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Fungi of post-harvest deterioration of carrot (*Daucus Carota* L.) and antifungal potential of essential oils of *Cymbopogon Citratus* and *Citrus Sinensis*

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Abstract

This work focused on the study of fungi associated with post-harvest carrot degradation **(***Daucus Carota L.***)** in the Dschang markets and the evaluation of the antifungal potential of essential oils **of** *Cymbopogon citratus* and *Citrus sinensis*. Samples were collected from Dschang markets and associated fungi were isolated and identified. The *in vivo* antifungal effect of *Cymbopogon citratus* (40 and 120 ppm) and *Citrus sinensis* (600 and 800 ppm) essential oils was evaluated on carrots inoculated with three fungal species. Thirteen fungal species, including *Botrytis cinerea* (23%), *Rhizoctonia carotae* **(**21%), *Cladosporium herbarium* (18%), *Verticillium albo atrum* (10%) and *Geotrichum candidum* (11%) were isolated from the decayed samples. *Botrytis cinerea*, *Rhizoctonia carotae*, *Cladosporium herbarium* and *Verticillium albo atrum* were pathogenic on carrot roots and lesions caused varied according to the fungal species from 8.83 cm^2 (*R. carotae*) to 5.48 cm^2 (*Botrytis cinereal*). *In vivo* results showed that the essential oils used significantly reduced the development of fungi on carrots compared to the control. Lesions developed on carrots inoculated with fungi and treated at different doses of *Cymbopogon citratus* and *Citrus sinensis* essential oils ranging from 1.1 to 1.77 cm^2 and 1.38 to 2.04 cm^2 respectively, were significantly lower than those developed on untreated control carrots (7 to 9 cm^2). *C. citratus* essential oil was found to be more effective than *Citrus sinensis*. Based on these results, the use of these essential oils can constitute a potential or suitable alternative to synthetic fungicides to fight against post-harvest fungi of carrots.

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Introduction

The carrot (*Daucus carota* L.) of the Apiaceae family (formerly umbellifers), is a vegetable that was first domesticated in Central Asia in the 10th century. Carrots were first used for medical purposes and gradually as food (Essing, 2013; Carlos and Dias 2014). This vegetable is an important source of bioactive compounds with beneficial effects on consumer health. They are consumed in different ways; they can be eaten raw or cooked. It is recognized as an important source of natural antioxidants, contains β-carotene, a precursor of vitamin A with anticancer activity (Speizer *et al.*, 1999). Carrots are a good source of carbohydrates and minerals like Ca, P, Fe, Na, Cu, Zn and Mg (Arscot and Tanumihardio 2010). They are also a good source of thiamine, riboflavin and vitamin C (Arscot and Tanumihardio 2010; Sharma *et al.*, 2012).

Each year, 35.5 million tons are produced, with 17 million tons per year coming from China, which is the largest producer of carrots, followed by Russia (1.6Mt) and the United States (1.3Mt). It is grown all over the world and is the third most consumed vegetable after potatoes and tomatoes (FAO, 2014). Comparatively, the yield of carrot in Cameroon is very low. Despite the very low yield produced, Cameroon exports carrots to Gabon and Equatorial Guinea (AGRI-STAT, 2012). The city of Dschang, with its abundance of marshy land, allows a strong development of market gardening. Indeed, more than ten hectares are cultivated on swampy land with a high production of vegetables such as black nightshade, tomatoes, carrots and green beans (Ntagmo *et al.*, 2012).

The most important factors that adversely affect the economic value of fruit and vegetables is the low shelf life, leading to many problems, most notably the pathogenic infection (Zhu, 2006). Fruit and vegetables are exposed to microbial infection when in contact with soil, water and dust during growth in the field or during harvesting, postharvest handling, processing, and distribution. They therefore carry a large amount of microorganisms included human and plant pathogens (Eni *et al.* 2010). Due to the rate of degradation of carrots, a large part of the crops is often lost during storage (Kouame *et al.*, 2016), due to abiotic (storage temperature and humidity) and biotic factors, (fungal attacks). The first report of fungi pathogens that affect the carrot due to poor storage conditions were *Botrytis cinerea*, *Pythium spp*, *Rhizoctonia carotae* and *Sclerotinia sclerotiorum* (Mercier *et al.* 1993; Gilbert, 2001; Allain-Boulé *et al.*, 2004; Arul *et al.*, 2010; Arikpo *et al.*, 2013). High percentage of carrots are lost annually to post-harvest degradation caused by fungal pathogens (Mahale *et al.*, 2008). The possibility of transmission of field fungus to the store through the contamination of containers with spores as well as the mismanagement after the harvesting process in terms of transport, storage and marketing can lead to the spread of a large number of fungi (Bukar *et al.* 2009). Few post-harvest technologies exist for the conservation of carrots, and when they do exist, they are too complex or inaccessible to farmers (Berinyuy, 2004). This is the case of UV light (Arul *et al.*, 2010), cold conservation of carrots (Le Cam *et al.* 1992) and the use of expensive chemicals with potential toxic effects. The use of biodegradable biopesticides from local plants that are of natural origin, would be appropriate and attractive to manage this problem of post-harvest carrot deterioration. Thus, the overall aims of this study is to contribute to the reduction of post-harvest carrot losses due to fungi through the use of essential oils of *Cymbopogon citratus* and *Citrus sinensis*. The study highlights the fungi associated with the carrot post-harvest deterioration and establishes the pathogenicity of isolated fungal species. It also evaluates the antifungal potential of essential oils of *Cymbopogon citratus* and *Citrus sinensis* against the fungal development on healthy inoculated carrots.

Materials and methods

Collection of carrot samples

Carrot samples of New Kuroda variety collected from farmers as well as retailers in Dschang markets were surveyed. Infected and uninfected carrot roots were collected separately in sterilized plastic containers,

then brought to the Phytopathology Laboratory of the University of Dschang. A physical examination by visual observation of carrot roots helped to determine which of the carrots to be considered in the sample. In the Laboratory, infected carrots were used for isolation of fungi and uninfected carrots were used for pathogenicity and *in vivo* tests.

Plant material collection and extraction

Leaves and stems of *Cymbopogon citratus* and fresh epicarp of *Citrus sinensis* were collected in February 2019 from the locality of Dschang and its environs. Their identification was confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang. Plant material collected was used for the hydrodistillation of the essential oils. Plant parts collected were washed three times under running tap water and rinsed with sterile distilled water. They were separately air-dried at room temperature for five days *Cymbopogon citratus* and two days (epicarp of *Citrus sinensis*) respectively before extraction. Extraction of essential oils was done by hydrodistillation process for about 5 hours, using a Clevenger apparatus. Oils recovered in a dark sterile glass were dried over anhydrous sodium sulfate and stored at +4 °C until used (Barnett and Hunter, 1998).

Isolation and Identification

The infected carrot roots collected were first surface sterilized by washing under running tap water and fragments of about 2 mm^2 were collected using a sterile scalpel blade from the lesions. These fragments were disinfected for 1 minute with 1% sodium hypochlorite solution, rinsed three times successively with sterile distilled water, and then dried by removing excess water by squeezing out with blotting paper. Petri dishes containing the PDA medium supplemented with chloramphenicol (250 mg/L), were aseptically inoculated with 5 fragments, and incubated at 27 °C ± 2. Three replicates were prepared for each sample. Observations were made daily and the visible fungal colonies around the fragments were separately transferred aseptically into fresh plates containing the medium used. After several replications, pure cultures were obtained and their identification was made with reference to standard textbooks such as (Barnett and Hunter, 1998).

Pathogenicity test

Fresh and healthy carrot roots were washed under tap water and superficially sterilized with 0.1% sodium hypochlorite solution for two (02) minutes followed by washing until the odor disappear.

The inoculation method by wounding carrot roots was used. Different fungal isolates were inoculated into wounds and closed with sterile cotton to prevent spores from spreading out. Control was used without fungal isolates (Fig.1). Treated and untreated tubers were placed separately in sterile plastic bags and incubated at 25 ± 2 °C for 10 days.

The extent of the lesions (following the bigger axis) and the size of the lesions (following the smallest axis), were measured using a graduated ruler following two perpendicular axes (Shuman, 2001). In order to determine the surface area of the lesions, assuming that the lesions grow in a circular manner their diameter (DL) was assessed as follows:

$$DL\ (cm2) = (Length\ of\ lesion - width\ of\ lesion)/2$$

In vivo antifungal activity of Essential Oils

Preparing the inoculum

The inoculum was prepared from the pure cultures of *Rhizoctonia carotae*, *Cladosporium herbarium* and *Verticillium albo atrum*, aged 7 days. The surface of the colony was flooded by 10 ml of sterile distilled water and then detached from the culture medium by lightly scraping using a “Pasteur pipette”.

The mycelial suspension was then filtered through 4 layers of muslin, to remove debris from the mycelium and culture medium and obtain a conidial suspension. After agitation of the spore suspension, the quantification was done on culture medium and then expressed as colony forming units (CFU/mL) (Addar *et al.* 2016).

Inoculation and treatment of carrots with essential oils

Fresh and healthy carrot roots were washed under tap running water and sterilized superficially with 0.1% sodium hypochlorite solution during two (02) minutes. After drying, the carrot tubers were wounded and inoculated with 5µl of conidial suspensions containing 104.10^6 CFU/mL, and then left for 30 minutes at laboratory room temperature. They subsequently received 40 ppm and 120 ppm concentrations of *C. citratus* essential oil and 600 ppm and 800 ppm of *C. sinensis* essential oil at the inoculated areas respectively (Chuku *et al.*, 2010). Areas inoculated and treated with essential oils were closed with sterile cotton. Control was used without essential oil treatment. Treated and untreated samples were placed separately in sterile plastic bags and incubated at 25 ± 2 °C for 10 days. The experiment was repeated 3 times. After 10 days of incubation, the surface of the lesions that developed on the tested carrot roots was measured using millimeter paper.

Statistical analysis

Data obtained for fungal lesions were submitted to analysis of variance (ANOVA). Means were separated by the Duncan's test at 5% probability threshold through the SPSS (Statistical Package for Social Science) software version 26.0.

Results and discussion

Isolated fungi

Isolation and identification revealed 13 fungal species from carrot samples. Among these fungi, the most common were: *Botrytis cinerea* (23%), *Rhizoctonia carotae* (21%), *Cladosporium herbarium* (18%), *Verticillium albo atrum* (10%) and *Geotrichum candidum* (11%). Other fungi belonging to the gener *Penicillium, Colletotrichum* and *Rhizopus* were isolated. *B. cinerea* (Fig. 2), was the major post-harvest pathogen of carrots, in relation to its wide range of hosts (virtually all vegetables and ornamental plants) and its extreme survival conditions in dying tissues and opening wounds (Droby and Lichter, 2007). Based on the work of (Moayad, 2018) in Baghdad, *Geotrichum candidum* (Fig. 3) and *Rhizoctonia carotae* (Fig. 4) which showed crater rot were isolated; their frequencies were 20% and 16.7% respectively.

These results largely coincide with the results of researchers interested in isolating fungi from the carrot in India (Khatoon *et al.*, 2016), in Nigeria (Ewekeye *et al.* 2013; Adebayo *et al.*, 2012), in Turkey (Kurt *et al.*, 2005) and Korea (Aktaruzzaman *et al.* 2014), who reported the presence of these fungi and established their involvement in the deterioration of carrots in post-harvest.

Table 1. Surface of fungal lesions on treated and untreated carrot roots.

Concentration (ppm)	Surface lesions (cm^2)		
	C. herbarium	*R. carotae*	*V. albo-atrum*
	Cymbopogon citratus		
0 (T)-	8.49±0.53[a]	8.83 ±0.21[a]	7.65±0.56[a]
40	3.35±0.52[b]	3.58 ±0.43[b]	3.22 ±0.51[b]
120	1.77±0.35[c]	1.67 ±0.13[c]	1.16±0.44[c]
	Citrus sinensis		
0 (T)-	8.49±0.53[a]	8.83 ±0.21[a]	7.65±0.56[a]
600	3.32±0.71[b]	3.87 ±0.67[b]	2.40±1.35[b]
800	1.90±0.86[c]	2.04±1.21[c]	1.38±0.11[c]

Means followed by the same alphabetical letter in the same column are not significantly different according to Duncan's test at P ≤ 0.05.

Pathogenicity test

The isolates of *Botrytis cinerea*, *Rhizoctonia carotae*, *Cladosporium herbarium* and *Verticillium albo atrum* were used for this test for an incubation period of 10 days. All the isolates were pathogenic on carrot roots (Fig. 5). Lesions caused by these fungi vary

according to the fungal species. *R. carotae* (8.83 cm²) and *C. herbarium* (8.49 cm²) were more aggressive, followed by *Verticillium albo atrum* (7.65 cm²) and *Botrytis cinerea* (5.48 cm²) compared to the control which did not show any symptoms.

These results are consistent with those of other researchers who have addressed this pathogenic aspect of fungi. (Aktaruzzaman *et al.*, 2014) showed that the postharvest gray mold rot of carrot was caused by *Botrytis cinerea*. It has been shown that *Geotrichum candidum* was responsible for post-harvest rotting of oriental melon, tomato, cucumber, potato, pumpkin and carrot (Yong-Ki *et al.*, 2011). Similarly, *Rhizoctonia carotae* was proven by (Kurt *et al.*, 2005) as responsible for the rot of carrots.

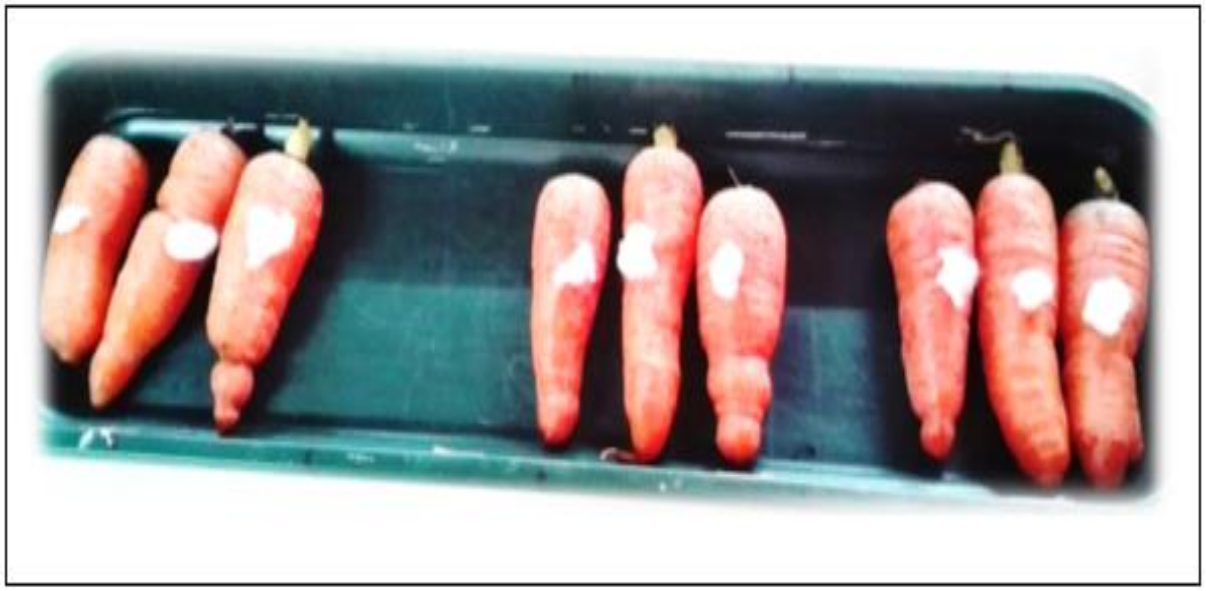

Fig. 1. Wounded carrot tubers inoculated and covered with sterile cotton.

This contamination of carrots by fungi is likely favored by their high levels of carbohydrates (sugars) and carotene (pre-vitamin A) and their high moisture content which encourages bacterial and fungal problems.

In vivo effect of essential oils of C. citratus and C. cinensis on the development of fungal species After 10 days of incubation, control treatments (without essential oils) had lesions with surface area ranging from 7 to 9 cm² (Table 1).

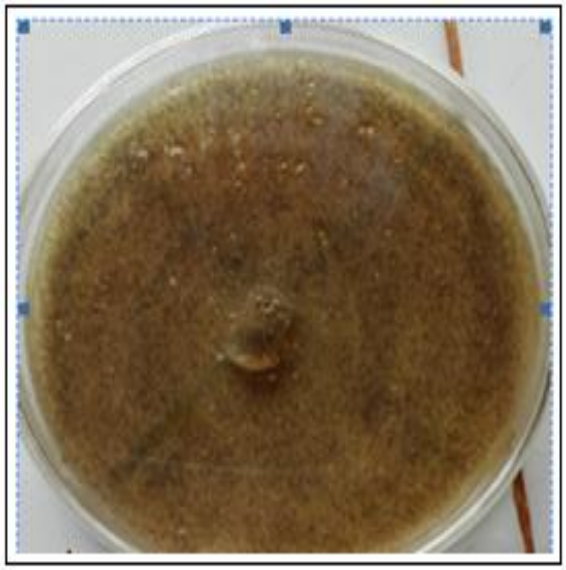

Fig. 2. *Botritys cinerea* X40.

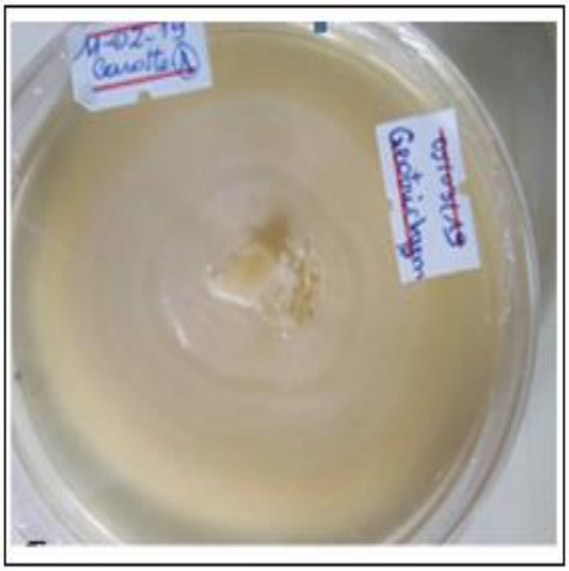

Fig. 3. *Geotrichum candidum* X40.

Lesions developed on carrots inoculated and treated at different doses of *Cymbopogon citratus* and *Citrus sinensis* essential oils were significantly lower than those developed on untreated control carrots (Fig.6). Carrots treated with *Cymbopogon citratus* essential oil (120 ppm) had lesion diameters ranging from 1.1 to 1.77 cm².

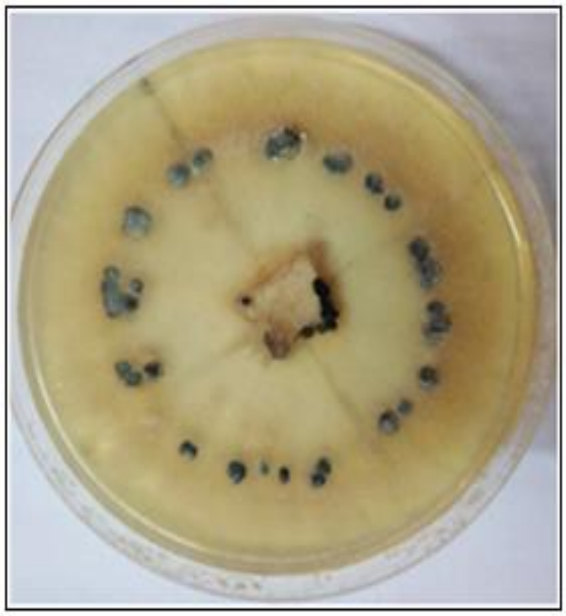

Fig. 4. *Rhizoctonia carotae* X40.

Those treated with *C. sinensis* essential oil (800 ppm) had lowest-diameter lesions ranging from 1.38 (*V. albo-atrum*) to 2.04 cm² (*R. carotae*). *In vivo* results showed that the essential oils used significantly reduced the development of fungi on carrots compared to the control. However, *C. citratus* essential oil was found to be more effective than *Citrus sinensis*. Nikos and Costas (2007) have shown that *Cymbopogon citratus* L. oil (ranging between 25 and 500 ppm) resulted in significant ($P < 0.05$) reduction on subsequent colonies development of *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *Aspergillus niger*.

In the highest oil concentration employed, fungal sporulation was completely retarded. Lemon grass oil reduced spore germination and germ tube length in *C. coccodes*, *B. cinerea*, *C. herbarum* and *R. stolonifera,* with the effect depend on oil concentration. According to a study by *Vitoratos et al. 2013*, lemon essential oils were reported to be significantly effective against the fungus *Botrytis cinerea* in tomatoes, strawberries and cucumbers.

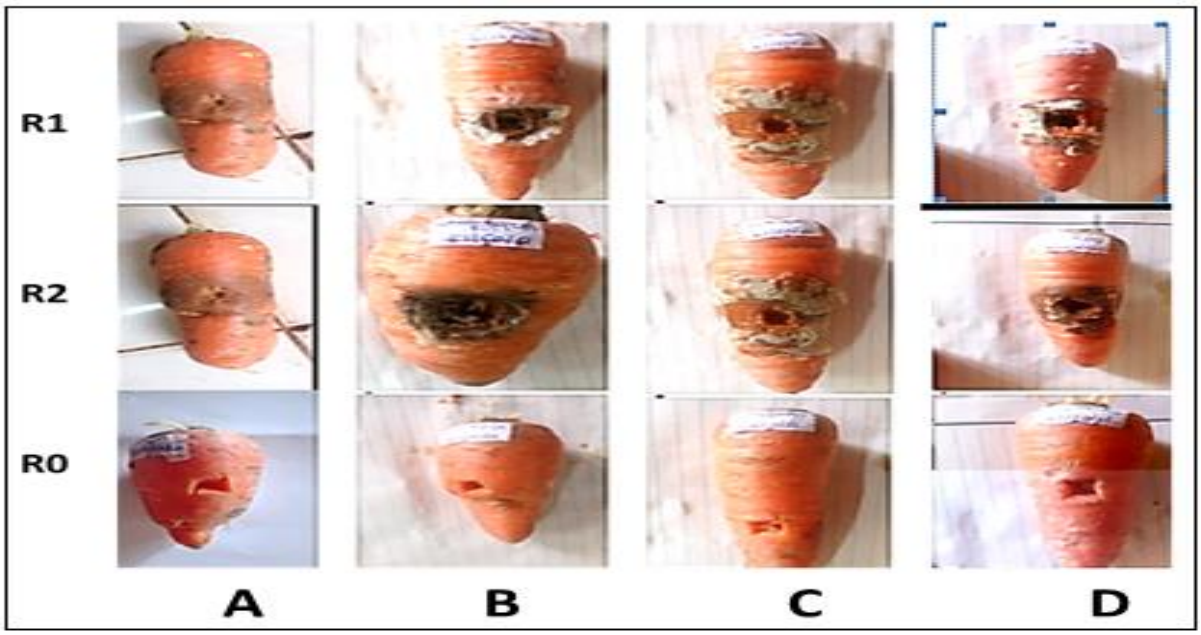

Fig. 5. Carrots infected with different isolates. A: *B. cinerea;* B: *V. albo-atrum* C: *C. herbarium;* D: *R. carotae* R1 and R2: Repeat 1 and 2; R0: Control.

The hydrodistilled essential oils of six different varieties of *C. sinensis* showed antifungal efficacy against some fungi among which *P. digitatum* (ED50 2389.9–1004.6 ppm) and *P. italicum* (ED50 5407.5–3142.2 ppm). Essential oil from peels obtained by cold-pressing method showed activity against *Mucor*

hiemalis, P. expansum and *F. proliferatum* having inhibition of 36.5%, 34.9% and 59.5% using the agar dilution technique (Van-Hung *et al*., 2013). Inhibition of fungal development can be justified by the fact that the essential oils used probably contain active compounds that could inhibit the growth of fungi or possibly help stimulate the carrot defense system. Concerning the compounds of these essential oils, terpenoids, especially sesquiterpenes and monoterpenes, have been considered to be the most possible antifungal candidates in *Citrus* essential oils (Caccioni and Guizzardi, *1994; Caccioni et al. 1998)*. Caccioni and Guizzardi (1994) found that citrus oxygenated monoterpenes exhibited the highest antifungal activity among examined compounds. Caccioni *et al.* (1998) reported that there was a positive correlation between the monoterpene content of the essential oils from various citrus species and the inhibition of pathogen fungi. More recently, an increasing body of research has found that the terpenoids in essential oils, including citral, geranial, eugenol, γ-terpinene, p-cymene, mircene, thymol and carvacrol, have a wide spectrum of fungitoxicity *(Souza et al., 2005; Vogt et al., 2013; Mesa-Arango et al., 2009 and Dharmawan et al., 2008)*.

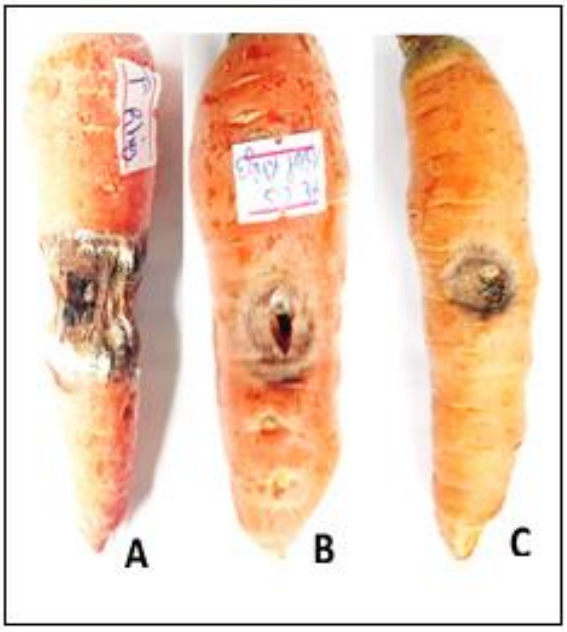

Fig. 6. A- Carrots inoculated with *R. carotae* and not treated with essential oil. B and C- Carrots inoculated with *R. carotae* and treated with essential oil of *C. sinensis*.

Among these compounds, citral and limonene have been suggested as the most significant antifungal candidates of *Citrus* essential oils. Citral is an acyclic α, β -unsaturated monoterpene aldehyde with two isomers, geranial and neral. The antifungal activity of citral has been demonstrated by several authors, either in Citrus or other plant essential oils (*Onawunmi, 1989; Caccioni and Deans, 1993)*. Souza *et al. (2005) also* determined the antifungal activity of different phytochemicals from lemon essential oils, and found that citral, eugenol and myrcene were fungitoxic. Overall, citral showed the best fungitoxicity. Wuryatmo *et al.* (2003) examined the inhibitory effects of vapor citral, its isomers geranial and neral, and related compounds (such as *R*-citronellal, *S*-citronellol, and Rcitronellic acid) on *P. digitatum, P. italicum, and Geotrichum candidum,* the major fungi responsible for post-harvest spoilage.

Conclusion

This study revealed that thirteen types of fungi were found to cause carrot root rot during the storage or transport and marketing operations in the city of Dschang. These pathogens lead to tremendous loss not only in terms of quantity but also reduce their economical and nutritional value. Some of these fungi are able to produce mycotoxins which constitute a health hazards for consumers. *In vivo* antifungal tests against the fungal development on carrots, revealed that the essential oils of *Cymbopogon citratus* and *Citrus sinensis* have very remarkable antifungal properties. Furthermore, their fungitoxicity may be attributed to the chemical compositions, molecular structure and concentrations of the bioactive compounds. However, *Cymbopogon citratus* essential oil was more active in this study. From the findings of this study, it is suggested the application of essential oils can reduce environmental pollution and constitute a suitable alternative to synthetic fungicides to fight against post-harvest fungi of carrots.

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