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Post-harvest conservation of orange (*Citrus sinensis* L.) using bacterial biocontrol agents isolated from the rhizosphere of the cashew tree (*Anacardium occidentale* L.) in Côte D'ivoire

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Abstract

Post-harvest fruit diseases, mainly due to fungi microscopic, constitute one of the main factors of fruit deterioration during their conservation. Unlike chemical control, biological control is more beneficial given its ecological and health nature for the consumer. However, this fight could have impacts on biochemical parameters and organoleptic of preserved fruits. The objective of this study is to highlight the inhibitory activity of bacterial biocontrol agents isolated from the rhizosphere of the cashew tree on the germs responsible for post-harvest spoilage of the orange. To do this, 300 oranges were collected from the fruit market in Côte d'Ivoire. The flora fungal spoilage of these oranges was isolated on PDA medium and conservation tests of the oranges with the supernatant of bacterial biocontrol agents were carried out. The results obtained made it possible to identify three (3) genera of fungi on spoiled oranges including *Colletotrichum*, *Alternaria* and *Lasiodiplodia* with respective isolation frequencies of 11%, 33% and 56%. Also, preserving the oranges with the supernatant of the bacterial biocontrol agents made it possible, over 14 days, to note an absence of growth of fungi and no sign of spoilage unlike the control oranges.

Keywords: Orange; Conservation; Alteration; Biocontrol; Côte d'Ivoire

1. Introduction

Citrus fruits are the fruits whose production is the second largest in the world with more than 115 million tons per year [1]. The citrus group belongs to the *Rutaceae* family. They originate from South Asia. Their distribution took place in the world through commercial exchanges [2]. Citrus fruits are classified into several groups namely oranges, lemons, limes, pomelos, clementines, tangerines etc. Orange is a citrus fruit very important in the world [3]. Global citrus production is around 99 million tons, of which 73% of production is consumed fresh, 26% is intended for processing and 9% for export. This production is divided into several varieties citrus fruits of which orange represents 57% [4]. In Côte d'Ivoire, the orange sector is largely dominated by the informal sector and constitutes a source of income for the rural population. Also, the orange has several nutritional properties in that it constitutes an important source of antioxidant agents including phenolic compounds, flavonoids and ascorbic acid. Indeed, these compounds have beneficial effects on human health because they possess numerous antioxidant, anti-inflammatory and antibacterial activities. These different properties of oranges protect and inhibit the harmful effects of free radicals on the human body [5]**.** However,

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the storage of Oranges remain a crucial problem for producers and traders in Côte d'Ivoire. Indeed, several environmental factors such as humidity, temperature and storage duration of oranges favor the growth of fungi such as the genera *Alternaria*, *Scleratonia*, *Colletotrichum*, *Fusarium* and *Penicillium*. These fungi are responsible for the rotting of orange during storage [6]. To overcome this problem, several conservation techniques including modified atmospheres, the addition of antioxidants (ascorbic acid, citric acid) and firming agents have been developed [7]. However, these techniques do not really satisfy producers who instead resort to synthetic pesticides. However, the use of chemical pesticides in the preservation of oranges could have harmful effects on the environment and the health of the consumer. Biological control is therefore necessary. Indeed, providing producers with organic material promoting the protection and conservation of fruits without having harmful effects on the consumer and the environment would be an adequate solution for the scientific and agricultural community. In this context, Studies have been carried out in particular on pineapple and mango. Indeed, the work of [8] made it possible to preserve pineapple over a period of two weeks (15 days) using *Pseudomonas fluorescens* CI. Also, [9] were able to preserve mango over a period of 15 days with *Bacillus subtilis* GA1. Despite all the advantages that biological control presents, it is clear that the organoleptic and nutritional characteristics of preserved fruits could undergo modifications. This could constitute a real obstacle to the marketing, processing and consumption of these fruits. Indeed, previous studies carried out have focused on preservation and without paying attention to their influence on organoleptic characteristics. Furthermore, in Côte d'Ivoire, there are rarely fields of orange trees under cultivation alone. Indeed, in the east of the country, the orange tree is cultivated in plantation with the cashew tree which shares the same rhizospheric flora. Therefore, this work sets the general objective of highlighting the inhibitory activity of bacterial biocontrol agents isolated from the rhizosphere of the cashew tree on postharvest spoilage germs of the orange.

2. Material and methods

2.1. Material

The material consisted of orange (*Citrus sinensis*). On the one hand, oranges healthy ones for conservation tests and on the other hand altered oranges for the search for spoilage molds were used (Figure 1). Four bacteria, two of which Gram-positive bacilli (*Bacillus*) and two Gram-negative bacilli (*Pseudomonas*) were used as biocontrol agents for orange. These biocontrol agents were isolated from the rhizosphere of the cashew tree in Côte d'Ivoire.

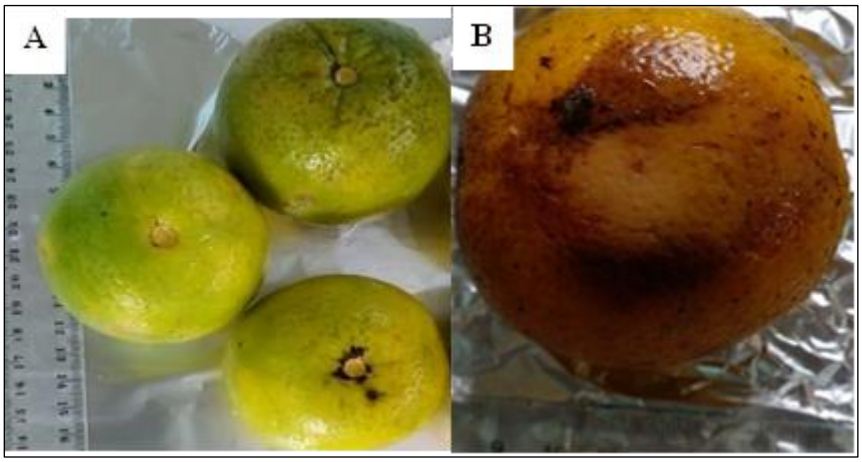

Figure 1 Study materials. (A- Healthy orange and B- Spoiled orange)

2.2. Methods

2.2.1. Sampling

The orange studied were collected from the fruit market in Abidjan (Côte d'Ivoire). In total, 30 orange fruit samples were taken during this study at a rate of 10 oranges per sample. Then the orange were transported in a cooler with cold accumulators, in order to maintain the temperature at 4 °C, to the Laboratory for analyses.

2.2.2. Isolation and identification of Orange spoilage fungi

The isolation of the fungi was carried out by direct contact on PDA agar (Potato Dextrose Agar) according to the method described by [10] (**Figure 2**). So the orange altered are washed, and cut into small fragments. The fragments obtained

are disinfected with 2% bleach for 2 min then rinsed twice with sterile distilled water with the aim of to eliminate exogenous microflora. Then three fragments are placed on Petri dishes containing the PDA agar using forceps previously sterilized under the conditions aseptic and the plates are incubated at 30 °C for 5 to 7 days. After 7 days incubation, the molds were purified by transfer of colonies on to PDA agar according to the method described by [11] and [12]. Thus, a filament of fungal colony was taken using sterile forceps then placed at a single point on the center of a Petri dish containing PDA agar in order to obtain typical development mold. Incubation is carried out at 30 °C for 7 days. This method is repeated until pure colonies are obtained.

Phenotypic identification of fungi was carried out based on observations macroscopic and microscopic according to the method of [11] and [12]**.** Identification was carried out according to the method of [11] by examining the culture on PDA agar. The cultural characteristics determined were the appearance of the colonies (fluffy, woolly, cottony, velvety, powdery or granular), the relief of the colonies (flat, convex, pleated, etc.), the color of the colonies (white, cream or colored, yellow, orange, brown, green, gray up to black), the size of the colonies (small, widespread or invasive) and growth (rapid or slow). It was carried out according to the method described by [13] by removing a filament using forceps then placed in a drop of heat-fixed methylene blue, placed on an object slide and covered with a coverslip then observed under an optical microscope with an X40 objective. The characteristics observed were the appearance of the mycelium (compartmentalized or not), the shape of the spores (oval, spherical, round, etc.), the shape of the conidial heads and the size of the conidiospores (short or long).

2.2.3. Conservation of orange

Supernatant production of bacterial biocontrol agents

A pre-culture of bacterial biopesticide is carried out by seeding 24-hour colonies in 25 mL of Yeast Extract Peptone Glucose (YPG) broth for 8 hours at 30 °C. The pre-culture was used to inoculate 4 broths of 200 mL YPG and incubated at 30 °C for 72 h. After 72 hours of incubation, centrifugation of the culture is carried out at 6000 rpm for 10 min at 4 °C [14]. The supernatant is then collected and stored at 4°C for conservation tests.

Inoculation of healthy orange with fungal and with the supernatant of the agents bacterial biocontrol

The healthy orange were disinfected with 70° ethanol and drained using paper household use, soaked in 70° ethanol. Then they were soaked in 100 ml of spores of pathogenic mold and allowed to dry at room temperature. Once dried, the orange inoculated with pathogenic mold spores were immersed in 100 ml of supernatant of biocontrol agents then preserved [15]. Witnesses consisted of orange inoculated with suspensions of spores of pathogenic strains without application of bacterial supernatant.

3. Results

3.1. Isolation and identification of orange spoilage fungi

The results of the analysis made it possible to show the involvement of fungi in the post-harvest rot of oranges. In total, 55 fungal isolates were identified. These identifications focused on the macroscopic and microscopic aspects of the said fungi. The results made it possible to highlight three (3) genera of fungi including the genera *Alternaria* sp, *Colletotrichum* sp and *Lasiodiplodia* sp. (Figures 2, 3 et 4).

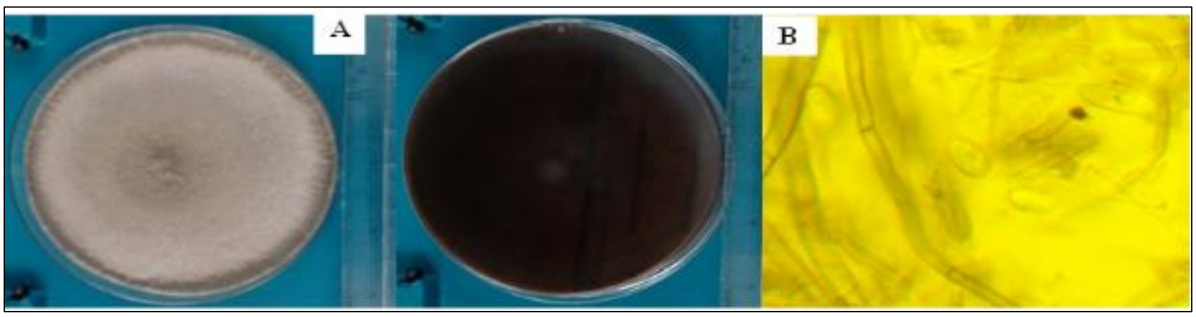

Figure 2 Macroscopic and microscopic aspects of *Colletotrichum* sp: (A- macroscopic appearance and B- microscopic appearance)

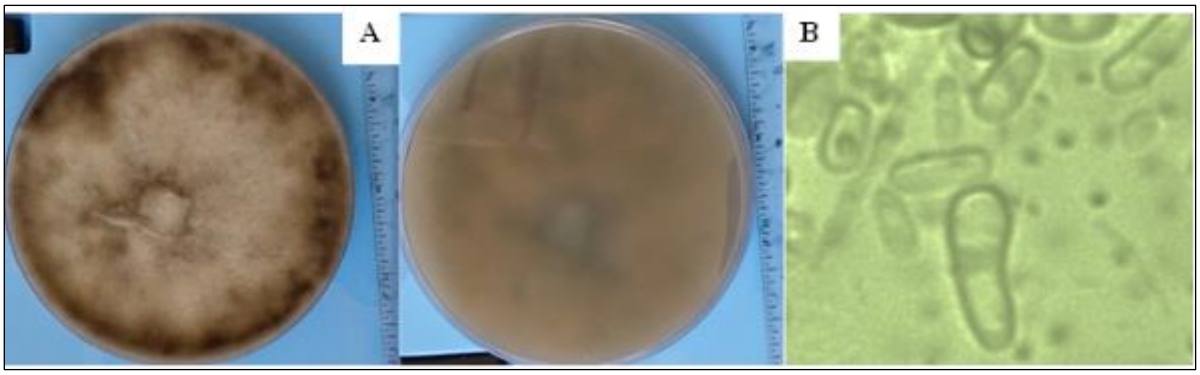

Figure 3 Macroscopic and microscopic aspects of *Alternaria* sp: (A- macroscopic appearance and B- microscopic appearance)

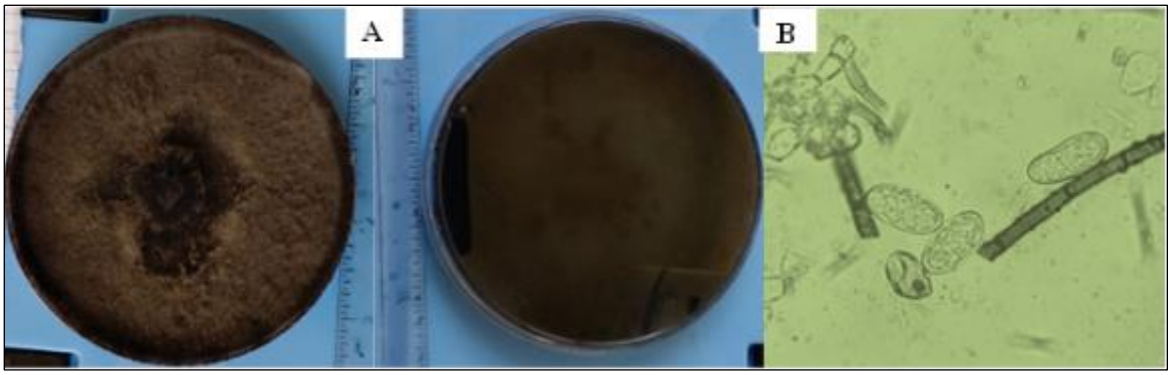

Figure 4 Macroscopic and microscopic aspects of *Lasiodiplodia* sp: (A- macroscopic appearance and B- microscopic appearance)

3.2. Frequency of Fungi isolation

In total, 55 fungal isolates were identified from spoiled orange samples. The 55 fungal isolates were grouped into three fungal genera, namely the genera *Lasiodiplodia*, *Alternaria* and *Colletotrichum*. However, the genus *Lasiodiplodia* presents a frequency of isolation superior to the two other genera which are *Alternaria* and *Colletotrichum*. These frequencies isolation are 56%, 33% and 11% respectively for the genera *Lasiodiplodia*, *Alternaria* and *Colletotrichum* (Table 1).

Table 1 Frequency of isolation of orange spoilage fungi

Isolated fungal genera	Number of isolates	Isolation frequency (%)
Lasiodiplodia	31	56 %
Alternaria	18	33 %
Colletotrichum	6	11 %
Total	55	100 %

3.3. Biocontrol test of orange with supernatants of bacterial biocontrol agents

The results obtained after conservation of the orange carried out with the supernatants of the bacterial biocontrol agents are presented in Figure 5. After 14 days of conservation, the oranges preserved with the bacterial supernatants did not present any major alteration inside and outside of the fruits (Figure 5 B, C, D et E). By against control oranges inoculated with fungus spores and not treated with supernatants, showed signs of deterioration from the 7th day of storage which were resulting in brown rot of oranges inside and out (Figure 5 A)

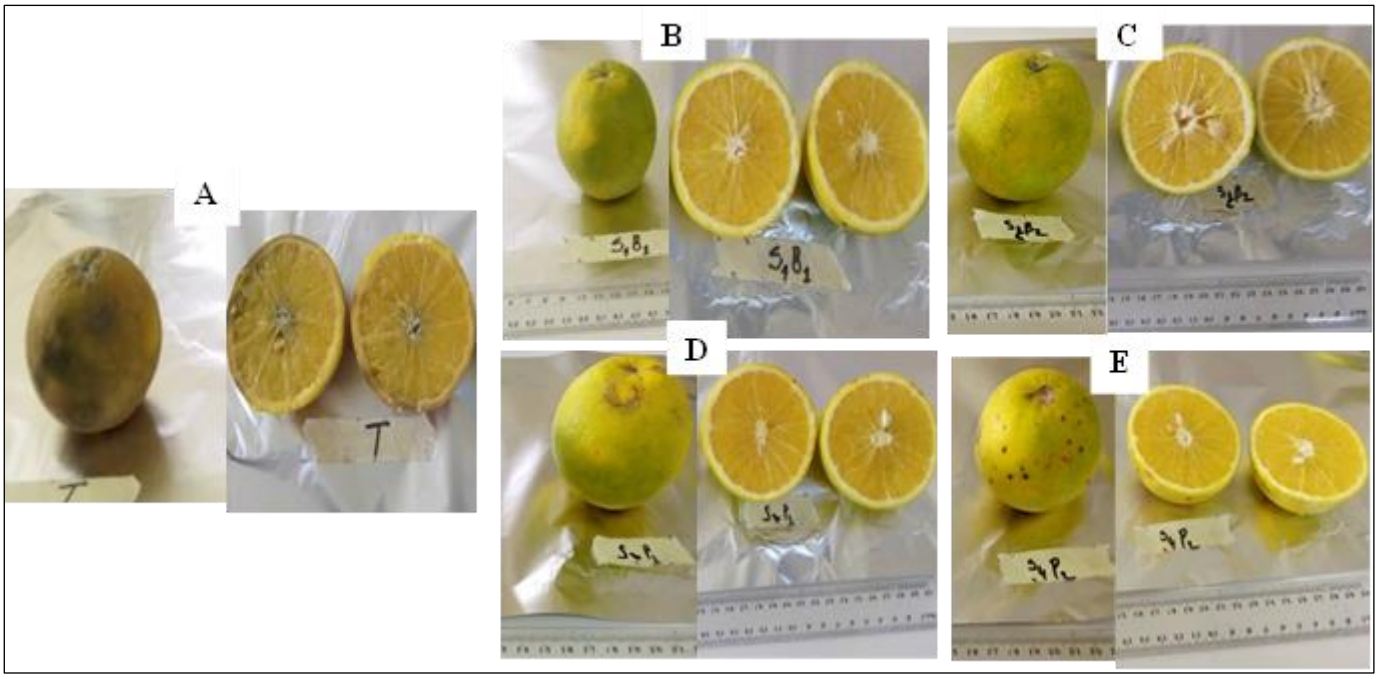

Figure 5 External and internal aspects of oranges preserved after 14 days

(A: Control orange; B: Orange with *Bacillus* sp 1; C: Orange with *Bacillus* sp 2, D: *Pseudomonas* sp1 and E: Orange with *Pseudomonas* sp 2)

4. Discussion

Oranges are fruits very vulnerable to microbial contamination since the phase of picking through its transformation through its conservation. In fact, the decline observed production would be due to competition from other markets and, mainly to the quality of orange which was greatly depreciated by the presence of diseases fungi including rot caused by *Phomopsiscitri*, *Lasiodiplodia natalensis*, *Colletotrichum* sp, *Alternaria* sp [16]. These fungal activities can also lead to mycotoxin contamination, and could represent a risk for the health of consumers [17]**.** Based on this analysis, it is therefore necessary to limit or even inhibit the action of these germs pathogens. Thus, the isolation and identification of pathogenic fungi involved in the alteration of oranges in Côte d'Ivoire made it possible to isolate three fungal genera. These three fungal genera are *Colletotricum*, *Alternaria* and *Lasiodiplodia* with respective isolation frequencies of 11%, 33% and 56%. The presence of these fungi could be explained by several environmental factors, such as aeration, pH, availability of water, nutrients and temperature that would favor their growth in agricultural products in conservation. The genus *Lasiodiplodia* was the majority in the spoiled orange fruit. These results are similar to the work of [18] who showed that the genus *Lasiodiplodia* was the most common with a frequency isolation rate of 83.33% in diseased citrus tissues. Also, [19] in their work highlighted the presence of these three fungal genera in rot citrus fruits and estimated that the genus *Lasiodiplodia* sp was the majority. Regarding the use of biocontrol agents, this study showed the capacity of these bacterial agents to inhibit the main fungal strains responsible for spoilage of orange. The preservation of oranges over a period of 14 days by the supernatant bacteria could be explained by the presence of substances produced by these bacteria during their metabolism in their respective growth environments. In fact, the presence of these substances would more or less limit the establishment of pathogenic germs on the skin of oranges. The production of lipopeptide molecules by these different bacteria including fengycin, surfactins and iturins produced by Bacillus sp promotes the bursting of the cell wall of fungi [20]. Also, in stationary phase of growth, *Pseudomonas fluorescens* CI produces several metabolites with high antifungal properties such as phenazines, pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol (2,4-DAPG) which inhibit the growth of pathogenic fungi [21]. These results are in agreement with several studies carried out on the biological control of orange pathogenic fungi such as that *Alternaria alternata* using biological control agents including bacterial and fungal. Also in *in vivo* conditions, use *Bacillus subtilis* at 3×10^{7} cfu/g and of *Bacillus megaterium* at 25×10^{6} cfu/g sprayed on the orange tree would decrease the incidence of rot [22]. According to [22], essays *in vitro* of *Trichoderma harzianum* and *Bacillus subtilis* showed an antagonistic action effect on a highly pathogenic isolate of *Alternaria citri*, with respective degrees of inhibition of 86.7% and 69.4%. At the same time, the work of [23] using *Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Paenibacillus polymxa, Pantoea agglomerans, Pseudomonas fluorescen*s and *Trichoderma harzianum* in the *in vitro* fight against *Alternaria alternata*

showed inhibition rates greater than 67%. Thus, [23] with the use of *Bacillus subtilis* GA1 in the post-harvest conservation of mangoes showed that this strain could inhibit the growth of fungal strains and bacterial pathogens of mangoes and to keep them for more than ten days.

5. Conclusion

The present study aimed to contribute to the fight against fungal alterations orange in Côte d'Ivoire using bacteria isolated from the rhizosphere of the cashew tree. The results of the microbiological analyzes obtained showed that orange are contaminated by fungi of the genus *Colletotrichum*, *Alternaria* and *Lasiodiplodia*. *Lasiodiplodia* was the most widely isolated fungus. The results concerning conservation tests of oranges with the supernatant of bacterial biocontrol agents showed good preservation of oranges in the presence of these bacteria over a period of 14 days.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] FAO. Joint FAO/WHO Food Standards Program. Codex Committee on Contaminants in Foods; seventh session, Moscow (Russia). 2013. 19 p.

[2] Tanaka K, Yamagishi N, Tanikaga R, Kaji A. Dianions of ethyl α-mercaptoacetate and ethyl α-mercapto propionate: a new method for an efficient synthesis of α,β-unsaturated esters from carbonyl compounds. Chemistry Letters. 1977; 6(5): 471 -474, https://doi.org/10.1246/cl.1977.471.

[3] Sarrou E, Chatzopoulou P, Dimassi-Theriou K, Therios I. Volatile Constituents and Antioxidant Activity of Peel, Flowers and Leaf Oils of *Citrus aurantium* L. Growing in Greece. Molecules. 2013; 18: 10639-10647; doi:10.3390/molecules180910639.

[4] USDA. Annual performance report. 2014. 64p.

[5] UNIDO. Meeting of Permanent Committee and Industrial Development Board. 1978; 5p.

[6] Haougui A. Mango tree dieback in Niger. Technical sheet. No. 615. Niger: ECA, 1 p.

[7] Tassadit D. Improvement in the conservation of 4th range mangoes by application of heat treatments and use of conservation under modified atmosphere. [Doctoral thesis]: University of Avignon and the countries of Vaucluse, Avignon (France). 2012; 149 pp.

[8] Koffi YF, Alloue-Boraud WAM, Ban KL, Dje KM (2017). Pineapple (*Ananas comosus*): alterations and means of control. International Journal of Innovation Sciences and Research. 2017; 6(2): 976-981.

[9] Alloue-Boraud M., Ban Koffi L., Dadie T., Dje K., Ongena M. Use of *Bacillus subtilis* GA1 for the fight against spoilage germs of mango in Ivory Coast. Journal of Animal & Plant Sciences. 2015; 25:3954-3956.

[10] Nguyen MT. Identification of mold species potentially producing mycotoxins in rice sold in five provinces in the central region of Vietnam, study of conditions that can reduce mycotoxin production. [Doctoral thesis], National Polytechnic Institute of Toulouse. 2007; 147p.

[11] Botton B, Breton A, Fevre M, Gauthier S, Guy PH, Larpent JP, Reymond P, Sarglier JJ, Vayssier Y, Veau P. Usefull moist and nusible: industrial importance. Paris. Milan. Barcelone: Masson. "Biotechnologies Collection (Paris)". 2nd edition. 1990. 52p.

[12] Fouzia L, Sara C. Isolation, identification and antibacterial activity of molds from a forest floor in Constantine. [Doctoral thesis], University of the Mentouri Constantine Brothers. 2015; 118p.

[13] Guiraud JP. Food microbiology. Dunod. Paris. 1998; 652p.

[14] Touré Y, Ongéna M, Jacques P, Guiro A, Thonart P. Role of lipopeptides produced by *Bacillus subtilis* GA1 in the reduction of grey mould disease caused by *Botrytis cinerea* on apple. Journal of Applied Microbiology. 2004; 96: 1151–1160.

[15] Gandomi H, Misaghi A, Basti AA, Bokaei S, Khosravi A, Abbasifa A, Javan AJ. Effect of *Zataria multiflora Boiss*. Essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. Food Chemical Toxicology. 2009; 47: 2397–2400.

[16] Azevedo FA, De Martelli IB, Polydoro DA, Pacheco C, Schinor EHA, Bastianel M. Positive relationship between citrus leaf miner and *Alternaria brown* spot. Ciência Rural. 2015; 45: 1160-1163.

[17] Koffi-Nevry R, Gohou G. Food hygiene and sustainable development, impact of the invisible (microscopic) world on poverty reduction. 2012; 20 p.

[18] Tedihou E, Kpemoua K, Tounou A. Dieback of mango and citrus trees in the central region of Togo and methods of control using fungicides. Journal of Applied Biosciences. 2017; 119: 11829-11838.

[19] Piccirillo G, Carrieri R, Polizzi G, Azzaro A, Lahoz E, Fernández-Ortuño D, Vitale A. In vitro and in vivo activity of QoI fungicides against *Colletotrichum gloeosporioides* causing fruit anthracnose in *Citrus sinensis*. Scientia Horticulturae. 2018; 236: 90-95.

[20] Ongena M. Molecular basis of biocontrol by *Bacillus* sp.: Lipopeptides playing in the game. Université de Liège – Gembloux Agro-Bio Tech – Unité de Bioindustries. 2010; 87 p.

[21] Haas D, Defago G. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology. 2005; 3(4): 307–319.

[22] Shehata A, Mohammed A, Mosa A, Ali M. Evaluation of some fungicides and biocontrol agents for controlling of alternaria rot on citrus fruits. Arab Universities Journal of Agricultural Sciences. 2018; 26: 691-699.

[23] Tekiner N, Kotan R, Tozlu E, Dadaşoğlu F. Determination of Some Biological Control Agents Against Alternaria Fruit Rot in Quince. Alinteri Journal of Agriculture Science. 2019; 34: 25-31.