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Determination of the prevalence of fungi associated with amaranth cultivation in the locality of Kombé, Republic of Congo

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Abstract

Amaranth is a nutrient-rich leafy vegetable. Phytopathogenic fungi are causing major losses in amaranth crop production in the Kombé area. The detection of these phytopathogenic fungi can play an important role. The aim of this study is to assess the abundance of endophytic and saprophytic fungi associated with amaranth cultivation in Brazzaville, with a view to recommending biological control of fungal diseases. The prevalence of fungi associated with amaranth began with a survey, then continued in the laboratory. During the survey, 15 amaranth and soil samples were then collected separately in different stomacher bags, labelled and shipped to the laboratory where they were stored in a refrigerator at 4°C for four (4) weeks for analysis. In the laboratory, endophytic and saprophytic fungi were isolated using the fresh-state inoculation method for endophytic fungi and the suspension-dilution method for saprophytic fungi. The isolated strains were identified by determining their macroscopic and microscopic characteristics. The phytosanitary survey revealed that the most dominant symptom on amaranth plants in Kombé was wilting (42.86%). The results obtained from the distribution of endophyte isolates by colour indicate the presence of 5 isolates of unidentified endophytic fungi obtained from the leaf organ. Through isolation and microscopic analysis of soil saprophytes, six genera of fungi were identified: *Botrytis cineara, Rhizoporus sp, Penicillium sp, Mucor sp, Scedospirium sp and Curvularia sp*.

Keywords: Leafy vegetable; Endophytes; Saprophytes; Amaranth

1. Introduction

Market garden crops are leafy vegetables, fruits, roots and tubers used for food and industrial needs [1]. In Africa, vegetables are an important component of daily diets [2]. They are also the most important component of the human diet at all stages of life [3].

In Central Africa, amaranth is widely grown in market gardens [4]. It can produce up to 40 tons (t) of fresh matter/ha in 3-5 weeks [3]. Amaranth is a popular leafy vegetable with a high economic value. According to market studies, it appears to be one of the main African leafy vegetables, probably the leading one in terms of both volume and area [5].

In Congo, amaranth is a widely consumed leafy vegetable with a high nutritional value [6,7]. However, amaranth production is not spared from pests attacks, which are limiting factors in production [2]. Biological pests can cause damage to parts of the plant or to the whole plant. Fungi are one of the main causes of disease in amaranth. They are responsible for around 70% of crop diseases [8]. Amaranth mould and damping-off are the main fungal diseases of Amaranthus. Amaranth mould is caused by the fungus *Choanephora cucurbitarum*, it has been causing necrosis on

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leaves, young stems and petioles of Amaranthus in Congo since 1990 [4]. The causal agents of damping-off in amaranth are cryptogams fungis *Pythium aphanidermatum* and *Rhizoctonia solani Kühn*. These two fungi attack many species of vegetable plants and are responsible for wilting the plant [6]. These fungi are a limiting factor in amaranth cultivation. These cryptogam fungi are a limiting factor in amaranth cultivation. Although attacks due to fungal diseases have been widely reported in market gardening sites in the Republic of Congo in general and in the city of Brazzaville in particular, very little work have been done on characterising the fungi responsible for these diseases, particularly in amaranth.

The aim of this study is to assess the abundance of endophytic and saprophytic fungi associated with amaranth cultivation in Brazzaville in order to recommend biological control of fungal diseases.

2. Material and methods

2.1. Plant material

The plant material consisted of local varieties of *Amaranthus hybridus*. These varieties are popular with the local population and are grown by market gardeners at the Agricongo centre in Kombé.

2.2. Laboratory equipment

The laboratory equipment consisted of Bacto* MALT EXTRACT and Agar agar for the preparation of the Malt Agar culture medium.

2.3. Determination of the prevalence of fungi associated with amaranth

A phytosanitary survey was carried out over three (3) days from August 26-28, 2021 among 25 market gardeners at the Agricongo centre in Kombé. The survey was carried out among 25 randomly selected growers on the site and who primarily grow amaranth. They were interviewed using a semi-structured questionnaire. The questionnaire focused on the following parameters: farm characterisation and health assessment at the survey site.

Each plot was carefully examined to assess the number of plants attacked by the diseases and the extent of the damage. Some plants showing symptoms of attack were taken for careful laboratory examination. A total of 15 samples of amaranth plants were collected from the site and stored in a refrigerator at 4°C for four (4) weeks for analysis.

For the amaranth plants and the soil, samples were taken from the available growing beds belonging to the growers selected during the survey. Each amaranth plant sample was followed by soil sampling using a spade in the horizon of the beds from which the amaranth plants were collected. Samples were collected from all the beds showing symptoms on the pigweed plants. After each amaranth sampling, the soil surface was cleared of debris and then sampled to a depth of 15 cm. Each plant and its soil were placed separately in different stomacher bags, labelled and shipped to the laboratory, where they were stored in a refrigerator at 4°C for four (4) weeks for analysis.

2.4. Isolation of endophytic and saprophytic fungi associated with amaranth cultivation

The associated fungi were isolated on Malt agar medium. For its preparation, 10 g of agar agar and 20 g of malt (Bacto* malt extract) were mixed in a 1000 mL Erlenmeyer flask. This mixture was then suspended in 500 mL of distilled water and autoclaved at 121°C for 15 minutes.

2.4.1. Isolation of endophytic fungi

A plant fragment of a plant taken was washed twice with sterile distilled water. It was then disinfected with 90°C alcohol. One minute later, the organ was rinsed again with sterile distilled water to remove any trace of alcohol and dried with sterile blotting paper. The leaves, roots and crown were then cut separately cut into small fragments with a sterile blade and placed in petri dishes containing Malt agar medium. The Petri dishes were placed in an inverted position and incubated in an oven at a temperature of 28°C for five (5) days [8].

2.4.2. Isolation of saprophytic fungi

The suspension-dilution method has been used to isolate soil fungi [9,10]. The principle involves suspending the soil in sterile water, then incorporating different dilutions of this suspension into the isolation medium [11].

To prepare the diluted suspensions, 10 g of soil was suspended in 90 mL of sterile distilled water. The solution was stirred for 1 hour on a magnetic stirrer and then left to settle for 2 minutes. The suspension obtained corresponds to

the stock solution (10^{-1} dilution) from which other dilutions were prepared up to 10^{-9} (decimal dilution). The transition from one dilution suspension to another was made by taking care of changing the cones or tips of the 1000 µL micropipette.

To incorporate the different dilutions of these suspensions into the isolation medium, the method used was spread plating. This involved depositing 0.1 mL of the inoculum on the surface of the agar and spreading it until homogenisation on the surface of the culture medium [12]. Only dilutions -1, -3, -5, -7 and -9 were used for inoculation. Using a sterile Pasteur pipette, the inoculum was spread on malt agar medium in three replicates. The Petri dishes were incubated in an inverted position at 28°C for seven (7) days until the fungi developed well.

After incubation, counting consisted of counting the colonies present on the working dilution. The count was carried out macroscopically, taking into account the characteristics of the colonies on its medium. The number of Colony Forming Units (CFU) was determined according to the formula:

$$\text{UFC} = \frac{\text{Number of colonies} \times \text{Final dilution factor}}{\text{Dry weight of soil}} \ldots\ldots\ldots\ldots\ldots (1)$$

The pure colonies obtained were transferred to screw tubes containing malt agar medium, inclined so as to obtain abundant colonies. Each tube was then labelled according to the information on the original Petri dish and stored in the refrigerator.

Isolates were identified using two classic techniques according to [13,14].

2.5. Characterization of the endophytic and saprophytic fungi associated with amaranth

2.5.1. Macroscopic identification of endophytes and saprophytes

This identification was based on several aspects that are visible to the naked eye:

- the appearance of the colonies: filamentous fungi form fluffy colonies with a thick, woolly, velvety or flaky texture,
- the relief of the colonies: they may be flat, raised, cerebriform or striated,
- the size of the colonies: they can be small, extensive or invasive,
- growth rate: colony diameter is measured at 7 days: rapid: ≥ 3 cm ¬ moderate: between 1 and 3 cm ¬ slow: ≤ 1 cm),
- colony colour (front and back): the most common colours are olive-green, brown or black, white, yellow or red,
- pigments: diffusible or localised in the mycelium.

2.5.2. Microscopic identification of saprophytes

Microscopic examination of fungi is based on several methods. The most commonly used methods are the adhesive tape method, the lactophenol method and the fresh state method. In our study, we used the fresh state method.

This method consisted of placing a drop of distilled water on the slide, then collecting and dissociating a sample of the colony to be identified in the drop, covering the slide with a coverslip and observing with an immersion microscope [15]. Observations were made using an immersion microscope at magnification (× 40). This type of identification is essentially based on morphological studies of the mycelium (absence or presence of partitions, colour, differentiation, etc.) and spores (shape, colour, texture of the walls, etc.) [16,17].

2.6. Statistical analysis

The data collected in the field were analysed using the computer-assisted statistical tool, using SPSS (Statistical Package For Social Science) software version 22.0 and R software version 4.1.3. The statistical methods used are related to the sampling methods and experimental design adopted. The normality of the residuals and the homogeneity of the variances were verified. Two-factor analysis of variances (ANOVA) and linear correlations were performed. Means were separated using the Student Newman and Keuls test and the Kruskal-Wallis test at the 5% probability threshold.

3. Results

3.1. Occurrence of fungi associated with amaranth cultivation

3.1.1. Plant health survey

Figure 1 shows that the most common symptom found on amaranth plants in Kombé is wilting (42.86%), followed by dry rot (P-dry) with a percentage of 23.81%. Other symptoms were yellowing (9.52%), rust spot (9.52%), mole rot (P-mole: 4.76%) and spider web (4.76%).

Statistical analysis shows significant differences between the symptoms encountered at the Kombé Agricongo site ($p < 0.05$). The analysis of variance revealed four (IV) homogeneous groups (a, b, d, bc), the most significant of which were wilt on boards amended with on poultry manure amended boards (group d) and dry rot (group bc) (Table I).

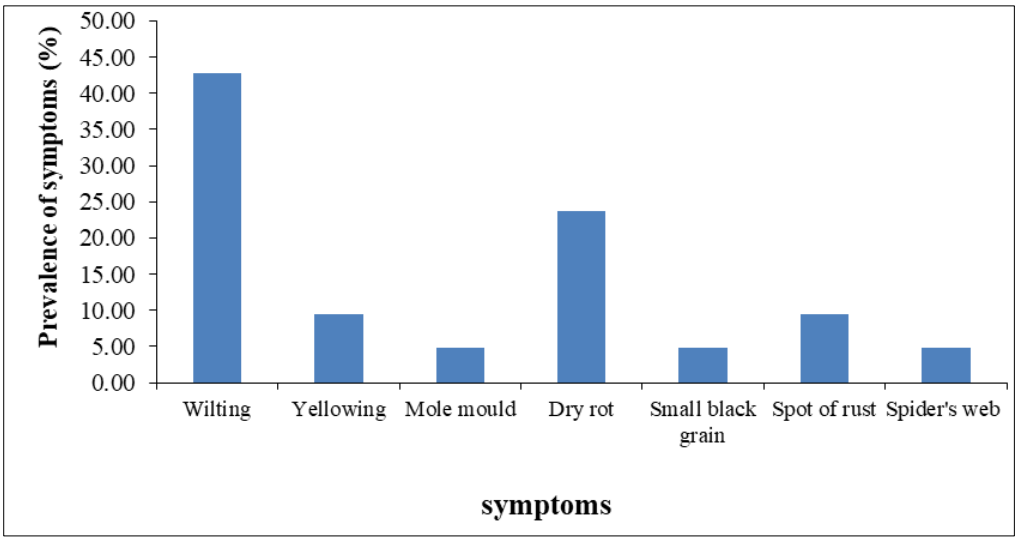


Figure 1 Symptoms observed on amaranth plants at the Agricongo site in Kombé

Table 1 Symptoms observed on amaranth plants at the Agricongo site in Kombé

Symptoms	N	Incidence (%)
Wilting	9	42.86^{d} ±0.01
Yellowing	2	09.52^{b}± 03
Mole mould	1	04.76^{a} ±0.21
Dry rot	5	23.81^{bc}±0.02
Small black speck grain	1	04.76^{a}±0.41
Spot of rust	2	09.52^{b}±0.34
Spider's web	1	04.76^{a}±0.71

N= number of plants per crop. Numbers followed by distinct letters are statistically different at the P>0.05 threshold. Otherwise, numbers followed by identical letters are not statistically different P > 0.05 according to the Student Newmann and Keuls test.

Table II shows that there is a weak positive correlation (0.083*) between the age of the plant and the symptoms encountered. In short, the age of the plants had very little influence on the appearance of symptoms. On the other hand, it is easy to observe a moderately positive correlation (0.33**) between density and the symptoms encountered, as well as between density and the age of the plant (0.34**). Age and density are parameters that can influence the appearance of symptoms (the disease).

Table 2 Linear correlation between survey variables (Symptoms, age of plants (DAS) and density)

	Symptoms	**Age of plants (DAS)**	**Density**
Symptoms encountered	1		
Age of plants (DAS)	0.083*	1	
Density	0.33**	0.34**	1

DAS= Days after semi *= low correlation; **= mean correlation; Endophytic fungi isolated from amaranth

The total number of isolates varied according to the organs inoculated during the analysis (Figure 2). The number of isolates was highest in leaves (25) compared to collars (23) and roots (7).

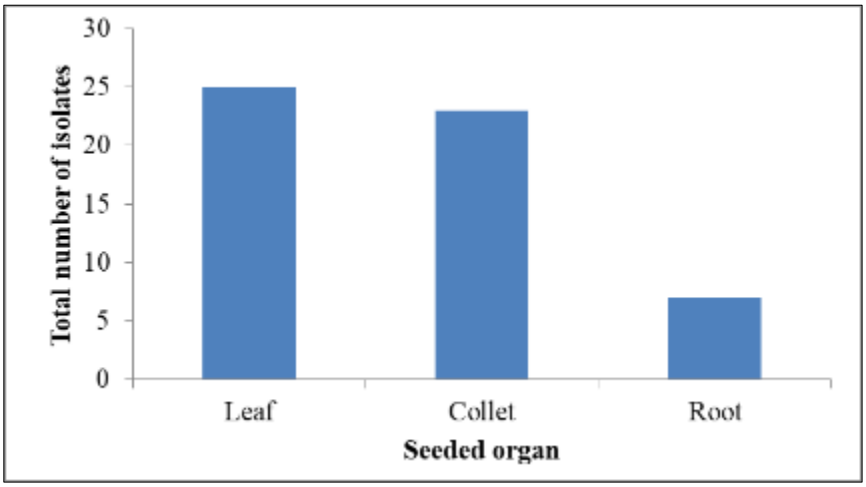


Figure 2 Isolates according to the organs seeded

3.1.2. Saprophytic fungi isolated from amaranth soil

The results shown in Figure 3 highlight eight (8) species of saprophytic fungi present in the soil of Agricongo in Kombé. The fungus ch1 was the most abundant in the soil, with an average value of 13333 x10^6, followed by ch6 (6667 x10^6), ch8 (6667 x10^6), ch2 (33 x10^6) and ch4 (33 x10^6). On the other hand, the fungi ch3 (4 x10^6), ch5 (10^6), ch7 (0.33 x10^6) were the least abundant in the soil. The statistical analysis shown in Table III reveals that there was no significant difference between the CFU concentration of the different types of fungi found in the soil of Agricongo in Kombé (p-value >0.05).

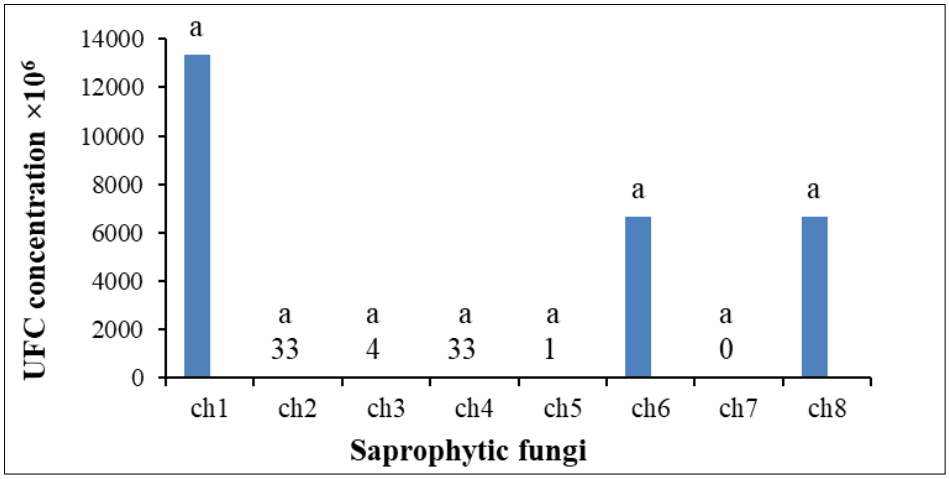


Figure 3 Abundance of saprophytic fungi isolated from soil samples

Ch: fungi (1, 2, 3, 4, 5, 6, 7, 8).

Table 3 Abundance of saprophytic fungi isolated from soil samples

Fungus	UFC ×10⁶	Standard deviation
ch1	13333.33 [a]	5.25
ch2	33.33 [a]	7.74
ch3	4.00 [a]	1.00
ch4	33.33 [a]	7.74
ch5	0.67 [a]	1,15
ch6	6666.67 [a]	4.01
ch7	0.33 [a]	0.58
ch8	6666.67 [a]	7.01

CFU×10^6 = number of fungal cells per fungus. Numbers followed by identical letters are not statistically different at the threshold of P > 0.05, p-value = 0.7002 according to Kruskal-Wallis; Ch: fungus (1, 2, 3, 4, 5, 6, 7, 8).

3.2. Characterisation of endophytic and saprophytic fungi

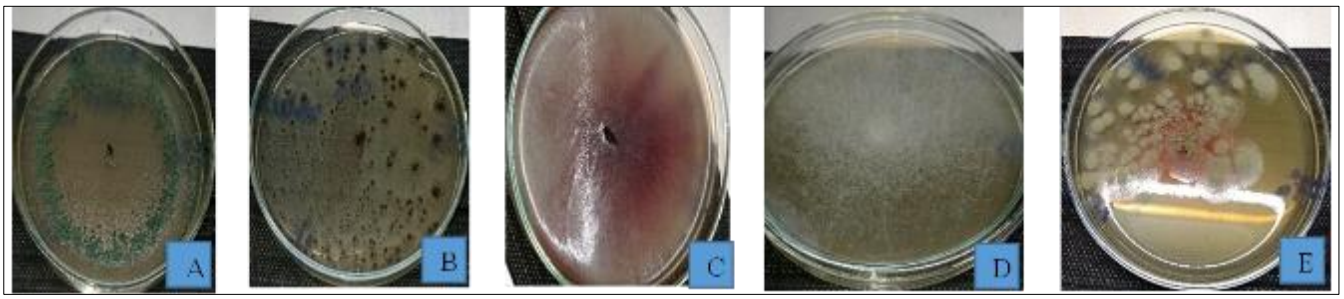
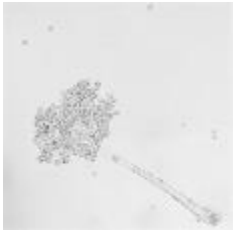
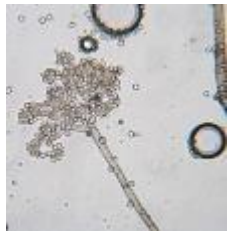
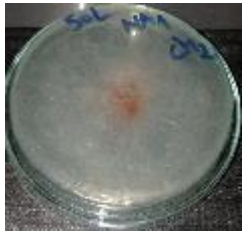
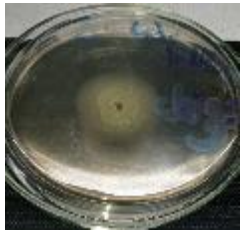
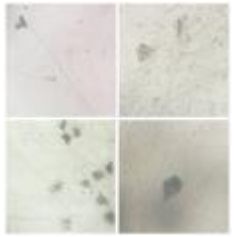
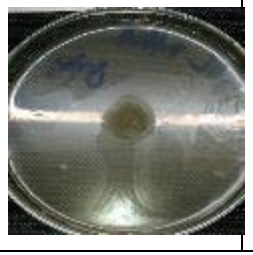
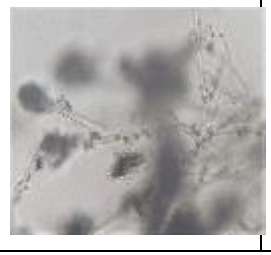
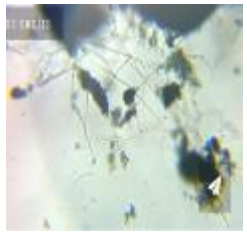


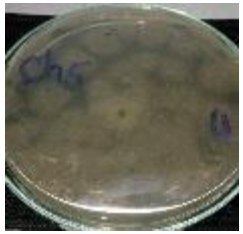
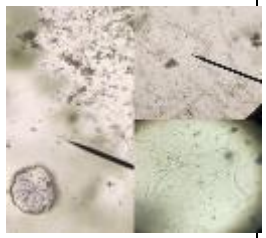
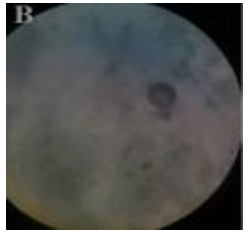
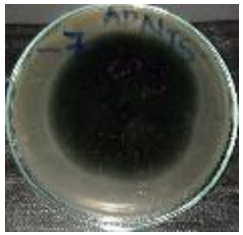
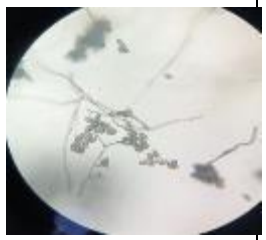
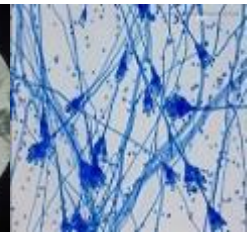
Figure 4 Macroscopic aspects of the five endophytic isolates

After incubation, a variety of mycoflora developed. Isolates were tested for purity by successive subculturing on malt agar medium. Subculturing involved removing a mycelial fragment with a sterile loop and transferring it to the malt agar medium. The inoculum was placed in the centre of the dish. The Petri dishes were incubated in an inverted position at 28°C for 72 hours.

Based on the colour of the endophytic isolates, Figures 4 and 5 show that the isolates were divided into five (5) groups according to the colour of the mycelial thallus and the type of organ sown. The group of green isolates contained the highest number of isolates compared to all other groups. The results of the same figure show that for the group of green isolates, the highest number of isolates was observed in the crown and the lowest number in the roots. As for the brown isolates, their numbers in the leaf and crown were similar and higher than in the roots. For the red-white isolates, the number at the level of the sown organs was as follows: leaf > crown > root. Next, the white group showed a total absence of isolates in the roots and a higher number of isolates in the crown. Finally, the grey group showed the highest number of isolates in the leaf and a similar number of isolates in the roots and crown.

Table 4 Macroscopic and microscopic characteristics of saprophytic isolates found in the soil

Strain code	Cultivation characteristics on malt agar			Microscopic aspects			Genres identified
	Side	Inverser	Description	Our results	Our results Photo reference microscope	Description	
Ch1			Speed rate: moderate colour: light blue-green white underside single, flat, powdery colony			Septate thallus Unicellular, globose and clear conidia	*Botrytis cineara*
Ch2			speed of growth: fast colour: white reverse white single colony with aerial mycelium, woolly and small black dots			Non-septic thallus. Presence of rhizoids, presence of stolons, globular sporocysts	*Rhizoporus sp*
Ch3			speed of growth: rapid colour: yellow with white edges pale yellow underside single, flat, woolly colony			Septate thallus Branched conidiophores Unicellular conidia arranged in a long, oval, clear, diverging chain	*Pénicillium sp1*
Ch4			speed of growth: moderate, colour: greyish green reverse white, single flat powdery colony			Septate thallus Branched conidiophores Unicellular conidia arranged in a long, oval, clear, diverging chain	*Pénicillium sp2*

Ch5	 	speed of growth: rapid, colour: dark brown reverse white, single flat granular colony	 	Non-septate thallus Conidia unicellular, round and dark brown	*Mucor sp*
Ch6		speed of growth: slow, colour: initially white, then blue-green, aged white single flat, flaky colony	 	Non-septate thallus, highly branched conidiophores with an ampuliform head and round, green conidia. Chlamydospores are round.	*Scedospirium sp*
Ch7	 	speed of growth: fast, colour: black reverse black, single flat colony	 	Septate thallus Curved conidia, multi-celled, multi-septate and black	*Curvularia sp.*
Ch8	 	speed of growth: moderate, colour: grey reverse grey, pigmented single, flat, powdery colony	 	Septate thallus Branched conidiophore Conidia oval, unicellular, and clear	*Penicillium sp3*

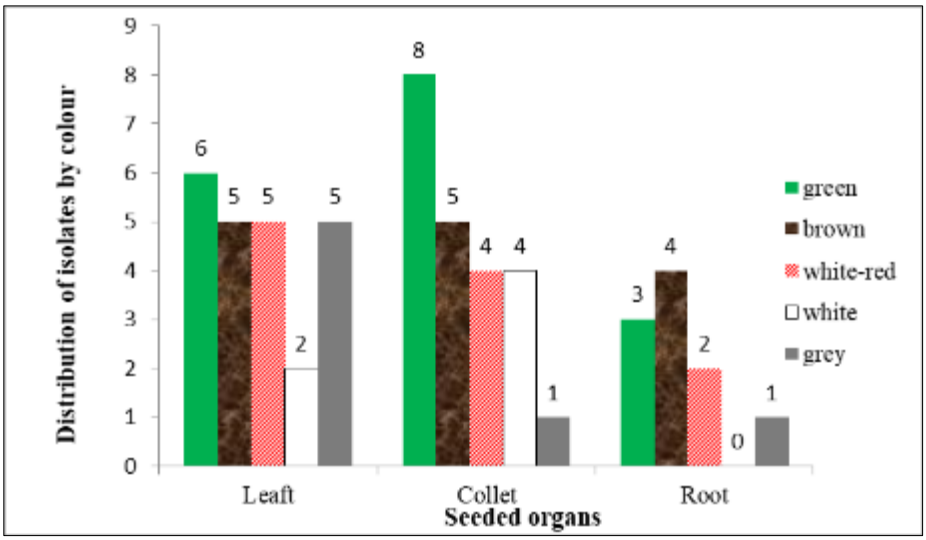


Figure 5 Distribution of isolates by colour of mycelial thallus according to sown organs

Based on macroscopic observations of the colonies, eight (8) saprophytic fungi were identified on the saprophytic isolates.

These fungi were identified according to the determination keys of [18]. On this basis, we defined the fungi representing six (6) genera: *Botrytis cineara, Rhizoporus sp*, *Penicillium sp*, *Mucor sp*, *Trichoderma sp* and *Curvularia sp*. The results are presented in the table below.

4. Discussion

The study assessed the abundance of endophytic and saprophytic fungi associated with amaranth cultivation in Brazzaville, with a view to recommending biological control of fungal diseases. The results of the survey showed that the most common and dominant symptom on amaranth plants in Kombé was wilt. There are several possible explanations for the appearance of this symptom on the observed plants. The first is that the same species are grown on the same plots. The results of the previous crops that we obtained after the surveys support this first hypothesis and confirm the hypothesis put forward by [19], which states that « in a good crop rotation, the health of the soil is preserved in the long term and the pressure of diseases and pests is kept at a low level ». The second hypothesis is the quality of the organic amendment. The addition of organic fertiliser enriches the soil with organic supplements and stimulates the development of micro-organisms. Unreasonable and uncontrolled use, without taking into account the different phenological stages of the crop and their needs, can lead to an upsurge in bio-aggressors [20]. Based on research into the impact of fertilisation and on plant resistance, [21,22] show that susceptibility to infestation increases with fertilisation. The remaining hypotheses are the sanitary quality of the plant material used (seed) and the presence of outbreaks in the environment of the plots. It should also be noted that the permanent presence of plant cover in neighbouring plots provides the most favourable conditions for the development of wilt pathogens. These results were almost similar to those of [23], who worked on integrated disease management. In his field, the symptoms manifested themselves as yellowing and wilting of the leaves, gradually leading to complete necrosis of the foliage.

The appearance of the symptoms observed on amaranth plants is weakly correlated with the age of the plants. This means that age cannot be a factor influencing the appearance or spread of the disease observed in young plants in the field. On the other hand, amaranth crops become more attractive and more exposed to pathogens (diseases) gradually as plant density increases. This high density can also facilitate the rapid spread of a contagious disease, as well as the intra-specific competition between plants that is responsible for their susceptibility to attack. These results corroborate those obtained by [24], which show a highly significant positive relationship between stomatal density and the severity of cercosporiosis at different phenological stages of the plant.

As for the isolation and characterisation of the isolates, the results of our analyses showed that the greatest number of isolates was observed at the leaf level. This high level of occurrence could be explained by foliar attacks by parasitic fungi. The results obtained from the distribution of endophyte isolates by colour indicate the presence of five undetermined fungal species in the samples taken at Agricongo in Kombé. This can be explained by the richness and

diversity of the fungal populations sampled. Our results are almost similar to those obtained by [25] who, following the isolation of endophytic fungi from date palm (*Phoenix dactylifera L.*), recorded a total of 485 fungal endophyte isolates from 630 root fragments used.

According to the results of the Kruskal-Wallis test, we then identified six (6) saprophytic genera from the analysis of the soil samples taken at Agricongo in Kombé: *Botrytis cineara, Rhizoporus sp, Pénicillium sp, Mucor sp, Scedospirium sp* and *Curvularia sp*. The presence of these fungal genera can be explained by the richness of the soil studied in organic matter, proteins and sugars provided by plant and animal residues. Our results are similar to those of [26], which revealed the presence of thirteen fungal genera in a soil sample from an orchard to know: *Aspergillus, Rhizopus, Alternaria, Ulocladium, Aureobasidium, Phoma, Mucor, Circinella, Torula, Memnoniella, Cladosporium, Bipolaris* and *Penicillium*.

5. Conclusion

The abundance of fungi associated with amaranth cultivation was assessed at the agriCongo centre in Kombé. The phytosanitary survey revealed that the most dominant symptom on amaranth plants in Kombé was wilting (42.86%). Isolation on malt agar medium revealed five (5) isolates of endophytic fungi undetermined obtained from the leaf organ. Eight (8) saprophytic fungi were found in soil samples collected at the Agricongo centre in Kombé. These fungal genera were statistically analysed using a Kruskal-Wallis test, which showed six (6) saprophytic genera to know: *Botrytis cineara, Rhizoporus sp, Pénicillium sp, Mucor sp, Scedospirium sp* and *Curvularia sp*.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans' subjects by any of the authors.

Authors' contributions

Joseph MPIKA designed the research project, executed the project and edited the manuscript. Horta Rovicia GACKOSSO conducted the field activities of the project and analysed the data. Dalcantara Liana ONGOUYA MOUEKOUBA wrote the manuscript and supervised the associated field work with Alaric MAKOUNDOU. ATTIBAYEBA is the head of the laboratory.

References

[1] Moustier, P. & Pagès, J. Peri-urban agriculture in Africa: on the fringes? INRA's Courrier of environnement.1997 ; 32 (32) : 87-96.

[2] Jame,B., Atcha-Ahowé, C., Godonou, I. et Baimey, H. Integrated pest management in vegetable production: a guide for extension agents in West Africa . International Institute of Tropical Agriculture. (IITA), Ibadan, Nigeria. 2010 ; 120 p.

[3] Saunders, R.M. et Becker, R. "*Amaranthus:* a potential food and feed resource." Advances in cereal science and technology. 1983; 7: 357-396.

[4] Makambila, C. et Goma, J-B. "*Choanephora cucurbitacearum*" Curr. a new fungal pathogen of amaranth in Congo. Agriculture Notebooks, 1993; 2(3) :217–219.

[5] Adjatin, A., Gbaguidi, A.A. et Sanoussi, F. Strategic action plan to promote the amaranth and bambara pea value chain in Benin. Master's thesis. Cotonou, Benin: University of Abomey-Calavi; 2016, 41p.

[6] Grubben, G.J.H. Growing amaranth, a tropical leafy vegetable, with special reference to South Dahomey. Dissertations & Thesis. Phytotechnics. Wageningen, Nederland: Mededelingen Landbouwhogeschool; 1975.

[7] Wouyou, A.D., Ahissou, E.A., Gandonou, C.B., Assogba, K.F., Houngbèmè, A., Gbaguidi F.A., Ahissou, H., Lagnika, L., Zanklan, S.A. and Lutts, S. Salinity increased vitamins concentration in *Amaranthus cruentus* leaves. African Journal of Biotechnology. 2017; 16(44) :2106-2111.

[8] Benhamou, N., Rey, P., Cherif, M., Hockenhull, J. Et Tirilly, Y. (1997) Teatment with the mycoparasite *pythium oligandrum* triggers induction of defence-related reactions in tomato roots when challenged with *Fusarium oxysporum f. sp. radicis-lycopersici*. Phytopathology, 1997; 87:108-121.

[9] Davet, P. Soil microbial life and plant production. Editions Quae ed. Paris, France: INRA edition ; 1996. 383p.

[10] Davet, P. et Rouxel, F. Detection and isolation of soil fungi. Quae ed. Paris, France : INRA edition; 1997. 203p.

[11] Rapilly, F. Mycological techniques in plant pathology. Annals of Epiphytia. INRA ed. Paris, France : INRA edition ;1968. 25-39p.

[12] Kouame D. & Kouakou P. Solid-state enumeration methods in food microbiology. Cours. Abidjan, Ivory Coast: Felix Houphouet Boigny University, Cocody; 2014, 32p.

[13] Cahagnier, B., Dragacc, S., Frayssinet, C., J.M. Frémy, Hennebert, G.L., Lesage-meessen, L., Multon, J.L., Richard-Molard, D. & 103 Roquebert, M.F. Mould in low-calorie foods. Tec & Doc-Lavoisier ed. Paris, France : Tec & Doc-Lavoisier edition ;1998. 225p.

[14] Guillaume Girault. Development of high-resolution molecular typing tools for the detection and differentiation of *Bacillus anthracis*. PhD thesis. Life sciences. Paris, France : AgroParisTech ; 2015, 283p.

[15] Carip, C. Microbiology hygiene microbiological basis of dietetics. Tec & Doc -Lavoisier ed. Paris, France : Tec & Doc-Lavoisier edition ; 2008. 153-675p.

[16] Harrigan, W. F. and McCance, M. E. 1976. Laboratory methods in food and dairy microbiology. Academic Press ed. London and New-York: Academic Press edition; 1973. 452p.

[17] Oteng-Gyang, K. 1984. Introduction to food microbiology in hot countries. Tec & Doc -Lavoisier ed. Paris, France : Tec & Doc -Lavoisier edition ;1984. 260p.

[18] Botton, B., Bretton, A., Fever, M., Gautier, S., Guy, Ph., Larpent, J.P., Reymond, P., Sanglier, J-J., Vayssier, Y and Veau, P. 1990. Useful and harmful moulds, industrial importance. Masson ed. Paris, France : Masson edition ; 1990. 512p.

[19] Saskia, H., Harm, B., Salomons, J., De Cara, M., Damsgaard, T.M., Vincent, M., Leendert M. et Michaela, S. Technical sheet. Crop rotation: practical information. file:///C:/Users/faou/Downloads/ROTATION%20DES%20CULTURES_%20INFORMATIONS%20PRATIQUES%20(1).pdf (Accessed at 22 December 2022).

[20] Abbas, D. Survey of phytosanitary sanitation programmes for a tomato crop in the Wilaya of Tipaza. Master's thesis. Natural and life sciences. Blida, Algeria: University Saad Dahlab De Blida. Faculty of Agroveterinary Sciences; 2012.

[21] Grechi, I., Hilgert, N., Sauphanor, B., Senoussi, R. et Lescourret, F. Modelling coupled peach tree-aphid population dynamics and their control by winter pruning and nitrogen fertilization. Ecological Modelling. 2010; 221(19): 2363-2373.

[22] Sauge, M.H., Grechi, I. and Poëssel, J.L. Nitrogen fertilisation effects on *Myzus persicae* aphid dynamics on peach: vegetative growth allocation or chemical defence. Entomologia Experimentalis and Applicata. 2010; 136 (2):123-133.

[23] Gouwie, C. Integrated disease management technical sheet - Brown Rhizoctonia. Rennes : Institut Technique de la Betterave (ITB). From: https://www.itbfr.org/fileadmin/user_upload/PDF/Fiches_Bioagresseurs/Gestion_integree_-_rhizoctone_brun_web.pdf (Accessed at 10 December 2022).

[24] Traoré, S., Kobenan, K., Kendia, K.E., Kone, D. et Traore, D. Relationship between stomatal density and response to black stripe disease in different genotypes of banana and plantain. African Agronomy. 2008 ; 20 (1), 37 – 47.

[25] Fadhela, M.M. Biological activities of endophytic fungi isolated from date palms (*Phoenix dactylifera L.*). Thesis Doctorate. Agronomic Sciences. Algeria: National Agronomic School of El Harrach; 2017.

[26] Ould Ahmed A. Isolation and identification of microscopic fungi present in the soil of a *Punica granatum L.* variety Messaad agrosystem in an arid climate. Master's thesis Specialty: Biotechnology and Plant Valorization. Mouloud-Mammeri University of Tizi Ouzou; 2019.