango, 2016 : Hashim, 2012).

Tiecouraet al. (2016) conducted the cultivation of *Volvariella volvacea*without the use of fungus seeds. According to him, Volvariella is a fungus that grows naturally on decaying palm trunk which is a cellulose rich medium.

Delmas(1989) work on mushroom production recognized the dead palm as a natural producer of *Volvariella volvacea*mushroom.

Conclusion:-

The study carried out on *Volvariella volvacea*, consisted in producing the mycelium of this fungus on a PDA medium and then on cereal seeds (corn, sorghum) and legumes (bean, soybean). From tissue fragments taken from a young carpophore from decaying palm trees, the culture was done on the different substrates. The results obtained showed that the mycelium of *Volvariella volvacea*grows better on PDA medium, also on cereals and legumes. From this study, it was found that sorghum, soybean and bean are better substrates for the culture of *Volvariella volvacea*seed. They obtained the best propagation speeds and lengths: Sorghum (14.1 cm), soybean (13.4 cm) and bean (12.9 cm). *Volvariella volvacea*mycelium growth is not efficient on maize as it showed lowest velocities and lengths (6.3 cm). However, seed production of *Volvariella volvacea*is susceptible to contamination.

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