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Full Length Research Paper

Investigation of sporulation and cost benefit of raw materials for the production of Curvularia pallescens

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An investigation was conducted on the pH, nutritional and cost benefit of the use of different substrates for the production of *Curvularia pallescens* Boedijn. The growth of the organism was examined at pH range between 5.5 and 8.6 and on different growth media, potato carrot agar (PCA), potato dextrose agar (PDA), sabouraud agar (SA), tap water agar (TWA), water hyacinth agar (WHA) and Czapek-Dox agar (ZA). Also, the growth of the organism was examined on different enrichment media "Czapek-Dox" with its sodium nitrate substituted with equal amount of different nitrogen sources (ammonium chloride, ammonium nitrate, glutamine and sodium glutamate). WHA produced significantly (P < 0.001) highest amount of mycelial growth (84.95 ± 0.05 mm) on day 6 than other media types. The spore concentration was highest, 6.6 E^{06} ± 3.33 E^{05} spores/ml with TWA. The colony size was significantly (P < 0.001) highest, 84.9 ± 0.1 mm on day 10 with sodium glutamate compared with other nitrogen sources. Spore count was only observed on ammonium chloride (1.67E^{06} ± 3.33E^{05} spores/ml), the optimal growth pH was 5.5. The cost of production of the organism per culture plate on WHA was 61.3 and 82% lesser than on PDA and SA respectively. The formulated water hyacinth agar medium appeared most economically feasible for the mycelial production of *C. pallescens* Boedijn.

Key words: *Curvularia pallescence* Boedjin, growth measurement, pH measurement.

INTRODUCTION

There are more than 30 species of *Curvularia* many are anamorphic states of the Loculoascomycete genera *Cochliobolus* and *Pseudocochliobolus* (Rossman et al., 19 87). Species of *Curvularia* Boedijn are saprophytes or phytopathogenic they are incident in tropical and subtropical areas, and are isolated from soil, air, organic matter, plants and animals, including humans (Farr et al., 1989; Travis et al., 1991).

A number of *Curvularia* species have been reported as causal agents of leaf spots, leaf blights, kernel rot, root rot, seedling blights, grain discoloration, grain lesions and rice grain deformation (Rashid, 2001; Parimelazhagan and Francis, 1999; Kim- Jisoo and Lee-DuHyung, 1998; Sisterna and Bello, 1998).

Some *Curvularia* species have been more extensively studied since they are known as cellulase producers (Banerjee, 1990; Banerjee, 1992; Freire, 1995; Jyoti and Sing, 1985; Nitharwal et al., 1991). *C. verruculosa* has a haloperoxidase enzyme system, which has been found to cause reductions in counts of some bacteria, yeasts and filamentous fungi. Hence, it has been shown to have an effective disinfectant property on contact lenses (Eva et al., 2003).

3 different species of *Curvularia* [(*C. affinis* Boedijn, *C. clavata* B.L. Jain and *C. penniseti* (*M. Mitra*) Boedijn] have been reported on *Eichhornia crassipes* in the United State of America and India (Evans and Reeder, 2001). Recently, we have isolated *C. pallescens* Boedijn from *Eichhornia crassipes* in Nigeria Okunowo et al., 2008. The phytopathogenicity screening of the organism as a poten-tial mycoherbicides or biocontrol agent for water hyacinth showed a negative result as it did not satisfy Koch's postulate (unpublished data). However, a previous study conducted on the organism showed that it is a good cellulase producer (unpublished data). Owing to this biotechnological importance, it was considered appropriate to investigate sporulation and cost benefit of raw materials

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Table 1. Preparation of buffer solution for microbial growth.

Vol. of Na_2HPO_4 stock(11.88g/litre)	Vol. of KH2PO4stocks(9.08g/litre)	*Approximate pH of buffer
0 ml	20 ml	4.6
2 ml	18 ml	5.8
12 ml	8 ml	6.7
19 ml	1 ml	7.8
20 ml	0 m	9.0

raw materials for the production of *C. pallescens*.

MATERIALS AND METHODS

Fungal isolate

A lyophilized strain of *C. pallescens* Boedijn isolated from water hyacinth in our previous study (unpublished data) was reconstituted in petri dishes. This was done by adding 20 ml sterile distilled water into a test tube containing a sample of the lyophilized organism. The test tube was agitated and an aliquot of the cell suspension was obtained with the aid of sterile syringe. A 2 ml suspension was added to a petri dish containing potato dextrose agar. And this was incubated for 7 days at 25°C.

Preparation of culture media

6 culture media in g/L of distilled water includes potato carrot agar (PCA) medium (potato 20 g, carrot 20 g and agar 20 g), potato dextrose agar (PDA) medium (Irish potato infusion 200 g, dextrose 15 g and agar 20 g), sabouraud agar (SA) medium (glucose 40 g, peptone 10 g and agar 15 g), tap water agar (TWA) medium (agar 18 g), water hyacinth agar (WHA) medium (dried powdered water hyacinth leaf 80 g, agar 18 g and fresh hyacinth leaf extract 100 g/l of distilled water) and Czapek-Dox agar medium (sodium nitrate 2 g, potassium nitrate 1 g, potassium chloride 0.5 g, magnesium sulphate 0.5 g, ferrous sulphate 0.01 g, sucrose 30 g and agar 20 g) were used to find out the most suitable media for mycelial growth of the fungus. Each culture medium was prepared in 1 l of distilled water and autoclaved at 120°C at 15 psi for 20 min. The different media for determining the effect of different sources of nitrogen on microbial growth were prepared from Czapek-Dox agar medium such that it contains one of the following nitrogen sources (2 g/l) ammonium chloride, ammonium nitrate, glutamine, sodium glutamate and sodium nitrate. These were also autoclaved as above.

More so, the medium for the determination of the pH effect on the microbial growth was prepared as follows using buffer solutions prepared by mixing different volumes of sodium dihydrogen phosphate solution and potassium dihydrogen phosphate solution as in Table 1. A 400 ml potato dextrose broth medium (PDB) was also prepared. The broth was divided into 5 equal portions of 80 ml and labeled A to E. The pH of A was adjusted to 4.6 with a pH meter (Cole- Parmer 60648, Chicago, USA) and 20 ml of the buffer of the same pH (Table 1) was added. The mixture was distributed (25 ml) into three 100 ml flasks and they were labeled A. Similarly, the pH of the medium lot B was adjusted to 5.8, C to 6.7, D to 7.8 and E to 9.0. The same operation was carried out, as in A. All the 15 flasks were autoclaved at temperature of 120°C at 15 psi for 20 min.

Growth measurement

The growth measurement was carried out by a slight modification of the method of Fawole and Oso (1988). The colony size method was employed for the mycelia growth of *C. pallescens* on media types and nitrogen sources. This was done as follows using a glass pencil, 2 diameters were drawn on the bottom part of each sterile petri dish (diameter = 85 mm) such that the point of interception is the center of the plate. The plates were poured and allowed to set. The plates were inoculated at the center with 8 mm disc of the resuscitated *C. pallescens* punched with a flame sterilized cork borer. These were placed in an incubator (Gallenkamp SG91/08 /717, Loughborough, UK) at 25°C. The colony size was estimated daily in mm by measuring along each of the 2 diameters. A replicate of 5 plates were made for each growth and nitrogen source medium. At the end (6^{th} day) of the growth study, the spore concentration was estimated for each medium using a Neubaurer haemocytometer slide.

pH measurement

2 agar plates of the reconstituted *C. pallescens* each received 10 ml sterile distilled water (SDW) containing 0.1% (v/v) Tween 80. The spore suspensions were aseptically pulled together in a sterile 100 ml flask. With the aid of a sterile pipette, 1 ml of this spore suspension was made each into the 15 flasks A to E (previously prepared). These were allowed to stand on a shaker at laboratory condition for 24 h. The content of each flask was harvested in a centrifuge (Surgifriend Medicals SM902B, England) at 3,500 g for 10 min. The pH of the supernatant was measured. The pellets were resuspended in the same volume of water (25 ml) and the optical density was measured at 530 nm in a spectrophotometer (Thermo Spectronic 4001/4, USA).

Cost of production of media types

The different media used in this study were compounded according to the established methods shown above except water hyacinth medium, which we have formulated. Analytical grade salts were used as supplied by Sigma- Aldrich Chemie, Germany. Hence, direct costing was made per gramme of salt used. The water hyacinth leaf used in media formulation was obtained at no cost at the Lagos Lagoon. Sterile reusable culture plates were used in this study to minimize cost, such that total cost excludes the cost of disposable plates.

Data analysis

Data were given as mean ± SEM of measurements from 5 replicates of plates containing the same strain of organism. Significant difference between time points (t = day 0 and t = day 1 to 10) were determined by Fisher's protected least significant different t-test with 2 tail probabilities of less than 0.05 considered significant. Statistical significant difference in the rate of microbial growth and

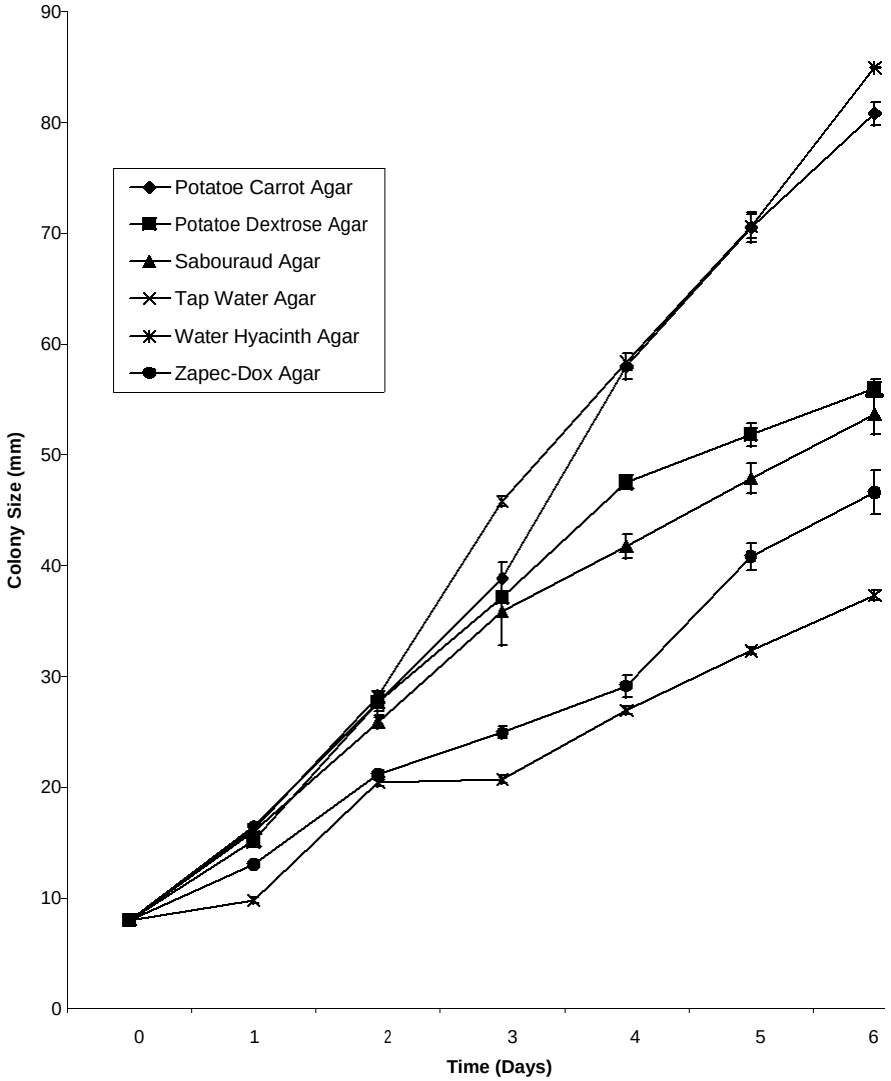

Figure 1. Growth profile of curvularia pallescens boedijn on different types of media. Values are in mean ± SEM for five replicate result.

final colony size between media types was assessed by a one-way analysis of variance. Statistical analysis was done on computer software programme (Microsoft Excel, 2000).

RESULTS AND DISCUSSION

Effect of media on the growth of *C. pallescens*

A graph of mean colony size of *C. pallescens* versus time was generated (Figure 1) . The microbial growth increase-ed significantly from day zero over the growth period in the media types ($P < 0.001$). The maximum growth was observed in water hyacinth agar medium (WHA) on day 6. Hence, the growth rate and final colony size on WHA were compared with the other media using a one-way analysis of variance. A statistically significant difference in the growth rate only exists with tap water agar medium (TWA) ($P < 0.05$). Also a significant difference ($P < 0.001$) in the final colony size was observed with other media. This is an indication that different media affects the mycelial growth of *C. pallscens*. A sharp increase in the growth was observed between day 2 and 3 in WHA medium. This suggests that day 3 signals the beginning of the exponential growth phase of *C. pallescens* and maximum utilization of the substrate. Similar drastic in-crease in the mycelial growth was observed with other media on the 3rd and 4th day except Czapek-Dox agar medium, which showed increase on the 4th and 5th day. The growth (mm) was least, 37.30 ± 0.42 with TWA and highest, 84.95 ± 0.05 with WHA on day 6. The growth of *C. pellescens* Boedijn on WHA was considerably higher than that of the other media types in this study, particularly

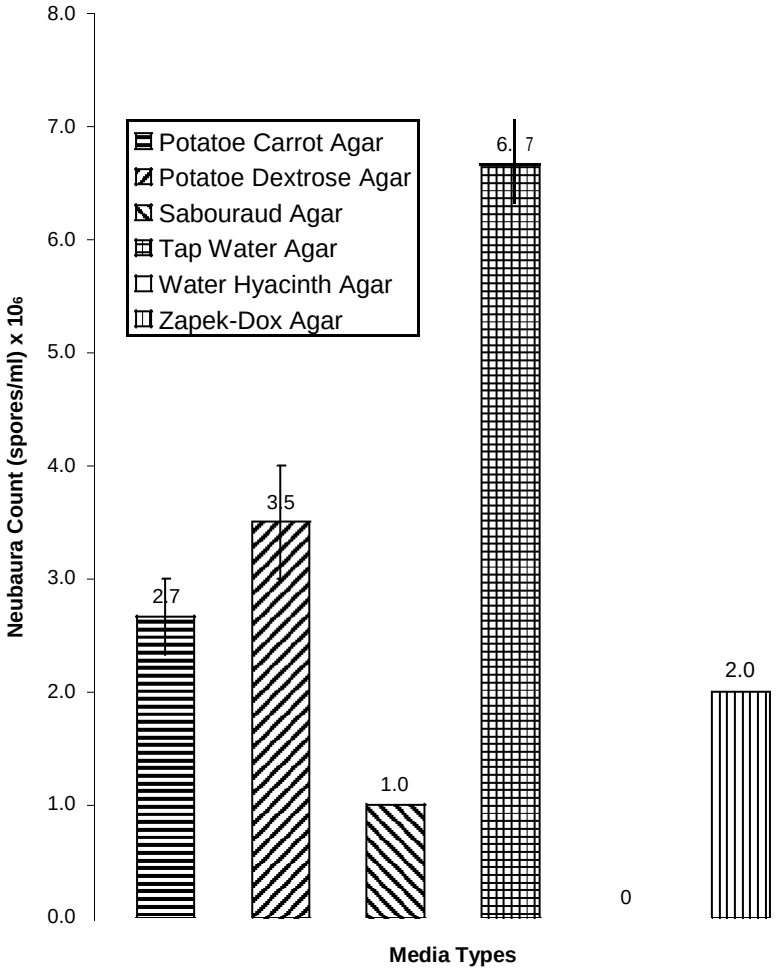

Figure 2. Neubaura count *c. pallescens* boedijn on different types of media on days 6. Values are in mean ± SEM for five replicate result.

on potato dextrose agar medium reported by Sonia et al. (1998) and Tabassam et al. (2003).

This considerable increase in the growth rate of the organism on WHA may be due to the right combination of nutritional requirement present in water hyacinth leaf, which may have made the plant a natural host for the organism. Proximate analyses of the water hyacinth leaves by Igbinosun et al. (1988) showed that it contains 14.7% dry matter, 12.4% ash, 22.75% crude protein, 15.0% crude fibre and 4.82% lipid.

Igbinosun et al. (1988) also reported the mineral composition of the leaf as 0.44% phosphorus, 4.28% potassium, 0.02% sodium, 2.63% calcium, 190.5 ppm magnesium, 77.3 ppm manganese, 77.3 ppm zinc and 1.62 ppm. The spore concentration of *C. pallescens* in WHA plate was not appreciable (Figure 2). The microbial spore concentration (spores/ml) was highest, 6.67 E^{+06} ± 3.33 E^{+05} with tap water agar medium (TWA), higher, 3.5 E^{+06} ± 5.0 E^{+05} with PDA, high, 2.00 E^{+06} ± 0.00 with Czapek-Dox, and low, 1.00 E^{+06} ± 0.00 with SA. This result infers that the sporulation of *C. pallescens* on WHA and TWA is inversely proportional to their mycelia growth. This also indicates that a less nutritive medium is needed for the sporulation of *C. pallescens*.

Effect of nitrogen sources on the growth of *Curvularia pallescens*

The *C. pallescens* growth in the nitrogen sources increased significantly as compared to day zero ($P < 0.01$). The maximum mycelial growth of *C. pallescens* was observed on the 10^{th} day in sodium glutamate medium (Figure 3). Its growth rate on sodium glutamate medium was compared to the other media using a one-way analysis of variance. The growth rate was significantly different ($P < 0.001$) compared to ammonium chloride and ammonium nitrate. A significant difference ($P < 0.01$) was also observed when compared to growth rate in glutamine medium.

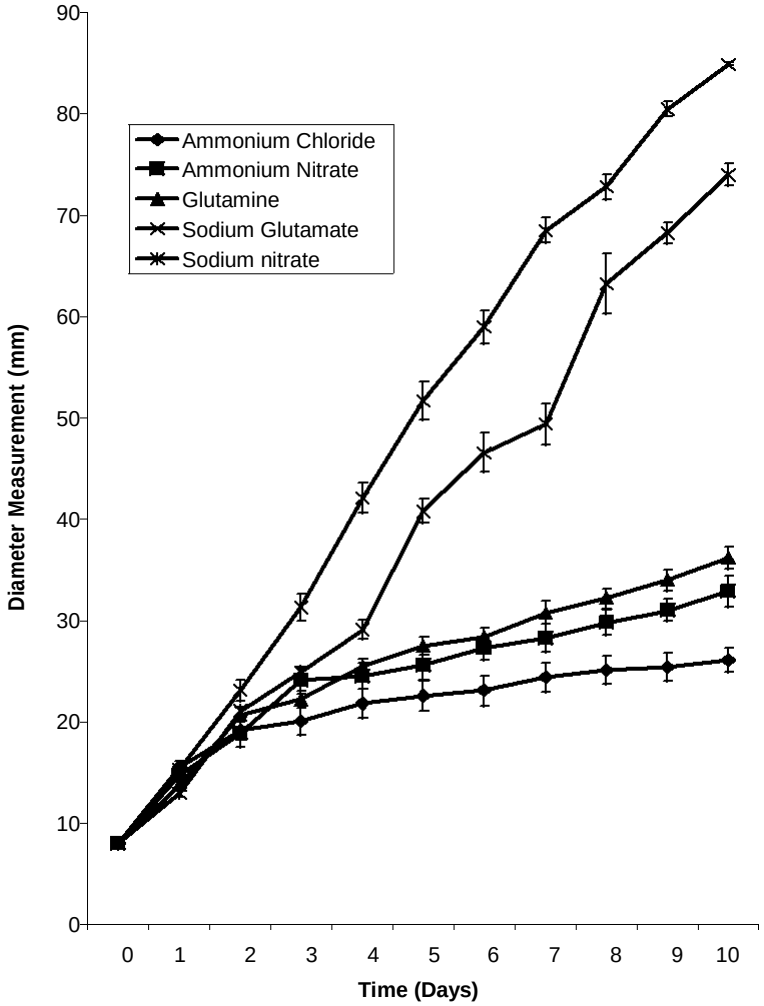

Figure 3. Growth profile of *C. pallescens Boedijn* on different types of nitrogen sources. Values are mean ± SEM for 5 replicate results.

There was no significant difference ($P > 0.05$) in the growth rate when compared to the notably used enrichment Czapek Dox medium. However, the growth rate on Czapek- Dox significantly differs from other nitrogen sources ($P < 0.01$). More importantly, the final mycelial growth (84.9 ±0.1 mm on day 10) on sodium glutamate significantly differs ($P < 0.001$) from other nitrogen sources. It is obvious in this study that the growth rate of *C. pallescens* on sodium glutamate and sodium nitrate significantly differs from other media. Both media contained sodium metal, suggesting that it is a very important element in the assimilation and growth of the fungus. It is also evident in this study that *C. pallescens* grew better in sodium glutamate medium as compared with sodium nitrate medium. This strongly points to the fact that an organic nitrogen source is needed for the assimilation and growth of *C. pallescens.*

On the other hand, there was no visible count in spores of *C. pallescens* on the different nitrogen sources except on ammonium chloride, which gave a concentration of $1.67E^{06} \pm 3.33E^{05}$ spores/ml.

Effect of pH on the growth of *Curvularia pallescens*

The turbidometry analysis was used to determine the pH effect on mycelial growth. Prior to the inoculation of the medium with the fungus, the initial pH of the autoclaved media (flasks A to E) changed from 4.6 to 5.5, 5.8 to 6.6, 6.7 to 7.4, 7.8 to 8.2 and 9.0 to 8.6. The optimal growth density of *C. pallescens* was highest at pH 5.5 and least at pH 8.6 (Figure 4). This is an indication that the hydro-gen ion concentration in the environment of a micro-organism has a significant effect on its growth. In this case, the pH range between 6.6 and 8.6 may be too high to activate the enzymatic machineries needed for nutrient breakdown and assimilation in *C. pallescens* . The optimal pH obtained in this study agrees with Sonia et al. (1998) who reported the growth of *C. pallescens* at pH 6.0.

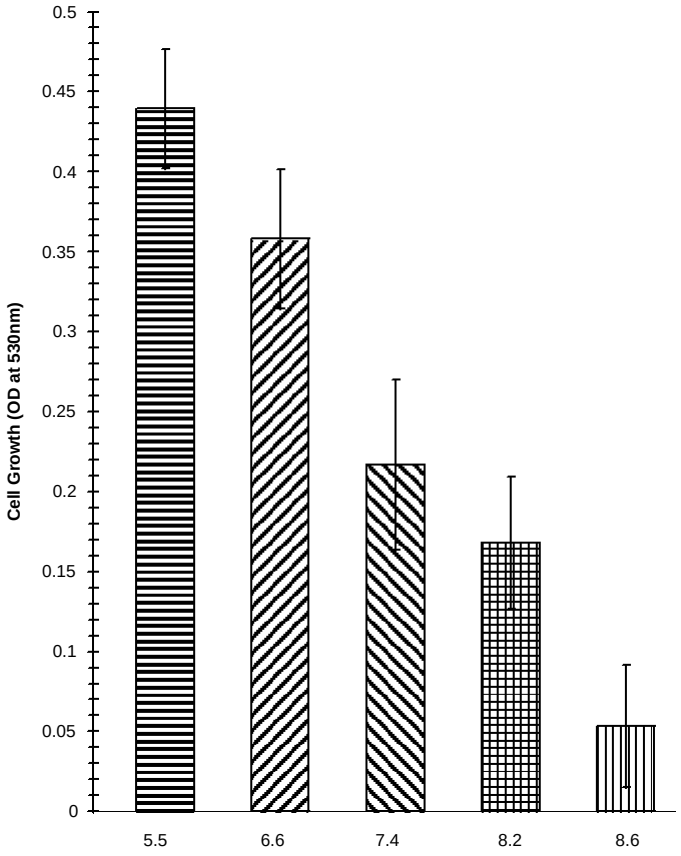

Figure 4. Optimal growth pH for *C. pallescens* Boedjin. Values are mean ± SEM for triplicate results.

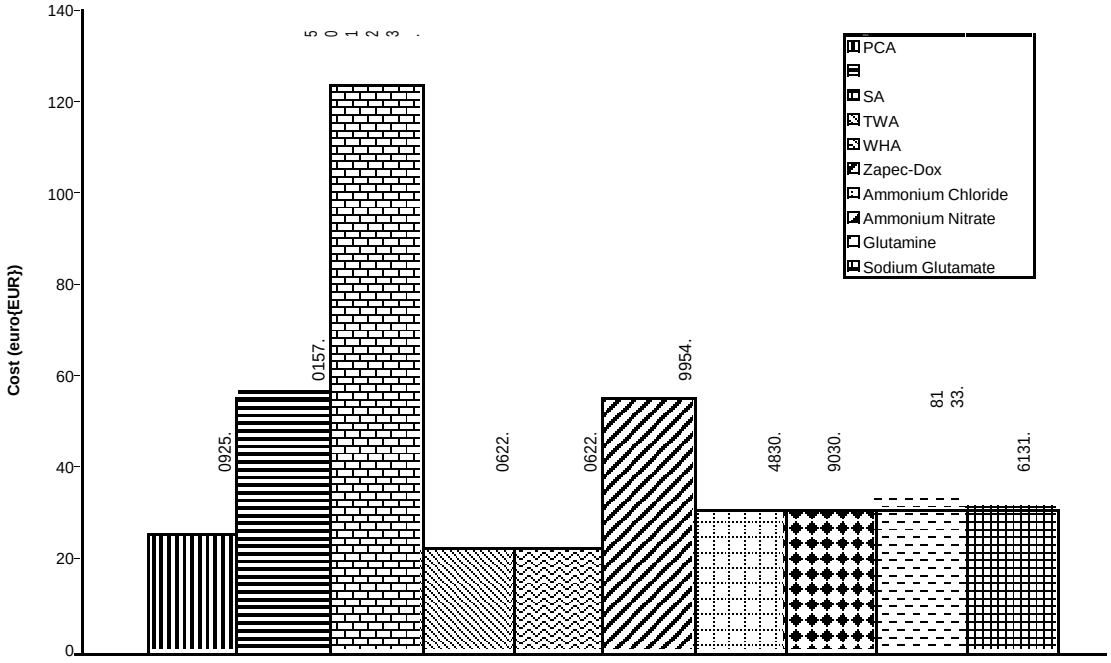

Figure 5. Cost analysis of different types of media per 1000 culture plates.

Cost benefit of different media and nitrogen sources

Since each petri dish contained 10 ml film of each media, the cost of production of the fungus was calculated as cost per litre divided by 100. The cost of the locally purchased raw materials such as the carrot and potato were converted to their euro (EUR) equivalent. Sabouraud agar appeared to be the most expensive medium followed by potato dextrose agar (PDA) and Czapek-Dox (Figure 5). The potato carrot agar (PCA), which was the second best medium for mycelial growth of *C. pallscence* appeared as second least expensive medium.

The least expensive media were water hyacinth agar and tap water agar. Although the sporulation of *C. pallescens* was poor on water hyacinth agar medium, however, it gave the highest mycelial growth. The reverse was the case with tap water agar medium. This suggests that either of these 2 media is very economical for use in the production of *C. pallescens* . This study, therefore recommend the use of water hyacinth agar for the propagation of *C. pallescens*.

In conclusion, this study has shown that water hyacinth leaf extract can be bio-utilized in the propagation of *C. pallescens* Boedijn. Thus, remains the most economical raw material for this purpose.

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