

Research Article

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Antifungal potential of *Eucalyptus saligna* and *Cupressus lusitanica* extracts against post-harvest fungi of green bean (*Phaseolus vulgaris* L.) pods decay in Dschang Western highland of Cameroon

Potentiel antifongique des extraits d'*Eucalyptus saligna* et *Cupressus lusitanica* contre les champignons responsables de la détérioration du haricot vert en post-récolte à Dschang, Ouest Cameroun

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ABSTRACT

This study aimed to evaluate the biodiversity of post-harvest fungi responsible of green bean pods decay and the antifungal potential of *Eucalyptus saligna* and *Cupressus lusitanica* extracts. Plant extracts were applied at different concentrations *in vitro* and on detached green bean pods simultaneously with pathogen inoculation. Infected and uninfected green bean pod samples were collected of Dschang locality. Fragments of green bean pods (including the symptomatic and healthy portions) were removed and inoculated on PDA media. After 7 days of incubation at $22 \pm 2^\circ\text{C}$, pure isolated fungi were identified according to the recommended references. The involvement of isolated fungi on post-harvest degradation of the quality of green bean pods was evaluated on uninfected green bean. *Colletotrichum lindemuthianum* and *Fusarium sp* were isolated on green bean pods and their involvement in quality degradation of the green bean at post-harvest was confirmed through lesions observed on inoculated green bean pods. Antifungal results showed that aqueous extracts of *E. saligna* completely inhibited the *in vitro* growth of *C. lindemuthianum* and *Fusarium sp* at the concentrations of 30 mg/ml while the ethanol extracts completely inhibited their growth at 8 mg/ml. Ethanol extract of *Cupressus lusitanica* completely inhibited the *in vitro* growth of the two fungi at 8 mg/ml. On detached green bean pods, aqueous extract of *E. saligna* completely reduced development of *C. lindemuthianum*. From these results, there is a possibility of using these extracts as an alternative control against post-harvest green bean decay.

Keywords: Green bean, Post-harvest fungi, *Eucalyptus saligna*, *Cupressus lusitanica*, Antifungal effect

RESUME

Cette étude avait pour but de caractériser les espèces fongiques responsables de la détérioration post-récolte du haricot vert et d'évaluer le potentiel antifongique des extraits d'*Eucalyptus saligna* et de *Cupressus lusitanica*. L'effet antifongique des extraits a été évalué *in vitro* à différentes concentrations et *in vivo* sur des gousses de haricot vert détachées, inoculées avec *Colletotrichum lindemuthianum* et traitées avec l'extrait aqueux d'*E. saligna* (40 mg/ml). A cet effet, des échantillons de haricot vert infectés et non infectés ont été collectés dans la localité de Dschang. Des fragments de haricot vert ont été prélevés et inoculés sur le milieu PDA et l'ensemble incubé au

laboratoire ($22 \pm 2^\circ\text{C}$) pendant 7 jours. Les champignons isolés ont été identifiés selon les guides de référence. La pathogénicité des champignons isolés a été évaluée sur des gousses de haricot vert non infectées. *Colletotrichum lindemuthianum* et *Fusarium sp* ont été majoritairement isolés sur des gousses de haricot vert et leur implication dans la dégradation de la qualité du haricot vert en post-récolte a été confirmée à travers des lésions observées sur des gousses de haricot vert inoculées. Les résultats antifongiques ont montré que les extraits aqueux de *E. saligna* ont complètement inhibé la croissance *in vitro* de *C. lindemuthianum* et de *Fusarium sp* à 30 mg/ml alors que les extraits éthanoliques ont inhibé complètement leur croissance à 8 mg/ml. L'extrait éthanolique de *Cupressus lusitanica* a complètement inhibé la croissance *in vitro* des deux champignons à 8 mg/ml. Sur les gousses de haricots vert détachées, l'extrait aqueux de *E. saligna* a complètement réduit le développement de *C. lindemuthianum* (après 10 jours d'incubation). À partir de ces résultats, il est possible d'utiliser ces extraits comme une alternative de lutte contre les champignons responsables de la détérioration post-récolte du haricot vert.

Mots clés : Haricot vert, Champignons post-récolte, *Eucalyptus saligna*, *Cupressus lusitanica*, Activité antifongique

1. Introduction

Green bean (*Phaseolus vulgaris* L.) pods are vegetables consumed fresh or processed. It is considered a highly perishable vegetable because it is harvested immature and has a high moisture content (El-Mogy and Kitinoja 2019). In 2016, the world's harvested area of green beans was 155.72 million ha producing 235.96 million metric tons (El-Mogy and Kitinoja 2019). Green bean is a good source of important nutrients and bioactive compounds. It contains proteins, carbohydrates, fibre, vitamins A and C, zinc and iron (Fabbri and Crosby 2016 ; Graham and Ranalli 1997 ; Santalla et al. 1999). The world export and import values were US\$ 789.23 million and US\$ 986.26 million, respectively. The Western highlands agro-ecological zone of Cameroon that covers the North West and West Regions is of high importance in the production of green beans. The Noun, Menoua and Mifi divisions of the West Region are greatly involved in the production of this beans (Christensen et al. 2007). Green beans faces a lot of problems at postharvest or during storage. In fact the most important constraints are biotic factors (fungi, viruses and bacteria) that cause diseases to plants and during harvest (Prachi et al. 2017). Seed-borne pathogenic fungi that cause losses of yield and quality of green bean worldwide include, *Macrophomina phaseolina* (Tassi) Goid., *Fusarium spp*, *Colletotrichum lindemuthianum* and *Rhizoctonia solani* Kühn (Schwartz et al. 2005 ; Naseri and Mousavi 2008). Anthracnose is a devastating seed borne disease of green bean which attacks leaves, stems and reduces the aesthetic value of bean pods at postharvest or in the market (Rava et al. 1993; Chamma Davide et al. 2009). Gray mould (*Botrytis cinerea*), white mould (*Sclerotinia sclerotiorum*), Rhizopus rot (*Rhizopus stolonifer*), and Pythium leak (*Pythium aphanidermatum* and *P. ultimum*) are the most common decays caused by postharvest fungal infections of green bean pods (El-Mogy and Kitinoja 2019). Most lead to decay during storage. Gray mould is considered as one of the most important fungal pathogens causing severe postharvest losses in vegetables (Aktaruzzaman et

al. 2017). Infection by *B. cinerea* often cannot be observed at harvest but symptoms develop quickly under moist conditions during refrigerated storage and transport, even at 0°C (Romanazzi et al. 2016). To fight against these fungi, many control measures have been used like the application of chemical fungicide (mostly used). Some work carried out elsewhere has reported postharvest treatment of green bean with potassium silicate, potassium thiosulfate, and potassium sulphate (El-Garhy et al. 2016). As a direct consumer food, the application of chemical fungicides on green bean pods to limit pathogen growth can be toxic to humans due to chemical residues. It seems very opportune to associate the use of plant extracts to this control measure as an alternative method. Previous studies showed the antifungal effects of plant extracts and some of them showed their *in vivo* effect by reducing the banana and avocado fruit rot caused by fungi (Yaouba et al. 2017). However, research on the evaluation of the *in vivo* effect of plant extracts against certain fungi responsible for the decay of the green bean in post-harvest especially in western Cameroon remains very limited. This work proposes to provide information concerning the fungal contamination of green bean in post-harvest at Dschang and assess the antifungal potential of plant extracts against the development of certain fungi on green bean pods in post-harvest.

2. Materials and Methods

2.1. Study area

Dschang is located in the agro-ecological zone of the Western Highlands at $5^\circ 26.617$ North latitude, and $10^\circ 04.814$ East longitude. The climate is Equatorial type with an average elevation of 1400 m. This climate is characterized by two seasons: a rainy season that lasts from mid-March to mid-November and a dry season that runs from mid-November to mid-March. The annual rainfall is around 1959 mm. The monthly temperatures are between 22°C and 36°C with an average of 28°C . Relative humidity is constantly high; the annual average is 83% (Hikersbay 2018).

2.2. Samples collection

Green bean pods presenting visible disease symptoms were collected in the Dschang main market (Market B), and transported in a clean white plastic paper to the Research Unit of Phytopathology and Agricultural Zoology (RU_PHYAZ), University of Dschang, Cameroon. This was done at February and March 2018.

2.3. Plant materials and extraction

Fresh leaves of *Eucalyptus saligna* and *Cupressus lusitanica*, selected for their antifungal efficacy as proven by previous studies and their easy availability (Yemo et al., 2017 ; Yaouba et al., 2015), were collected in march 2018 from Dschang locality. Their identification were confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang. Plant parts collected were washed three times with running tap water and rinsed with distilled sterile water. They were separately air-dried at room temperature (Plant pathology laboratory) and ground in a mortar. One hundred grams of the resulted dried powder were macerated in 500 ml of distilled water or ethanol and mixed thoroughly. For aqueous extract the mixture was allowed to rest in maceration for 48 hours and the supernatant passed through whatman's N° 1 filter paper to obtain the extract. Concerning ethanolic extract, after maceration for 8 hours in a warring blender (Warring International, New Hartford, CT, USA), the macerate was passed through Whatman's N° 1 filter paper and evaporated using a Rota vapour at 40°C water bath temperature (Heidolph). Extracts were preserved aseptically in a brown bottle at 4°C until further use (Souza et al. 1995).

2.3. Isolation, Identification and Characterization of fungal pathogens associated with green bean pods.

The collected green bean pods were washed with running tap water and fragments of about 5 mm in diameter (including the symptomatic and healthy portions) were removed. These pods fragments were surface disinfected in 3% of sodium hypochlorite solution for 5 minutes. After drying with sterile paper towel, they were plated onto chloramphenicol amended Potato Dextrose Agar (PDA). After incubation at 22 ± 2°C, daily observations were carried out and fungal growth was assessed microscopically. Cultures of the isolates were transferred to a new culture medium plated on Petri dishes, from where pure cultures were obtained. Identification of the isolates was based on morphological characteristics, described in the 1998 illustrated genera of fungi by Barnett and Hunter (1998) and with literature on the identification of pathogenic fungi by Dugan (2006).

2.4. Pathogenicity test of isolated fungi

This test was done to confirm the identity of the fungi isolated from diseased green bean pods.

Disease-free green bean pods disinfected as mentioned above were used for this part of the work. The method of inoculation by wound of bean pods was used according to Rivera-Vargas et al. (2006). The inoculated green bean pods with pure culture of *C. lindemuthianum* and *Fusarium sp* were kept in laboratory conditions (22 ± 2° C) for seven days. Data were collected on the lesions developed by the fungus and the identity of fungi checked. After the incubation period, the symptoms obtained were compared to those from which these fungi were isolated (Úrbez-Torres and Gubler 2009a).

2.5. In vitro antifungal activity of plant extracts

The antifungal effect of plant extracts were evaluated on *Colletotrichum lindemuthianum* and *Fusarium sp* isolated from green bean pods. The in vitro antifungal activity was assessed according to the agar dilution method (Sharma and Trivedi, 2002 ; Yaouba et al. 2017) on PDA (DIFCO). Plant extracts were dissolved in dimethylsulphoxide (DMSO) and diluted to give serial dilutions that were incorporated into growth media. Concentrations of 2 ; 4 ; 6 and 8 mg/ml for ethanol extracts and 10; 20 and 30 mg/ml for aqueous extracts were used. PDA medium supplemented with different concentrations of the extracts were inoculated with 6-mm diameter (plugs) of the test fungus cut from the margin of 5-day-old cultures. The plates were incubated in duplicates over a period of 10 days for *Colletotrichum lindemuthianum* and *Fusarium sp* at 22 ± 2°C. The radial mycelia growth was measured daily and the fungi toxicity was expressed as percentage inhibition of radial mycelia growth (Dohou et al. 2004) using de following formula:

$$PI = \frac{(DT-D)}{DT} \times 100$$

Where: DT and D, are the radial mycelia growth measurements in the control and treatment plates respectively.

2.6. In vivo effect of aqueous extracts of *E. saligna*

The method of wound inoculation of bean pods was used according to Rivera-Vargas et al. (2006). Green bean pods inoculated with pure culture (spore suspension) of *C. lindemuthianum* were left for 30 minutes on the slap. Subsequently, 3 drops of the aqueous extract of *E. saligna* (40 mg/ml) were deposited on the inoculated portions. The whole was stored in laboratory conditions (22 ± 2 °c) for seven days. The control without treatment with the plant extract was carried out and stored under the same conditions. The observations revealed whether lesions developed following fungal infection or not.

2.7. Statistical analysis

Data obtained on percentage (%) inhibition were subjected to analysis of variance (ANOVA) and averages were separated using Duncan's multiple comparison test at 5% probability level using the

software SPSS (Statistical Package for Social Science) version 21.0

3. Results

3.1. Isolated fungi and effect on green bean pods

Colletotrichum lindemuthianum and *Fusarium* sp were isolated on green bean pods that were collected from the main market of Dschang (Fig 1 and Fig 2). The pathogenicity test of these two fungi confirmed their involvement in the degradation of the quality of the green bean in post-harvest. Lesions were observed on the inoculated green bean pods (Fig 3 and Fig 4). *C. lindemuthianum* is known as a seed

borne disease of common bean plant (*Phaseolus vulgaris* L.) and it is the pathogen responsible for beans anthracnose at post-harvest (Mota et al. 2016). Whereas *Fusarium* spp is responsible for wilt incidence in common bean plant which was basically observed on the pods at post-harvest (El-Mougy, 2001). *C. lindemuthianum* was proven by many researchers to be responsible for common beans pod anthracnose (Padder et al. 2010), so was considered to be the causal agent of anthracnose. It is a seed borne disease which is the main source of distribution during propagation of seeds (Tu, 1983). On the other hand, anthracnose also attacks the leaves of common bean in the field (Hall, 1994).

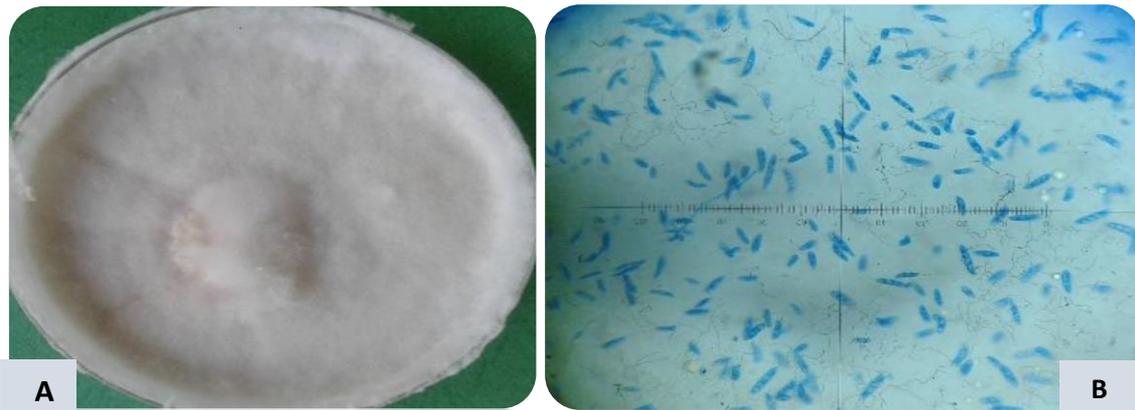


Figure 6: *Colletotrichum lindemuthianum*; (A) 10 days old pure mycelia culture and (B) spores and mycelium at 400x.

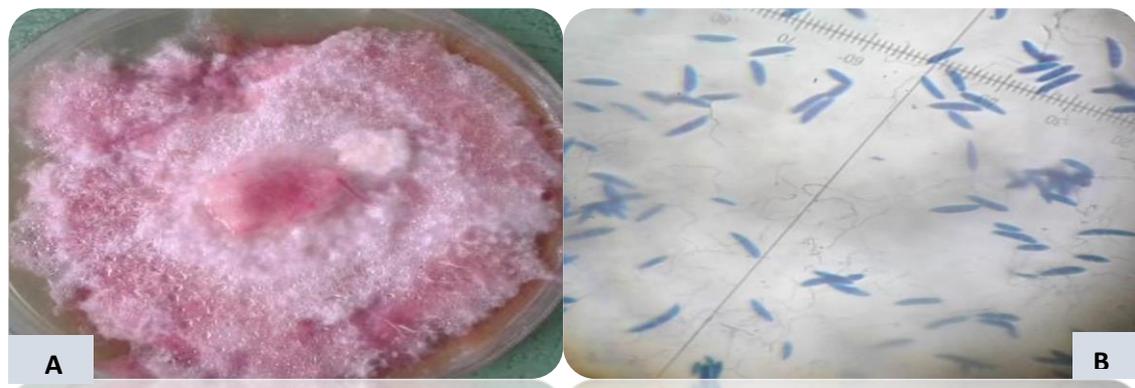


Figure 2: *Fusarium* sp, 10 days old pure culture (A) and (B) spores and mycelium under a microscope at 400x

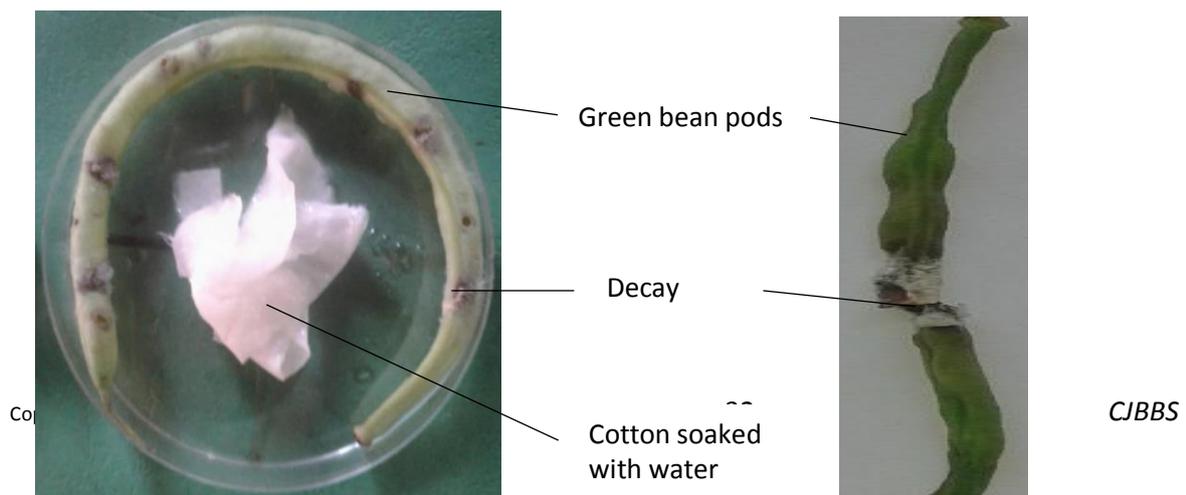


Figure 3 : Green bean pods inoculated with

Figure 4: Green bean pods

3.2. Antifungal properties of ethanol extracts

Antifungal effects of ethanol extracts of *E. saligna* and *C. lusitanica* on fungal growth are presented on Table 1. There were significant differences in the mycelial growth inhibition of plant extract supplemented samples compared with the negative control (ANOVA and Duncan Multiple Range Test, $P < 0.05$). The effect of the two plant extracts varied with the increase in concentrations, which lead to a gradual inhibition of the growth of *C. lindemuthianum* and *Fusarium sp.* It was noted that the ethanolic extracts of the two plants totally (100%) inhibited the growth of the two fungi at a concentration of 8 mg/ml.

It is also noted that the aqueous extracts of the two plants showed an inhibitory effect on the growth of the fungi tested (Table 2). The observed inhibition depended on the concentration and the plant species used. There were significant differences in the mycelia growth inhibition of plant extract supplemented samples compared with the negative control. Complete growth inhibition (100%) of *C. lindemuthianum* and *Fusarium sp* was observed at the concentration of 30 mg/ml by *E. saligna*. As for the aqueous extract of *C. lusitanica*, the concentrations applied did not allow 100% inhibition of mycelial growth. However, at 30 mg/ml, 86 and 88% inhibition of growth of *C. lindemuthianum* and *Fusarium sp*, respectively, were observed.

3.3. Antifungal effect of aqueous extracts

Table 1: Percentage (%) inhibition of fungi by ethanolic extracts of *E. saligna* and *C. lusitanica*

| Plant extracts | Concentration (mg/ml) | Fungal species | |
|-----------------------------|-----------------------|----------------------------|-----------------------------|
| | | <i>C. lindemuthianum</i> | <i>Fusarium sp</i> |
| <i>Eucalyptus saligna</i> | T- | 0.00 ± 0.00 ^d | 0.00 ± 0.00 ^e |
| | 2 | 63.73 ± 4.13 ^c | 46.863 ± 3.59 ^d |
| | 4 | 71.76 ± 0.59 ^b | 61.373 ± 5.77 ^c |
| | 6 | 97.84 ± 0.68 ^a | 71.176 ± 1.019 ^b |
| | 8 | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |
| | T+ | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |
| <i>Cupressus lusitanica</i> | T- | 0.00 ± 0.00 ^e | 0.00 ± 0.00 ^e |
| | 2 | 77.84 ± 0.34 ^d | 78.235 ± 1.18 ^d |
| | 4 | 80.78 ± 1.23 ^c | 81.765 ± 0.59 ^c |
| | 6 | 83.53 ± 1.18 ^b | 86.275 ± 1.18 ^b |
| | 8 | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |
| | T+ | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |

Values followed by same letters in the same column are not significantly different according to Duncan test ($P \leq 0.05$). T- = negative control (distilled water) and T+ = positive control (Mancozeb).

Table 2: Percentage (%) inhibition of fungi by aqueous extracts of *E. saligna* and *C. lusitanica*

| Plant extracts | Concentration | Fungal species | |
|---------------------------|---------------|---------------------------|---------------------------|
| | | <i>C. lindemuthianum</i> | <i>Fusarium sp</i> |
| <i>Eucalyptus saligna</i> | T- | 0.00 ± 0.00 ^d | 0.00 ± 0.00 ^d |
| | 10 mg.ml-1 | 70.59 ± 0.59 ^c | 48.63 ± 2.07 ^c |
| | 20 mg.ml-1 | 83.33 ± 1.23 ^b | 89.96 ± 0.68 ^b |

| | | | |
|-----------------------------|------------|----------------------------|----------------------------|
| | 30 mg.ml-1 | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |
| | T+ | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |
| | T- | 0.00 ± 0.00 ^c | 0.00 ± 0.00 ^c |
| <i>Cupressus lusitanica</i> | 10 mg.ml-1 | 77.45 ± 1.80 ^d | 78.82 ± 2.12 ^d |
| | 20 mg.ml-1 | 80.39 ± 0.34 ^c | 81.96 ± 0.68 ^c |
| | 30 mg.ml-1 | 86.08 ± 0.90 ^b | 88.24 ± 1.18 ^b |
| | T+ | 100.00 ± 0.00 ^a | 100.00 ± 0.00 ^a |

Values followed by same letters in the same column are not significantly different according to Duncan test ($P \leq 0.05$). T- = negative control (distilled water) and T+ = positive control (Mancozeb).

3.4. *In vivo* effect of *E. saligna* aqueous extract

The results of the *in vivo* activity of the aqueous extract of *E. saligna* on the development of *Colletotrichum lindemuthianum* and *Fusarium sp* were recorded after 10 days. The green bean pods

inoculated with *C. lindemuthianum* and *Fusarium sp*, and supplemented with drops of the aqueous extract of *E. saligna*, did not present any fungal development after 10 days (Figure 3).

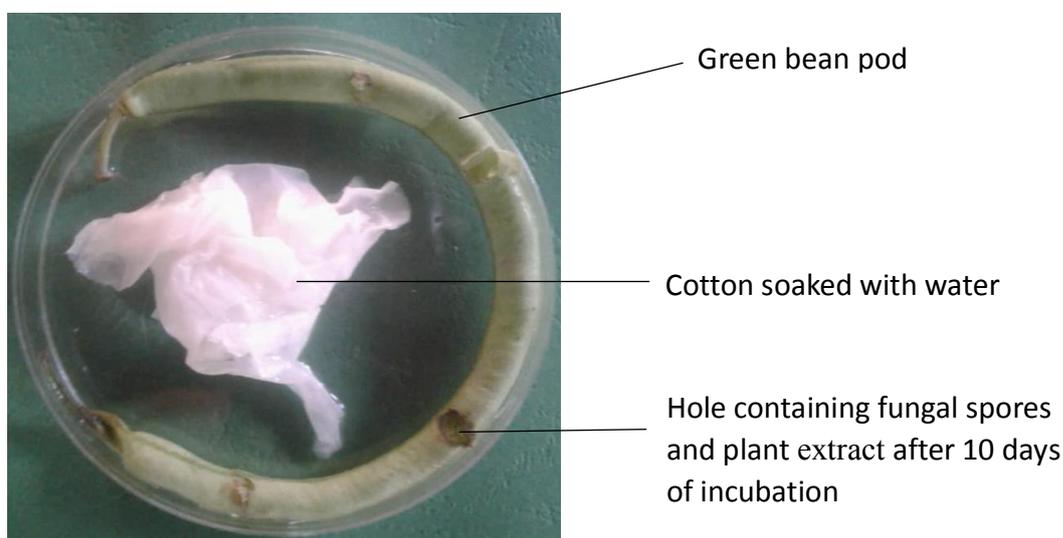


Figure 3. Green bean pod inoculated with *C. lindemuthianum* and treated with aqueous extract of *E. saligna*

4. Discussion

In this study, *Colletotrichum lindemuthianum* and *Fusarium sp* isolated on green bean pods, confirmed their involvement in the degradation of the quality of the green bean in post-harvest by causing decay. Lesions were observed on the inoculated green bean pods. These results are similar to those found by Mota et al. (2016) and El-Mougy, (2001), which showed that these fungi are responsible for the deterioration of the green bean in post-harvest. On the other hand, the antifungal effect of the aqueous and ethanol extracts of *Eucalyptus saligna* and *C. lusitanica* were evaluated against *Colletotrichum lindemuthianum* and *Fusarium sp* under both *in vitro* and *in vivo* conditions. The findings showed that the effect of plant extracts on *C. lindemuthianum* and

Fusarium sp radial growth and disease development on green bean pods vary depending on the type of plant species used, method of extraction and concentration of the extracts applied. These extracts were found to have considerable effect on inhibition of fungal radial growth *in vitro*. They equally possess characteristic of reducing disease development on detached green bean pods. However, *Eucalyptus saligna* extracts were the most effective in reducing the radial growth of *C. lindemuthianum* and *Fusarium sp*.

These findings further confirm the fact that plants are rich sources of potentially useful antimicrobial products for the development of new chemotherapeutic agents (Mousavi et al. 2009; Musyimi et al. 2008 ; Ferreira et al. 2009; Safary et

al. 2009). Many reports are available on the antifungal, antibacterial, antiviral, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Dey and De, 2010; Mahesh and Satish, 2008; Samy and Ignacimuthu, 2000; Palombo and Semple, 2001). Some of these observations have helped in identifying the active compounds responsible for such activities and in the development of drugs for therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection. Results obtained from *Eucalyptus saligna* extracts correspond to work done by Yemo et al. (2017) who reported the antifungal activities of this plant against *Colletotrichum kahawae* isolated from green coffee berries in Cameroon. Similar results were reported by Amsalu et al. (2011) who tested the effectiveness of these extracts against *Colletotrichum kahawae* isolated from green coffee berries in Ethiopia. Results obtained with *C. lusitanica* extract are similar to those reported by Yaouba et al. (2015) and Tsopmbeng et al. (2014) who showed that this extract inhibited the development of *Phytophthora megakarya* on cocoa pods as well as *P. Colocasiae* on detached leaves of taro respectively. Extracts of *E. saligna* have been reported to contain eucalyptol, an antifungal substance that prevents or hinders the growth of many fungi (Enyiukwu et al. 2014). In addition, leaf extracts of *Carica papaya* contain proteolytic enzymes which are known to have antifungal activity against *Colletotrichum gloeosporioides* (Bautista-Banos et al. 2002) and alkaloids which have fungicidal activity against *Colletotrichum* and *Fusarium* species (Oliva et al. 2003). The results of the work of Eliane et al. (2017) indicate that even if terpenes are present in the extract of *E. saligna*, the phytotoxicity of the extract is most assured by phenolic compounds.

5. Conclusion

This study identified the fungi associated with green beans and established the responsibility of *Colletotrichum lindemuthianum* and *Fusarium* sp in the quality loss in post-harvest conditions. The antifungal effect recorded leads to suggest the possible use of *E. saligna* and *C. lusitanica* extracts as an alternative means of green bean post harvest decay management and provides information that could constitute a base for future tests under natural conditions. It would also be very interesting to do fragmentation of these plant extracts and test the antifungal effect of the different constituents.

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