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Evaluation of the antifungal activity of the extracts of some medicinal plants on the strains of *Alternaria alternata*, *A. solani* and *Fusarium sp.* in Kisangani (DR Congo)

Manya W[1], Etobo K[2], Nshimba S[3], Onautshu O[2*]
[1]Medical Biology Section, Kisangani Higher Institute of Medical Technology (ISTM-KIS), Kisangani, DR Congo
[2]Department of Biotechnology, Laboratory of Microbiology and Phytopathology, Faculty of Science, Kisangani University, Kisangani, DR Congo
[3]Department of Ecology and Plant Resource Management, Faculty of Science, Kisangani University, Kisangani, DR Congo

***Corresponding author:** Onautshu O
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Abstract

With the intention of selecting plants for use as a fungicide, a study was conducted on the *invitro* activity of 10 medicinal plants in the Kisangani area. The activity of 3 types of extracts (concentrated crude, ethanolic and ethereal) was tested on 3 phytopathogenic strains (*Alternaria alternata*, *Alternaria solani* and *Fusarium* sp.). The method of inhibition of mycelial growth on solid medium (PDA) in Petri dishes was used to study the activity of extracts of medicinal plants towards strains. 9 plants showed satisfactory activity for at least one type of extract on at least one of the fungal strains. However, the statistical evaluation concluded a lack of significant difference between the different extracts, and highly significant differences between different plants. *Alchornea cordifolia* is the only plant that has shown no efficacy on all fungal strains tested.

Keywords: Antifungal activity, extracts plants, medicinal plants, strains, Kisangani.

INTRODUCTION

Agriculture is the basis of the economy of several African countries, especially those of Central Africa. Unfortunately this agriculture is subject to several constraints, mainly of abiotic and biotic orders. Producers are confronted with a variety of diseases that attack crops. Many foodand income crops are ravaged by parasites, the most notorious of which are phytopathogenic fungi. Inthe midst of these, species of the genus*Fusarium* attack crops such as tomato [1], rice [2], wheat [3], banana [4], cotton [5], earth [6, 7].

Attacks of crops by species can have many direct and indirect negative impacts on the nutrition and the income ofpeasants, therefore the food insecurity and poverty [8].

The chemical fight is most commonly used for the control of these fungal parasites. However, the fungicides currently used are phytotoxic, persistent with adverse effects on the environment [9]. To avoid the frequent appearance of resistant strains during these fungicidal treatments by the use of chemical pesticides and to limit the environmental impacts due to their repetitive application, it is essential to develop and exploit medicinal plants.

The therapeutic use of the plants is very present in some countries of the world and especially in developing countries, because of the absence of a modern medical system [10].

Since antiquity, natural products, especially those of plant origin have always been an important source of therapeutic agents. Currently, around 25-30% of all drugs available for the treatment of diseases are derived from natural products (plants, animals, bacteria and fungi) [10].

This work was undertaken to evaluate the antifungal activity of extracts of some medicinal plants used in human medicine, on *A. alternata*, *A. solani* and *Fusarium* sp. in Kisangani (D.R.Congo).

MATERIALS AND METHODS

Field of Study

This work was done in the city of Kisangani in the Democratic Republic of Congo; it is the chief town of the Tshopo provinceand is located in the part of the Congolese central basin at 0° 31'North and 25° 11'E at an altitude of 396m. In the Kisangani region, rainfall is abundant but irregularly distributed over the year; the annualaverage rainfall calculated for a period of 50

years (1956 to 2005), shows 1724 mm, for an average annual temperature of 25.3 ° C, the monthly precipitation height is greater than 60 mm [11].

Plant Material

The plants in this study are: *Aloe vera*, *Moringa oleifera*, *Alium cepa*, *Basella alba*, *Newbouldia laevis*, *Alchornea cordifolia*, *Alium sativum*, *Artocarpus altilus*, *Mitracarpus villosus*, *Ageratum conyzoides*. The taxonomy of these plants is shown in Table-1.

Table-1: Taxonomy of tested plants

Family	Genus	Species
Xanthorrhoeaceae	*Aloe*	*A. vera*
Moringaceae	*Moringa*	*M. oleifera*
Lilliaceae	*Allium*	*A. cepa*
Basellaceae	*Basella*	*B. alba*
Bignoniaceae	*Newbouldia*	*N. laevis*
Euphorbiaceae	*Alchornea*	*A. cordifolia*
Lilliaceae	*Allium*	*A. sativum*
Moraceae	*Artocarpus*	*A. altilis*
Rubiaceae	*Mitracarpus*	*M. villosus*
Asteraceae	*Ageratum*	*A. conyzoides*

Fungal Material

The fungal strains studied in this work are *A. alternata*, *A. solani* and *Fusarium* sp. These strains were isolated in the laboratory of the University of Ghent (Belgium) and sent to the laboratory of Microbiology and Phytopathology of the Faculty of Sciences of the Kisangani University (DRC) for their tests on the plants of the region.

Methods

Preparation of Concentrated Crude Extracts

The crushed fresh vegetable matter is pressed to collect a juice. 10 ml of each juice are taken and concentrated by evaporation (at a temperature not exceeding 50 ° C) until an amount of about 2 ml [12].

Preparation of Ethanolic and Ethereal Extracts

The ethanol (96%) and the petroleum ether served as the extraction solvent. 50 ml of each solvent were poured into the jars in which 10 grams of crushed fresh vegetable material were placed each time. The mixtures were macerated for 48 hours and then filtered. The filtrates were finally concentrated by evaporation to 2 ml of extract in each tube [13-15].

Activity of Plant Extracts

The method of inhibiting mycelial growth on a Petri dish in a solid medium was used to study the activity of extracts of medicinal plants compared to the thinner. This consists in depositing in the center of each Petri dish containing the PDA (Potato Dextrose Agar) medium (39gr / l) to which a test extract has been spread, a 5mm diameter mycelial explant obtained after perforation with a carrier piece. Incubation was made at 25° C under permanent white light.

Mycelial growth was monitored regularly for 7 days by measuring each explant under different extracts at the rate of three repetitions per plant extract studied.

Data Processing

The reduction of mycelial growth (RMG) or percent inhibition of mycelial growth compared to the untreated control is determined by the formula:

$$RMG(\%) = \frac{C_o - C_c}{C_o} X\ 100 \ [16]$$

With C_0: growth (mm) of the fungus on the culture medium without extract of the tested plant,

C_c: growth (mm) of the fungus on the culture medium at a concentration c of extract of the tested plant

The degree of activity of the extract is determined according to the following scale. The extract is said:

- Very active and the very sensitive fungal strain for an inhibition of between 75 and 100%,
- Active and the sensitive strain for an inhibition of between 50 and 75%,
- Moderately active and the intermediate strain for an inhibition of between 25 and 50%,
- Little or no active and the strain insensitive or resistant to inhibition <25% [16].

To achieve the assigned objectives and thus verify the hypotheses, the analysis of the variance was chosen as a statistical test. It was made possible by the R 2.10.0 Software [17].

RESULTS AND DISCUSSION

Crud Concentrated Extract

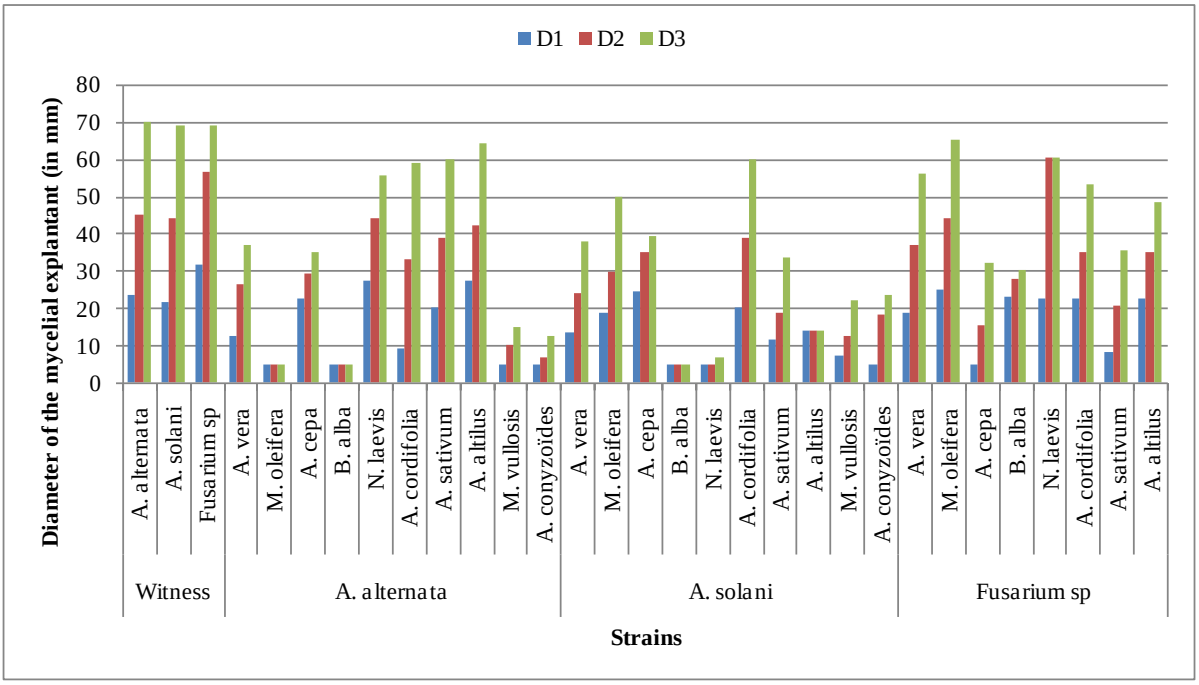

Fig-1: Evolution of the diameters of the mycelia explantants of strains on different media with and without concentrated crude extracts

It is observed from this illustration that all the plants tested showed an inhibitory activity against the strains tested according to the evolution of mycelial explantant growth relative to the witness strain. The maximum diameters attained by the strains under the action of the extracts of the plants make it possible, according to the degree of activity, to spread out 6 plants with a very active concentrated crude extract to the Alternaria strains. Of which (4) *M. oleifera*, *B. alba*, *M. villosus* and *A. conyzoides* for *A. alternata* and (3) *B. alba*, *N. laevis* and *A. altilus* for *A. solani*. While *B. alba* inhibited both Alternaria strains, no plants acted very actively on *Fusarium* sp. for which (4) the *B. alba* extract was only active as well as the extracts of *A. cepa*, *A. sativum* and *A. conyzoides*. On the other hand, *A. vera* and *A. cepa* (2) were for the strain of *A. alternata* while *A. sativum*, *M. villosus* and *A. conyzoides* showed antifungal activity on *A. solani*. The 3 fungal species show variable reactions in the presence of plant extracts tested as was the results of Bouazza and Hassikou [16].

As active as ours result, Kabore et *al.*, [18] have shown and explained the effectiveness of *M. oleifera* seeds in water disinfection by the clarification and elimination of pathogenic microorganisms in Burkina Faso. The process before flocculation with the seed powder of this plant is as follows: the active ingredients contained in the powder (basic polypeptide, more specifically a set of polyelectrolytes) are released in water and meet the colloids present, thenthey give rise to a phenomenon of flocculation. These positively charged polyelectrolytes neutralize colloids in murky waters because the majority of these colloids have a negative charge [19, 20].

Rakotoniriana et *al.*, [21] compared coagulants of *M. oleifera* and chemical (Al_2 $(SO_4)_3.18H_2O$) in water treatment in Madagascar. The turbidity value decreases from 403 to 4TNU (Turbidity Nephelometry Unit) with the use of $Al_2(SO_4)$ $_3.18H_2O$ coagulant chemistry while that after treatment by the plant decreases from 403 TNU to 5 TNU, two values that comply with the potability standard [22, 23]. Regarding the post-treatment conductivities and salinities, the results of treatment with alumina sulphate are more contaminant than Moringa compared to the value of conductivity and salinity [22, 24]. This effect is observed in this work for *A. alternate* strains about concentrated crude extracts.

Bouazza and Hassikou [16] tested the antifungal activity of the leaf pulp of *A. vera* on seven species of fungi (*Botrytis cinerea*, *Curvularia lunata*, *Penicillium expansum*, *Trichophyton rubrum*, *A. alternata*, *Aspergillus fumigatus* and *Trichoderma viride*) and compared to a few trade fungicides. They concluded that the *A. vera*'s pulp used in the concentrated state is very active on the pathogenic species studied. These results corroborate those of this study on *A. alternata* strain.

Like us on fungi strains, several studies have investigated the effects of different garlic extract

preparations on bacterial growth. Either by the indirect method, the viable cell count [25, 26, 27]. It is the enumeration of the colonies to which the viable bacteria present in the medium spread on solid medium.Also by the spectrophotometry (OD 600 nm) for the monitoring of the measurement of the biomass during a bacterial culture [28, 29]. This non-informative method for cell viability, however, has the advantage of being faster [30].

Bouras and Benhamza [31] tested the impact of 2 plant extracts, basil (*Ocimum basilicum*) and garlic (*A. sativum*) in the fight against tomato leafminer *Tuta absoluta* on six varieties of tomato *Solanum lycopersicum* under plastic shelters The results of the larvicidal effect of the two plant extracts on larvae L3 and L4 of *T. absoluta* tomato leafminer show that the two extracts studied are not very effective against *T. absoluta*. The dose 2.5 is shown to be the most lethal for both extracts with a mortality rate of 43.3%.For the dose 5% garlic extract is more effective than basil extract with respective mortality rates of 36.66 and 6.67%. The crude *A. sativum* extract of this work also showed similar effects on *A. solani* and *Fusarium* sp.

Ethanolic Extract

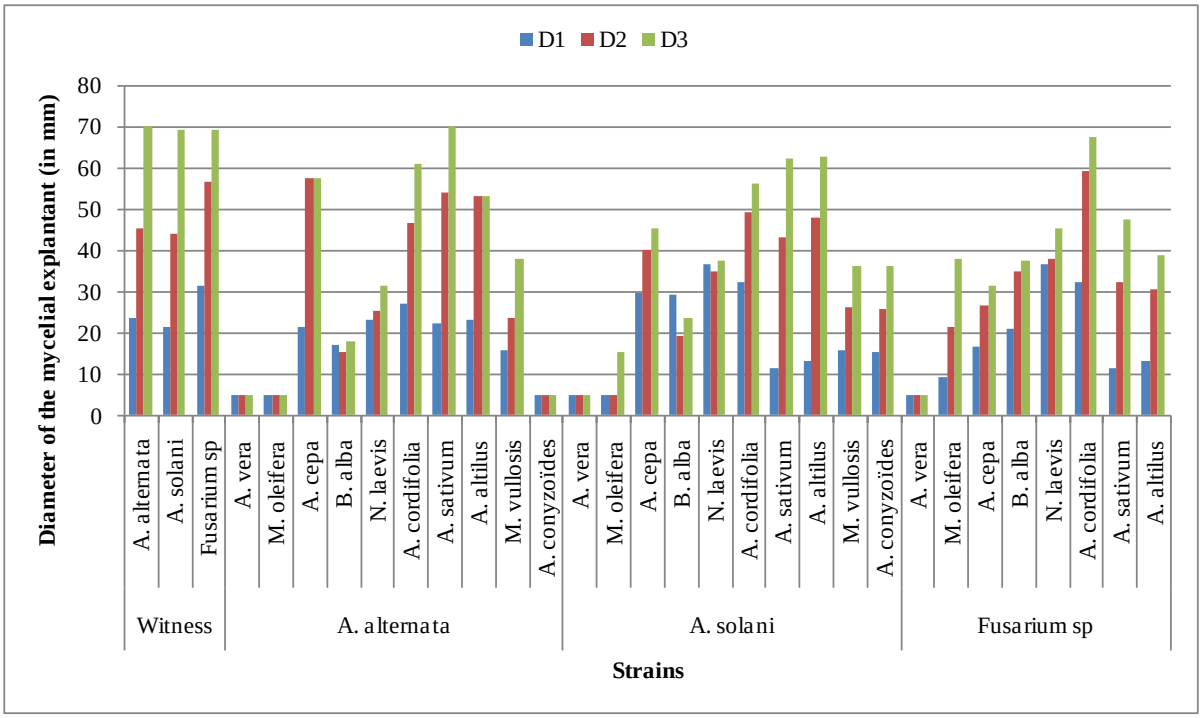

Fig-2: Evolution of the diameters of the mycelian explantants of the strains on different media without and with ethanolic extracts

Reading of this figure reveals that some plants with low activity have seen under the action of ethanol the improvement of activity and a change of class of activity according to the maximum diameters reached by the mycelian explantant. This is how the extract of *N. laevis* which had or had little activity in the concentrated crudeon *A. solani* state improved. As stated by Bouxid Hanae [32], the method of preparation of an herbal product may have an effect on the amount of active ingredient present. The timing and season of the harvest, as well as the type of soil where it grows, can also influence its effectiveness. Alilou [33] in a phytochemical and antifungal study of two plants from southern Morocco: *Asteriscus graveolens subsp. odorus (Schousb.) Greuter* and *Asteriscus imbricatus (Cav.) DC.* had extracted the leaves and flowers of these two plants in three solvents with increasing polarities including petroleum ether, ethyl acetate and methanol. A difference in yield of these extracts was thus noted. This difference could be due to the extraction capacity of each solvent. Each of these can extract well-defined families of secondary metabolites existing in the different parts of the plants studied. Indeed, according to Cowan [34], petroleum ether could extract alkaloids, terpenoids and coumarins. As for the solvent of ethyl acetate, it makes it possible to extract the glycosides. Methanol, the most polar solvent of the three, allows the extraction of tannins, saponins, terpenoids, anthocyanins, xanthoxylins, quassinoids, lactones, flavones, phenones and polyphenols. The modification of the action of *N. laevis* of this work would be its mode of preparation; that is to say a concentration of the active ingredient of the plant that would be due to the extracting solvent (ethanol).

Ethereal Extract

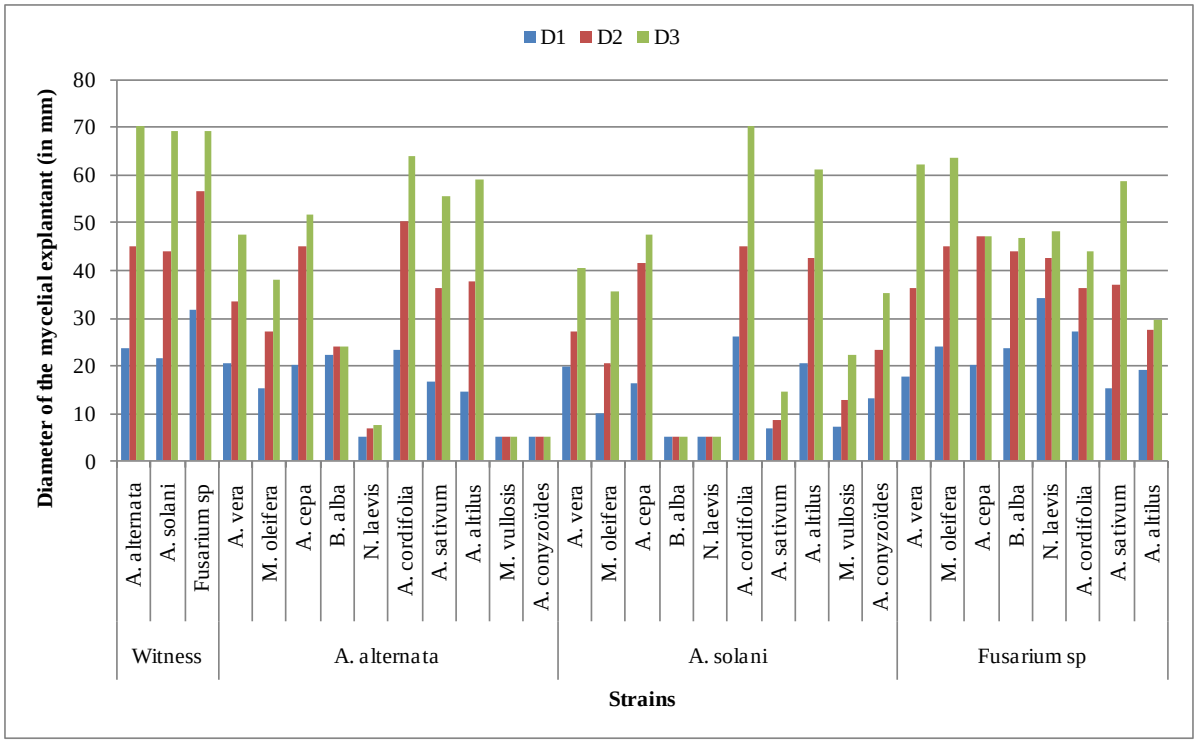

Fig-3: Evolution of the diameters of the mycelian explantants of the strains on different media without and with ethereal extracts

The observation of Fig-3 shows a decrease in the number of the plants with antifungal activity on the tested strains compared to the concentrated and ethanolic crude extracts. Only strains of *A. solani* kept a high sensitivity for more than half of the plants tested. This corroborates Alilou's [33] claim in his study that petroleum ether and ethyl acetate extracts from leaves and flowers appear to have the same activity against *Penicillium digitatum*. However, remain significantly lower than those of methanol extracts. The alkaloids, terpenoids and coumarins extracted by petroleum ether as well as the glycosides extracted by ethyl acetate would be a reliable argumentative support for this difference [34]. In fact, the statistical study has shown that the petroleum ether and ethyl acetate extracts have a very marked specificity for *P. digitatum* more than *P. expansum* for both the leaves and the flowers of *A. graveolens subsp. odorus*. In addition, the antifungal effect of the three extracts of *A. imbricatus* studied does not show an inhibition of *P. expansum*, unlike *P. digitatum* which seems to react significantly to their antifungal effect.

Statistical analyzes to compare the effects of the extracts and the various plants analyzed reveal that there are no significant differences between the various extracts (Pr> 0.3244) while there are highly significant differences between the different plants tested. Because P value (Pr <2.2e-16 ***)

CONCLUSION

In the present work, we are interested in the in vitro study of the antifungal activity of 3 types of extracts (concentrated crudes, ethanolic and ethereal) of 10 medicinal plants from the Kisangani region on 3 phytopathogenic strains (*A. alternata*, *A. solani* and *Fusarium* sp.) with the intention of selecting those that can serve as an *in situ* test source. Experimentation of the activity of the different extracts of the plants tested allowed selection according to the maximum diameters reached on media containing the tested plant extracts that:

9 plants (*A. vera*, *M. oleifera*, *A. cepa*, *B. alba*, *N. laevis*, *A. sativum*, *A. altilus*, *M. villosus*, *A. conyzoides*) showed concentrated crude extracts to react very actively or actively on one or the other fungal strain except *A. cordifolia* which did not act actively on any strain. *B. alba* and *A. conyzoides* were very active or active on the 3 fungal strains tested.

8 plants (*A. vera*, *M. oleifera*, *A. cepa*, *B. alba*, *N. laevis*, *A. altilus*, *M. villosus*, *A. conyzoides*) gave ethanolic extracts reacting very actively or actively on one or the other fungal strainexcept two plants: *A. cordifolia* and *A. sativum*. The extracts of *A. vera* were very active on all fungal strains tested.

7 plants (*M. oleifera*, *B. alba*, *N. laevis*, *A. sativum*, *A. altilus*, *M. villosus*, *A. conyzoides*) provided ethereal extracts reacting very actively or actively

except *A. vera*, *A. cepa*and *A. cordifolia*. The ethereal extracts of *N. vullosis* were for all the strains tested.

A. cordifolia is the only plant that has shown no efficacy on the fungal strains tested. Statistical analysis showed that there was no significant difference between the different plant extracts, which allows to continue the search for concentrations that totally inhibit the growth of fungal strains. Moreover, the difference is highly significant between the plants tested, thus reflecting the quintessence of plant activity and not the action of solvents used in the preparation of different extracts; fact that completely eliminates the *A. cordifolia* plant compared to the strains tested.

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REFERENCES

1. Hibar, K., Daami-Remadi, M., & El Mahjoub, M. (2007). Effect of some Chemical and Biological Fungicides on Mycelial Growth and Disease Severity of *Fusarium oxysporum f. sp. radicis-lycopersici* (in French). *Tropicultura*, *25*(3), 146.
2. Zehhar, G., Touhami, A. O., Badoc, A., & Douira, A. (2006). Effet des *Fusarium* des eaux de rizière sur la germination et la croissance des plantules de riz. *Bulletin-Societe De Pharmacie De Bordeaux*, *145*(1/4), 7.
3. Lauzon, M., Dion, Y., & Rioux, S. (2007). Fusariose de l'épi chez le blé et l'orge. CEROM. *Bulletin Technique, Phytopathologie*, (2), 01.
4. Moore, N. Y., Bentley, S., Pegg, K. G., & Jones, R. D, (1995). Maladies des *Musa*. Fiche technique n° 5. La fusariose du bananier, Réseau international pour l'amélioration de la banane et de la banane plantain. Montpellier: Parc Scientifique Agropolis.
5. Assigbetse, K., Henni, J., & Boisson, C. (1990). Pathogenicity variations of three pathogens of tomato and cotton: *Verticillium dahliae* Klebhan, *Fusarium oxysporum f. sp. lycopercisi* (Sacc.) Snyd. and Hans. and *F. oxysporum f. sp. vasinfectum* (Atk.) Snyd. and Hans.
6. Soltner, D. (1979). Les grandes productions végétales. Phytotechnie spéciale. 10éme Edition, 427.
7. Hassen, A. (1999). Production de pomme de terre. Maison arabe de L'édition et la distribution, 446.
8. Soro, D., Koné, M. W., Koné, D., & Kamanzi, K. (2010). Evaluation de l'activité antifongique par bioautographie de quelques plantes médicinales de côte d'ivoire contre deux formes spéciales de *Fusarium oxysporum*. E.J.S.R. 40(2): 307-317.
9. Mabsoute, L., Meskine, M., Bouznad, Z., & Kharrat, M. (1996, November). Résultats des surveillances sur les maladies cryptogamiques des principales légumineuses alimentaires dans le Maghreb. pp: 43-50. In *Proceeding du symposium régional sur les maladies des céréales et des légumineuses alimentaires* (pp. 11-14).
10. Tabuti, J. R., Lye, K. A., & Dhillion, S. S. (2003). Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *Journal of ethnopharmacology*, *88*(1), 19-44.
11. Kahindo, J. M. (2011). Potentiel en Produits Forestiers Autres que le Bois d'œuvre dans les formations forestières de la région de Kisangani. Cas des rotins *Eremospatha haullevilleana* De Wild and *Laccosperma secundiflorum* (P. Beauv.) Küntze de la Réserve Forestière de Yoko (Province Orientale, RD Congo). *Unpublished PhD thesis, Faculty of Science, University of Kisangani*.
12. Mbuyi, M., Bigawa, S., Kumbukama, L. B., Tchatchambe, W. B. & Omande, B. (1989). Recherches sur l'activité antibactérienne de quelque espèces végétales utilisées dans le traitement des diarrhées infectieuses en médecine traditionnelle zaïroise in Ann. Fac. Des Sciences. Unikis (6), 97.
13. Harbone, J. B. (1983). Photochemical methods. Chapman Et hall. London, 28.
14. Bouret, J. C. (1984). Le défi de médecine par les plantes. France-empire, Paris, 345.
15. Janovska, D., Kubikova, K., & Kokoska, L. (2003). Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. *Czech journal of food sciences*, *21*(3), 107-110.
16. Bouazza, F., & Hassikou, R. (2011). Activité antifongique *in vitro* de la pulpe foliaire d'*Aloevera*, *Bull. Soc. Pharm. Bordeaux*, 150(1-4), 95-106.
17. Buysse, W., Stern, R., & Coe, R. (2004). Genstat Edition Discovery pour Usage quotidien, 3ème édition, traduit de l'anglais par Amini Mutaganda. ICRAF Nairobi, Kenya, 122.
18. Kabore, A., Savadago, B., Rosillon, F., Straore, A., & Dianou, D. (2013). Optimisation de l'efficacité des graines de Moringa oleifera dans le traitement des eaux de consommation en Afrique sub-saharienne: cas des eaux du Burkina Faso. *Revue des sciences de l'eau/Journal of Water Science*, *26*(3), 209-220.
19. Foild, N., Makkar, H. P., & Becker, K. (2002). Potentiel du Moringa oleifera pour les besoins agricoles et industriels. *Potentiel de Développement Pour Les Produits de Moringa. Actes de l'Atelier International de Dar-es-Salam Saint-Sauveur, Appora, Besse et Fuglie eds*, *29*.
20. Ndabigengesere, A., Narasiah, K. S., & Talbot, B. G. (1995). Active agents and mechanism of coagulation of turbid waters using Moringa oleifera. *Water research*, *29*(2), 703-710.

21. Rakotoniriana, H. J., Randriana, N. R., Ramaroson, J. D., Randrianarivelo, F., Herihajaniavo, A. M., & Andrianaivo, L. (2015). Etude comparative des coagulants dans le traitement des eaux. Mada-Hary, 4, 67-77.
22. OMS. (2006). Normes de l'OMS sur l'eau potable - Lenntech, www.lenntech.fr/.../normes/normes-oms-eaupotable.h...
23. Norme de potabilité Malagasy. (2015). Décret n°2004-635 du 15/06/04. Paramètres, Organoleptiques, Odeur,Couleur, Saveur, Désagréable., http://www.jirama.mg
24. Aquatechnique. (2013). conductivitéhttp://aquatechnique.pagespersoorange.fr/Techniques/page_%20conduc.htm,
25. Ross, Z. M., O'Gara, E. A., Hill, D. J., Sleightholme, H. V., & Maslin, D. J. (2001). Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Applied and environmental microbiology*, *67*(1), 475-480.
26. Bakri, I. M., & Douglas, C. W. I. (2005). Inhibitory effect of garlic extract on oral bacteria. *Archives of oral biology*, *50*(7), 645-651.
27. Pranoto, Y., Salokhe, V. M., & Rakshit, S. K. (2005). Physical and antibacte rial properties of alginate-based edible film incorporated with garlic oil. *Food research international*, *38*(3), 267-272.
28. Nakagawa, H., Tsuta, K., Kiuchi, K., Senzaki, H., Tanaka, K., Hioki, K., & Tsubura, A. (2001). Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. *Carcinogenesis*, *22*(6), 891-897.
29. O'Gara, E. A., Hill, D. J., & Maslin, D. J. (2000). Activities of garlic oil, garlic powder, and their diallyl constituents against Helicobacter pylori. *Applied and environmental microbiology*, *66*(5), 2269-2273.
30. Meddebwiem. (2008). Etude des effets des rayonnements ionisants sur les propriétés biochimiques et biologiques de l'ail (*Allium sativum*), Mémoire de Mastère des Substances Naturelles Thérapeutiquement Actives, Fac des sciences de bizerte, Université 7 novembre, Carthage, Maroc, 78.
31. Asma, B. O. U. R. A. S., & Somia, B. E. N. H. A. M. Z. A. (2013). *Impact de deux extraits végétaux, le basilic Ocimum basilicum et l'ail Allium sativum, dans la lutte contre la mineuse de la tomate Tuta absoluta sur six variétés de tomate Lycopersicum esculentum sous abris plastique à l'ITDAS de Hassi Ben Abdellah-Ouargla* (Doctoral dissertation).
32. Hanae, B. (2012). *Les plantes médicinales et diabète de type 2 (à propos de 199 cas)* (Doctoral dissertation, thèse de médecine: faculté de médecine et de pharmacie-Fès).
33. Alilou, H. (2012). Etude phytochimique et antifongique de deux plantes du Sud du Maroc: *Asteriscus graveolens subsp. odorus* (Schousb.) Greuter et *Asteriscus imbricatus* (Cav.) DC.
34. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.