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Identification of Growing Media Favorable for the Growth of *Pythium aphanidermatum*, a Telluric Pathogen of Papaya (*Carica papaya* L.) in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pawpaw (*Carica papaya* L.) is an economically important plant in Côte d'Ivoire. However, its cultivation is subject to numerous biotic constraints, including root and crown rot, caused by several species of *Pythium*, *Phytophthora*, and *Rhizoctonia*. Of all these fungi, *Pythium aphanidermatum* is

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the most dreaded in Ivorian plantations, causing root, crown, trunk, and fruit rot. This causes enormous production losses, reducing farmers' incomes. The present study was initiated with a view to finding a suitable culture medium for the development of *P. aphanidermatum*, which is responsible for root and crown rot of papaya trees, through the study of its morphology and biology. To this end, the mycelial growth of *P. aphanidermatum* was evaluated on different culture media (PDA, V8, CMA and Papaya). The results showed that all these culture media were favorable for the mycelial growth of *P. aphanidermatum*. However, in terms of propagule production, PDA and V8 culture media were the most favorable. The CMA medium produced fewer propagules than the PDA and V8 media. Papaya culture medium inhibited oospore production. Sporocysts and chlamydospores were observed in all media. The culture medium did not influence the mycelial growth of *P. aphanidermatum* but rather oospore production.

Keywords: Pythium aphanidermatum; oospores; sporocysts; chlamydospores; culture media.

1. INTRODUCTION

Research into ways of combating plant pathologies has made enormous strides. These control methods include the characterization of the pathogen, which will enable effective means of control. Today, pathogen characterization is easier with molecular techniques [1,2]. As for control methods, the integration of biological methods (antagonistic organisms and plant extracts) is gaining ground, not only for its effectiveness against these pathogens but also for its ability to preserve the environment [3,4,5]. These methods have been made possible by synthetic media, which enable pathogens to be extracted from their natural environments and preserved in the laboratory [6]. Despite this, difficulties remain, as many fungi, although cultivated, do not produce spores or grow very slowly. Some microorganisms, such as fungi, require a specific medium or substance to grow or sporulate [7]. Without the right culture medium (substance), there is no growth. This often makes it difficult to characterize and implement appropriate control measures.

In Côte d'Ivoire, the problem of identifying and characterizing phytopathogenic fungi of cultivated plants is still in its infancy. Therefore, it is important to know the culture media favorable to the development and growth of pathogenic fungi. The aim of the present study was to identify a suitable culture medium for the development of *P. aphanidermatum*, which is responsible for root and crown rot in papaya, by studying its morphology and biology.

To this end, we evaluated the mycelial growth of *P. aphanidermatum* on four different culture media and compared the number and size of spores of this pathogen on the same culture media.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fungal material

The fungal material used was a strain of *Pythium aphanidermatum* kept in the mycotheque of the Plant Health Unit of the Plant Production Biology and Improvement Laboratory of NANGUI ABROGOUA University (Fig. 1). This strain was isolated from papaya plants from a plantation in Toumodi (Côte d'Ivoire) and showed typical rot symptoms.

Fig. 1. Top view of the macroscopic appearance of the 7-day-old *Pythium aphanidermatum* strain used

2.1.2 Culture material

The culture material used in this study consisted mainly of potato puree, D-glucose, agar, V8 vegetable puree, ripe papaya fruit, and corn powder. This material was purchased commercially from specialist laboratory equipment retailers.

2.2 Methods

2.2.1 Preparation of different culture media

2.2.1.1 Preparation of Potato Dextrose Agar (PDA) culture medium

Potato Dextrose Agar (PDA) was prepared by adding 20 g of potato puree, 20 g of D-glucose, and 20-g Agar-Agar. The entire mixture was made up to 1 L with distilled water. The resulting solution was autoclaved at 121°C for 30 min under a pressure of 1 bar and poured after cooling to 45°C into sterile 90-mm-diameter glass Petri dishes in a laminar flow hood in the presence of a flame [8].

2.2.1.2 Preparation of V8 culture medium

To prepare the V8 culture medium, 250 ml of V8 commercial vegetable juice was added to 250 ml of sterile distilled water. The mixture was thoroughly mixed and filtered. Next, 5 g of agar was added, and the mixture was autoclaved at 121°C for 30 min at a pressure of 1 bar [9]. After cooling to 45°C, the V8 culture medium was poured into sterile 90-mm-diameter glass Petri dishes in a laminar flow hood in the presence of a flame.

2.2.1.3 Preparation of Corn Meal Agar (CMA) culture medium

Corn Meal Agar (CMA) was prepared by weighing 29.5 g of corn powder, which was poured into an Erlenmeyer flask containing 250 ml of distilled water. 5 g of agar and 5 g of glucose were added to this mixture. The Erlenmeyer flask containing the preparation was closed and autoclaved at 121°C for 30 min at a pressure of 1 bar [10]. After cooling to 45°C, the medium was poured into sterile 90-mm-diameter glass Petri dishes under a laminar flow hood in the presence of a flame.

2.2.1.4 Preparation of Papaya culture medium

One ripe papaya was washed, peeled, and cut. Then, 50 g of papaya was ground in 250 ml of distilled water, and 5 g of D-glucose and 5 g of Agar-Agar were added. The mixture was then autoclaved at 121°C for 30 min under a pressure of 1 bar and poured into sterile 90-mm-diameter glass Petri dishes in a laminar flow hood in the presence of a flame.

2.2.2 Sowing and measurement of mycelial radial growth of the *Pythium aphanidermatum* strain in different culture media

The daily radial growth of the thallus of the *P. aphanidermatum* strain on each culture medium was measured every 24 h after seeding. Two perpendicular axes were drawn on the reverse side of the Petri dishes. Fungal inoculum (0.4 cm in diameter) was deposited at the intersection of the two perpendicular axes using a sterile punch. Measurements on each type of culture medium were performed and stopped as soon as the mycelium had colonized the entire surface of the available medium.

2.2.3 Macroscopic and microscopic observations of the *Pythium aphanidermatum* strain in different culture media

2.2.3.1 Macroscopic observations of the Pythium aphanidermatum strain in different culture media

Macroscopic observations of the fungal strain in fully colonized Petri dishes of the thallus enabled the description of the color and appearance of the mycelium.

2.2.3.2 Microscopic observations of the Pythium aphanidermatum strain in relation to culture media

To observe the microscopic characteristics of the *P. aphanidermatum* strain, a slide and coverslip were mounted. A small portion of the mycelium was removed and placed in a drop of distilled water on the slide. The preparation was then closed with a slide and observed under a light microscope. These observations provide information on the vegetative and reproductive structures of the *P. aphanidermatum* strain.

2.2.3.3 Counting of Pythium aphanidermatum strain propagules according to culture media

Thirty observations were made per type of culture medium. For each culture medium, the propagules observed were counted using an optical field. The averages of these observations were compared.

2.2.3.4 Measurement of Pythium aphanidermatum oospore size in different culture media

The diameter of the oospores produced on each culture medium was measured using a micrometer. Thirty observations per type of culture medium were made. The experiment was repeated three times.

2.2.4 Statistical analysis

STATISTICA software version 7.1 was used to analyze the quantitative values recorded in the various experiments. To compare the averages of the mycelium and oospore growth measurements obtained, the transformed growth rates were used. ANOVA 1 analysis of variance was performed to compare means using the Newman-Keuls test with a threshold of 5%.

3. RESULTS

3.1 Macroscopic and Microscopic Characteristics of the *Pythium aphanidermatum* Strain in Different Culture Media

3.1.1 Macroscopic characteristics of the *Pythium aphanidermatum* strain in different culture media

The mycelium of the *P. aphanidermatum* strain was raised, cottony, and had a white coloration on all four culture media (Fig. 2). The mycelium was stiff to the touch and difficult to pick, and the underside was milky white. The growth fronts were fibrous and almost invisible. Mycelial growth occurred in three phases. In the first phase, mycelial growth was actively rapid, enabling it to occupy the entire surface of the available culture medium. Growth at this stage was rarely aerial. During the second phase, the mycelium grew upward, occupying the entire volume of the Petri dish. A third phase of growth was observed, consisting of the collapse of the aerial thallus, leaving a characteristic smooth patch in the medium. This phase occurs 1–2 weeks after aerial growth (Fig. 2).

3.1.2 Microscopic characteristics of the *Pythium aphanidermatum* strain in relation to culture media

Microscopic observations revealed that the *P. aphanidermatum* strain had a cylindrical mycelium with thin walls. The mycelium is branched and unpartitioned. It produces oospores, orgones, and sporocysts on PDA, V8, and Papaya culture media, and chlamydospores on CMA culture medium (Fig. 3).

3.2 Radial Mycelial Growth of *Pythium aphanidermatum* on Different Culture Media

The *P. aphanidermatum* strain grew well on the four culture media tested (PDA, V8, CMA and Papaya). However, differences were noted in the time taken to colonize all the available surface areas of the culture media on which it was seeded.

On the PDA culture medium, it grew rapidly. After 24 h of growth, the diameter occupied by the fungus was 5.10 cm. In contrast, on the other culture media, after 1 day (24 h) of incubation, mycelial radial growth was 3.70 cm on V8 culture medium, 2.70 cm on CMA culture medium, and 1.7 cm on Papaya culture medium. After 48 h of incubation, the *P. aphanidermatum* strain occupied the entire surface area of the PDA and V8 culture media. Mycelial radial growth diameters were 4.30 and 3.60 cm in CMA and Papaya culture media, respectively. However, it was only after 72 h (3 days) of culture on the CMA medium that the *P. aphanidermatum* strain occupied the entire surface of the culture medium. On the other hand, on Papaya culture medium, on which growth was very slow, it was after 96 h (4 days) that the fungus occupied the entire surface of the Petri dish. Fig. 4 shows the average mycelial growth diameter of *P. aphanidermatum* in the four culture media. Analysis of this figure shows two groups of mycelial development of the *P. aphanidermatum* strain on the four (4) culture media. The first group consists of culture media on which mycelial growth is rapidly initiated; these are PDA and V8 culture media. On these two media, within 48 h, the mycelium reached the periphery of 90-mm-diameter Petri dishes. However, among these culture media, a small difference was noted. The rate of mycelial growth on day 1 was higher in the PDA culture medium. On this medium, the growth rate was estimated to be 5 cm/d, whereas it was 3.50 cm/d on the V8 culture medium. On the second day, the growth rate on the V8 medium reached more than 5 cm/d. On these two culture media, the average speed was approximately 0.17 cm/h. The second group of culture media was CMA and Papaya, on which the *P. aphanidermatum* strain grew slowly in the first 48 h, estimated at 1.70 and 3.70 cm/d, respectively. From day 2 to day 3, mycelial growth was higher. It ranged from 3.50 to 4.00 cm/d. The *P. aphanidermatum* thallus covered the entire surface of these culture media on the fourth day of incubation.

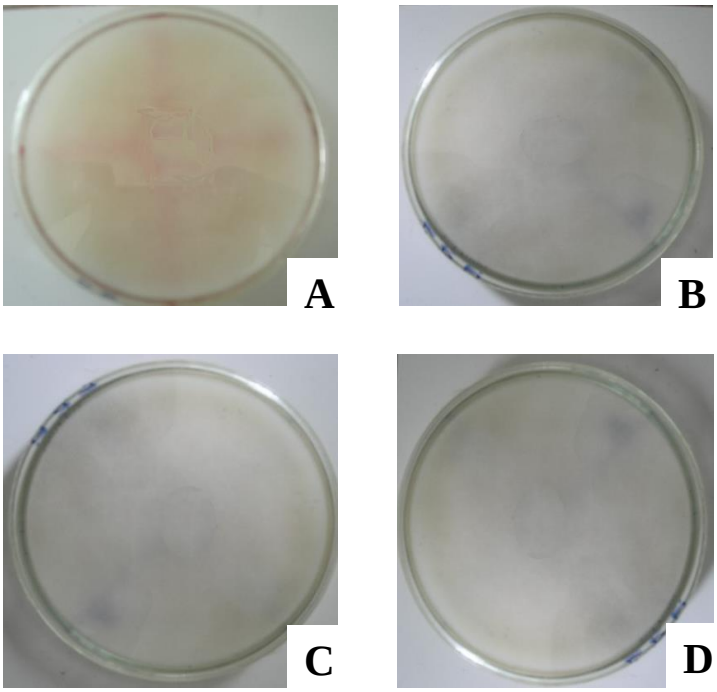

Fig. 2. Macroscopic aspects of the *Pythium aphanidermatum* strain on different culture media A: Mycelial appearance on PDA culture medium, B: Mycelial appearance on V8 culture medium, C: Mycelial appearance on CMA culture medium and D: Mycelial appearance on Papaya culture medium

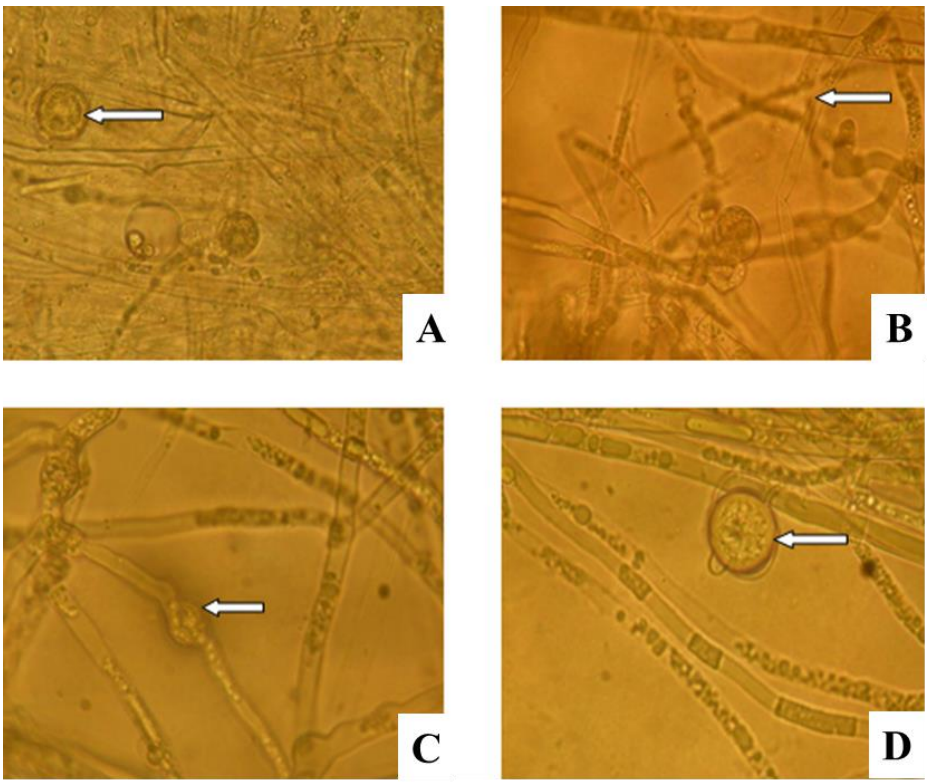

Fig. 3. Microscopic aspects of the *Pythium aphanidermatum* strain on different culture media A: sporocyst; B: mycelium; C: chlamydopore; D: oospore

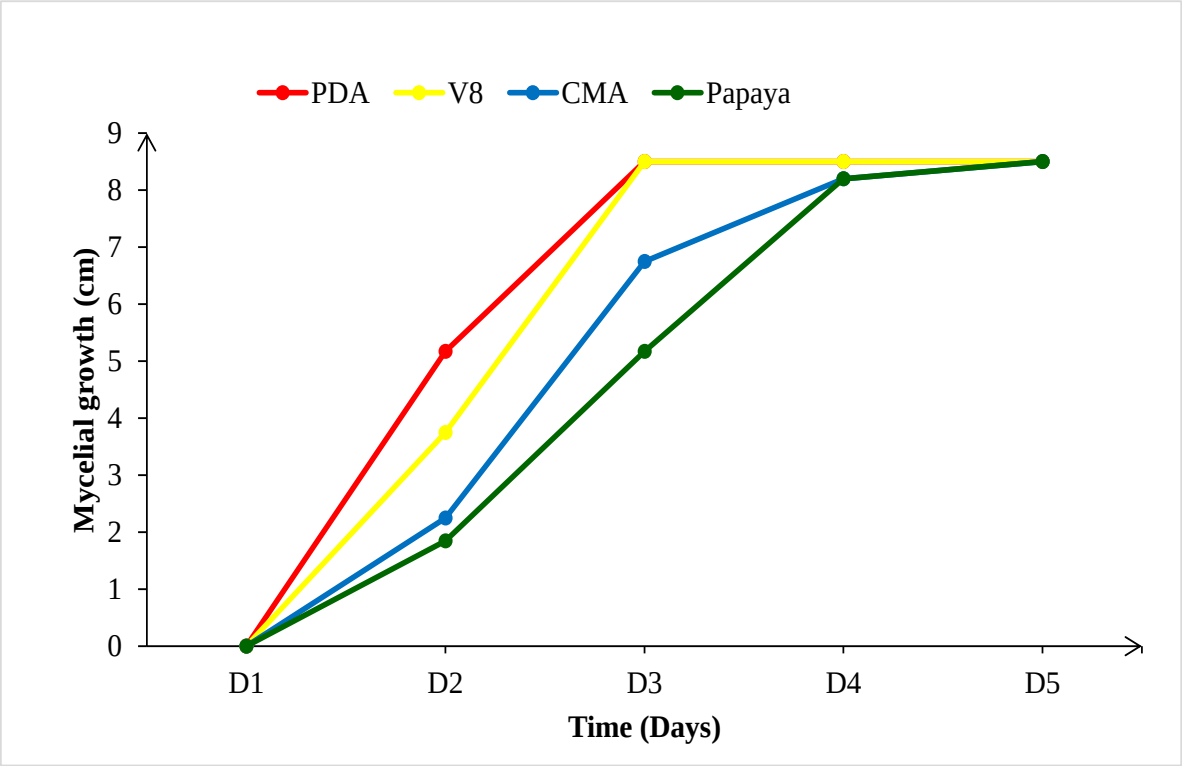

Fig. 4. Radial mycelial growth of *Pythium aphanidermatum* in the four culture media as a function of time

3.3 Effects of Different Culture Media on Oospore Number and Size

3.3.1 Effects of different culture media on oospore numbers

The number of propagules produced on different culture media is shown in Table 1. On average, 30 oospores were counted per optical field on the V8 medium, compared with 33 on the PDA medium. These values are not statistically different. On the CMA culture medium, oospore production was not as high as that on the V8 and PDA culture media. On average, 13 oospores were counted per optical field in the CMA medium. On Papaya culture medium, microscopic observations showed that oospore production averaged 2 oospores per optical field (Fig. 5).

3.3.2 Effects of different culture media on oospore diameter

Measurement of *P. aphanidermatum* oospore size on each culture medium is shown in Table 1. On PDA, CMA, and Papaya culture media, the oospore size ranged from 28.80 to 29.86 µm. On the V8 culture medium, oospores developed very rapidly. The average oospore diameter on the V8 culture medium was around 35.3 µm. There was no statistical difference between the oospore diameters in the PDA, CMA, and Papaya culture media. However, the mean oospore diameters of these media were statistically different from those of the V8 culture medium (Fig. 5).

Table 1. Number and diameter of *Pythium aphanidermatum* oospores in different culture media

Culture media	Number of oospores	Oospore diameter
PDA	33.00 a	29.73 a
V8	30.00 a	35.33 b
CMA	13.00 b	29.86 a
PAPAYA	2.00 b	28.80 a
Moyenne	19.50	30.93

Means affected by different letters are significantly different according to the Newman-Keuls test at the 5% threshold.

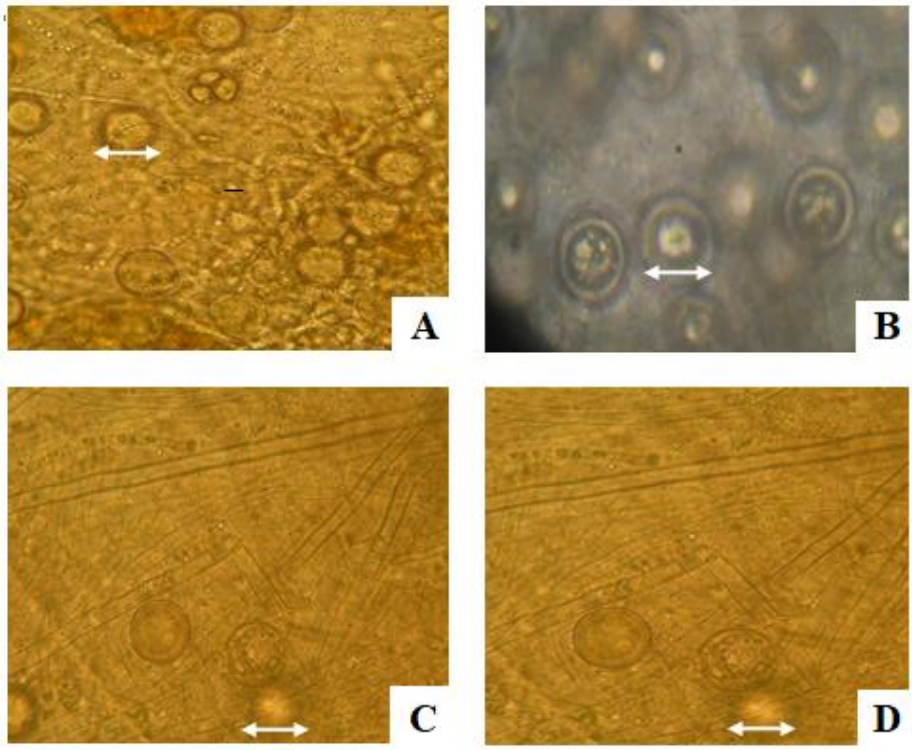

Fig. 5. Microscopic aspects of *Pythium aphanidermatum* oospores on different culture media (Gx400)

A: *Microscopic appearance of oospores on PDA culture medium,*
B: *Microscopic appearance of oospores on V8 culture medium,*
C: *Microscopic appearance of oospores on CMA culture medium,*
D: *Microscopic appearance of oospores on Papaya culture medium.*

4. DISCUSSION

The cultural characteristics of the *P. aphanidermatum* strain on the four different culture media (PDA, CMA, V8 and Papaya) were identical: aerial, silky-white thallus. The underside is whitish. The fungus developed the same macroscopic characteristics on all four culture media because of their high sugar content. This result is similar to that of Fortin [11]. According to this author, in pure culture, *P. aphanidermatum* forms a dense thallus with abundant, white, silky aerial mycelium on sugar-containing media. The *P. aphanidermatum* strain produced vegetative and reproductive structures on various culture media. These included abundant, branched mycelium, oospores, sporocysts, and chlamydospores. This was due to the high glucose content of the culture media. This result is in agreement with that of Fortin [11], who showed that *P. aphanidermatum* on synthetic medium produced orgones, sporocysts, chlamydospores, and oospores. However, growth rates were not identical in all media. These differences in fungal growth rates could be explained by differences in the nutrients contained in each culture medium [12,13]. The Potato Dextrose Agar (PDA) culture medium is based on potato puree, which is rich in starch (and therefore carbohydrates), whereas the V8 culture medium is composed of various vegetables such as cabbage and tomato, which contain glucose, potassium, and iron [14]. These elements are highly valued by *P. aphanidermatum*. These elements are highly valued by *P. aphanidermatum*. Papaya culture medium contains papain, iron, ascorbic acid, carbohydrates, and phosphorus, which inhibit the growth of the fungus. This inhibition could be explained by the presence of papain in the medium. According to Koul et al. [15], papaya fruits are not attacked by *Phytophtora* and *Pythium* fungi because of a particular substance.

Evaluation of in vitro mycelial radial growth of the *P. aphanidermatum* strain on different culture media (PDA, V8, CMA, and Papaya) showed that the fungus grows faster on PDA and V8 culture media. On the Papaya culture medium, the mycelium grows very slowly. However, analysis

of variance for the mycelial growth character of the fungus on the other three culture media showed that there was no significant difference in the mycelial growth variable between these culture media. The absence of interaction between these culture media indicates that the culture medium had no influence on the mycelial growth of the fungus. This lack of interaction occurs because these three culture media contain the nutrients required by the fungi for their growth. These include proteins, carbohydrates, manganese, magnesium, phosphorus, copper, lipids, and B vitamins. Therefore, these are the standard media on which most phytopathogenic fungi grow. According to Botton et al. [16], these culture media allow the proliferation and isolation of many fungi. The quantity of sugar, carbohydrate, water, protide, and especially vitamin B in PDA and V8 culture media, and the acidity of these media, could explain the rapidity of mycelial growth on these media. This result is close to that of Iacob et al. [17]. According to these authors, the growth of certain fungi such as *Phytophthora* spp. on culture medium is rapid when a source of cellulose is added to the medium, which, as it degrades, releases additional glucose that is easily assimilated by the fungus. Unlike the V8 and PDA culture media, the Papaya culture medium did not promote rapid mycelial growth of the *P. aphanidermatum* strain. The results of macroscopic observations of mycelial growth on different culture media are linked to those of the oospores (and oogones) observed on these different culture media during microscopic observations. Indeed, on culture media on which the fungus grows rapidly (V8 and PDA), abundant development of oospores and orgones was observed. On the Papaya culture medium, however, oospores were rare. This could be due to the chemical constituents of papaya, as papaya fruit contains papain, ascorbic acid, carbohydrates, fiber, calcium, phosphorus, iron, and especially thiamin [18]. The increase in oospore size observed on the V8 culture medium could be explained by the fact that this is the ideal culture medium for oomycetes [16]. The scarcity of propagules on the Papaya culture medium could be explained by the presence of an inhibitor of oospore growth and development in papaya; this inhibitor could be papain. According to Koul et al. [15], papaya fruits are not attacked by *Phytophtora* and *Pythium* fungi because of a substance specific to papaya. The papaya used as a growing medium could therefore be treated with phytosanitary products during production, transport, and marketing to combat pathogens, including fungi. According to Azouaoui-Ait et al. [19], glyphosate from treated fruit inhibits the growth of *Pythium* and *Fusarium* fungi. The absence of biotin in papaya fruit could also explain the scarcity of propagules on this medium, since according to Wu et al. [20], biotin promotes fungal sporulation.

5. CONCLUSION

The study of the *in vitro* development of the *P. aphanidermatum* strain on different culture media (PDA, V8, CMA and Papaya) showed that this phytopathogenic fungus develops better on the PDA and V8 culture media than on the other two culture media. On PDA and V8 culture media, mycelium develops rapidly and abundantly. Examination of the fungus on these four culture media also revealed the production of propagules. A delay in fungal development was observed in Papaya culture medium. Inhibition of oospore formation was also noted. Therefore, the V8 and PDA culture media are ideal for the growth of *P. aphanidermatum*. These two culture media can be recommended in programs to identify and characterize pathogens of the *Pythium* genus. This study should be continued to determine the precise papaya constituent that inhibits propagule formation. In addition, the determination of inhibition threshold doses should be explored with a view to integrate them into control and prevention programs.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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