

BIOLOGICAL CONTROL POTENTIAL OF ESSENTIAL OILS OF DIFFERENT PLANT SPECIES ON *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) PENZ. & SACC., THE PATHOGENIC AGENT OF MANGO ANTHRACNOSE

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ABSTRACT: Mango plays an economically strategic role in the horticultural sector of many countries around the world, particularly in Africa. In Senegal, it represents 60% of fruit production and largely dominates the export market. However, mango production is facing many phytosanitary constraints of phytopathological or entomological origin the main of which is anthracnose caused by *Colletotrichum gloeosporioides*. For the control of this fungal pathogen, synthetic fungicides are often used with the known negative impact on human, animal and environment. The essential oils of various aromatic plants are reported to have great potential on fungal pathogens. Some of them, extracted from *Eugenia caryophyllata*, *Mentha piperita*, *Cymbopogon citratus* and *Eucalyptus camaldulensis* have been used to test their *in vivo* and *in vitro* antifungal activity on *C. gloeosporioides* in order to improve mango fruit quality, shelf life and market value. The *in vivo* efficacy of these essential oils was tested on Keitt variety already showing symptoms of anthracnose in field and then on local variety "bouko diekhal" inoculated with strains of *C. gloeosporioides*. The treatment consists by soaking mangoes in different concentrations of essential oils and determining incidence and severity. The essential oil of *E. caryophyllata* (clove) showed the best efficacy with total inhibition of mycelial growth of *C. gloeosporioides* at the concentration of 800ppm. Mangoes inoculated with the fungus showed a severity rate of $10.22\% \pm 8.37\%$ with the concentration of 500ppm while for the control mangoes, treated with distilled water displayed $39.44 \pm 12, 36\%$ severity. Treatment of the fruits with the essential oils of *Mentha piperita* (peppermint) and *Cymbopogon citratus* (lemongrass), at the respective concentrations of 3000ppm and 2300ppm, led also to a complete inhibition of mycelial growth of the fungus. These results are promising with respect to the potential use of essential oils for the biological control of pathogenic fungi causing diseases of mango fruit.

KEYWORDS: *Colletotrichum gloeosporioides*, essential oils, biological control, Senegal

I. INTRODUCTION

Mango is the sixth most produced fruit in the world with more than 50 million tons produced in 2017 on more than 50 million hectares (FAOSTAT., 2019). In Senegal, its production is exposed to many phytosanitary constraints due to pests and diseases (Diedhiou et al., 2007; Ndiaye et al., 2008). Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc (Arauz, 2000, Zakaria et al., 2015), remains the most important fungal disease of mango in pre and post harvest stage with greater losses in the post harvest stage (Nelson, 2008, Pardo De la Hoz et al. ., 2016). In Senegal, anthracnose can cause losses ranging from 42 to 90% depending on climatic conditions (Mbaye, 2006; Diédhiou et al., 2014). Because of the environmental and health problems caused by synthetic fungicides, the search for alternatives becomes necessary and essential oils represent a promising way for the biological control of this fungal disease (Cissé et al., 2020). The effectiveness of certain essential oils on many phytopathogenic agents has already been reported (Glitho et al. (2004), Pandey et al (2012), Ba (2014), Vidal et al. (2018), Cissé et al. (2020)). This study was therefore carried out with the aim of testing the antifungal efficacy of the essential oils of *Eugenia caryophyllata*, *Mentha piperita*, *Cymbopogon citratus* and *Eucalyptus camaldulensis* on mango anthracnose caused by *C.gloeosporioides*.

II. MATERIAL AND METHODS

Fungal material : Strains of *Colletotrichum gloeosporioides* were isolated from infested mangoes of Keitt variety from Casamance.

Essential oils : The essential oils of cloves (*Eugenia caryophyllata*), and the fresh leaves of *Eucalyptus camaldulensis* were extracted by steam distillation. *Mentha piperita* and *Cymbopogon citratus* essential oils were

obtained from France. The ready for use essential oils after extraction or upon arrival were stored in tinted bottles to avoid denaturation by light.

In vitro activity of essential oils on mycelial growth of *Colletotrichum gloeosporioides* : Preparation of fungal cultures :The essential oils were incorporated into the PDA (Potato Dextrose Agar) culture medium after autoclaving at a temperature of 121 °C, and 1 bar pressure for 25 minutes. After cooling down to ≈50 °C the sterilized culture media was poured into Petri dishes of 9 cm in diameter at a rate of 20 ml each. For the tested concentration of essential oils, 3 PDA plates representing 3 replicates were inoculated with 3 mm diameter of agar disc colonized by a pure culture of *Colletotrichum gloeosporioides* aged of 7 days. An incubation period of 8 days, corresponded to time interval taken by the fungus to fill the non-treated Petri dishes, was considered.

Evaluation of mycelial growth : Mycelial growth was assessed every 48h by calculating the average colony diameter of perpendicular measurements passing through the middle of the fungal colony. Three repetitions are performed for each concentration. Growth inhibition rates (GIR) were determined according to the formula from Doumbouya et al. (2012):

$$\text{GIR (\%)} = (T - E) * 100 / T; \text{ where}$$

GIR = Growth inhibition rate

T = Average diameter of fungal colony in the control treatment (in cm)
E = Average diameter of fungal colony in treated plates (in cm)

In vivo activity of essential oils on *Colletotrichum gloeosporioides*

Preparation of spore's suspension and inoculation : Pure culture of a 10 days old strain of *Colletotrichum gloeosporioides* was used. 10ml of sterile distilled water is poured in the petri dish containing the pure culture. The mycelium was then scraped with a scalpel blade and filtered in order to have a spore suspension free from any mycelial fragments. Inoculation of the pathogen was done by creating two wounds with a depth of 1.5mm, one at the level of the peduncle and another at the apex of the mango fruit. Subsequently, 50 µl of the *C. gloeosporioides* spore suspension is inoculated by using a micropipette. After inoculation, mangoes are kept at room temperature (about 25°C) for 5 minutes for drying before the tests by soaking.

Post-harvest treatment of mangoes infected in field (Keitt variety) :Six treatments were carried out and for each treatment 12 mangoes were soaked in the solution for 3 minutes then air dried. The different treatments considered were hot water at 51°C during 5 minutes, Prochloraz (reference control), *Eugenia caryophyllata*, *Mentha piperita*, *Cymbopogon citratus*, and *Eucalyptus camaldulensis*.

Post-harvest treatment of mangoes artificially inoculated by *C.gloeosporioides* : After inoculation with spore suspension of *Colletotrichum gloeosporioides* and drying at room temperature (25°C) during 10mn, the mangoes are soaked one by one with nine mangoes per treatment for 2 minutes in different concentrations of essential oils added with 5 ml of Tween 80 (0.1%). After soaking, the mangoes are left at room temperature.

Table 1: Different concentrations used for treatments

Treatment	oncentration (ppm) with Keitt	Concentration (ppm) with bouko diékhal
<i>E.caryophyllatta</i>	5000	500
<i>E.camaldulensis</i>	5000	5000
<i>C.citratus</i>	Not used	2000
<i>M.piperita</i>	5000	3000
Prochloraz	5000	Not used
Hot water 51°C (5mn)	0	Not used

Evaluation of incidence and severity of anthracnose : After treatment, the incidence and severity of anthracnose were assessed. The evaluation was carried out every 48 hours for the Keitt variety and every week for the local variety “bouko diekhal” during 15 days.

The incidence (I) was determined according to the following formula:

$$I = \frac{n}{N} * 100$$

Where n = number of mango showing symptoms and N = total number of mangoes

Disease severity was assessed using the following scale: 0 to 4 is used with (0) = no symptoms; (1) = 1 to 25% of mango surface showing symptoms; (2) = 26 to 50% of mango surface showing symptoms; (3) = 51 to 75% surface showing symptoms; (4) = 76 to 100% of mango surface showing symptoms

The severity of the disease in each replication of a treatment was determined according to the following formula:

$$S = \frac{\sum(n_i \times s_i)}{N \times Z} * 100$$

Where ni = number of mango of severity i; si = partial severity i; N = total number of mango; Z = the greatest severity index (4)

Statistical analyses : The data was processed with R.3.2.3 software (R Core Team, 2015). A two-factor analysis of variance (ANOVA) was performed using the aov function of the agricolae package (de Mendiburu, 2015).

III. RESULTS

Activity of essential oils on mycelial growth of Colletotrichum gloeosporioides : The different essential oils used demonstrated good antifungal activities. An inhibition of mycelial growth of *C. gloeosporioides* was noted at various concentrations (Figure 1). With the extract of *E. caryophyllata*, the growth inhibition of the mycelium of the fungus varied from 52% at 500 ppm to 100% at 800ppm, showing thereby a strong effectiveness. The sametrend was observed with lemongrass and mint extracts, only the effective concentration were higher, 2300ppm and 3000ppm respectively. *E. camaldulensis* essential oil was less effective with the highest inhibition rate of 58.03 obtained at 10.000ppm.

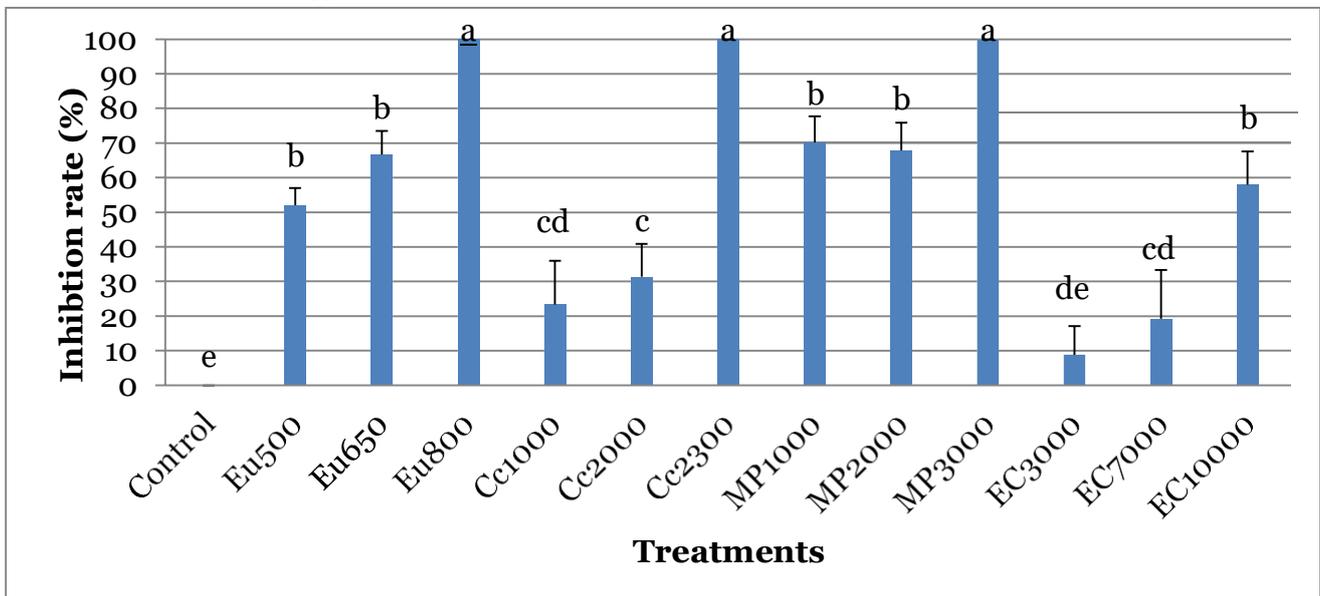


Figure 1: Activity of essential oils on the mycelial growth of *Colletotrichum gloeosporioides* (n=39, p=9, 61e-16), Control = Distilled water + Tween 80 (0.1%), Eu = *Eugenia caryophyllata*, Cc= *Cymbopogon citratus*, MP=*Mentha piperita* and EC=*Eucalyptus camaldulensis*

Activity of essential oils on naturally field infested mangoes : With the essential oils of *Eugenia caryophyllata*, *Mentha piperita* and *Eucalyptus camaldulensis* disease incidence and severity were reduced down as compared to the absolute control and the reference control (Figure 2). The disease incidence reached a maximum value of 41.66% with the treatments with hot water (51°C during five minutes) and essential oil of *E. camaldulensis*. The mangoes treated with essential oils of *E. caryophyllata*, *M. piperita*, and the reference control have respective incidence of 25%, 33.33% and 25%. The average severities obtained with the absolute control, hot water and the reference control are respectively $19.16 \pm 26.44\%$, $15.83 \pm 22.34\%$ and $8.33 \pm 12.63\%$. Essential oil of *E. caryophyllata* shows the greatest efficiency with an average severity of $7.5 \pm 14.22\%$ followed by the essential oil of *M. piperita* with $8.95 \pm 17.69\%$ and last comes the essential oil of *Eucalyptus camaldulensis* with $13.41 \pm 21.82\%$. Moreover, *Eugenia caryophyllata* has a most effective antifungal activity than the synthetic fungicide, Prochloraz.

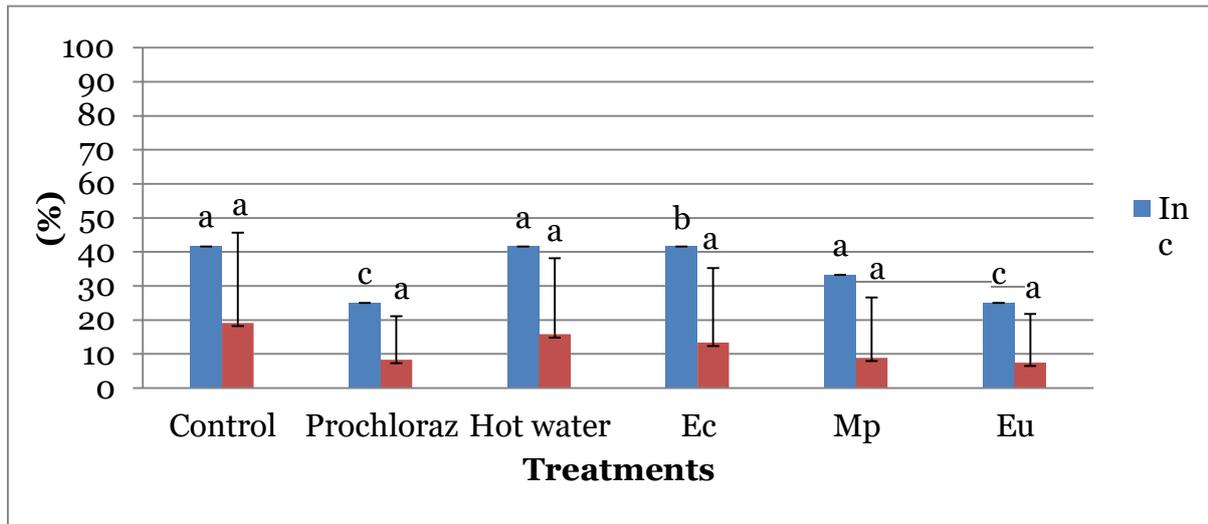


Figure 2 : Incidence and severity of mango anthracnose for infested fruits on field (n=72, $P_{Inc} = 2e-16$, $P_{Sev} = 0.673$), Inc = incidence, Sev = Severity, Control = Distilled water + Tween 80 (0.1%), Prochloraz= Synthetic fungicide as reference control, Ec = *Eucalyptus camaldulensis*, Mp= *Mentha piperita*, Eu= *Eugeniacyrophyllata*.

Activity of essential oils on inoculated mangoes : The essential oils used show good efficacy on inoculated mango by *C. gloeosporioides*. A relatively high incidence of anthracnose was noted with all the treatments (figure3). With *E. camaldulensis* and *C. citratus*, an incidence of 100% is obtained at the respective concentration of 5000 and 3000ppm. Essential oils of *M. piperita* and *E. caryophyllata* have respectively incidence of 88.88% and 55.55%. For the severity, 39.44 ± 12.36% is noted for the control. The essential oil of *E. caryophyllata* demonstrated the greatest efficiency followed by *M. piperita* and *C. citratus* with respective severities of $10.22 \pm 8.37\%$, $12.66 \pm 7.95\%$ and $15.55 \pm 9.16\%$. *E. camaldulensis* is the least effective where the severity of symptoms reaches $21.11 \pm 6.50\%$. The analysis of variance performed on the incidence and severity of the disease shows that the effect of treatment is statistically significant (P value <0.05).

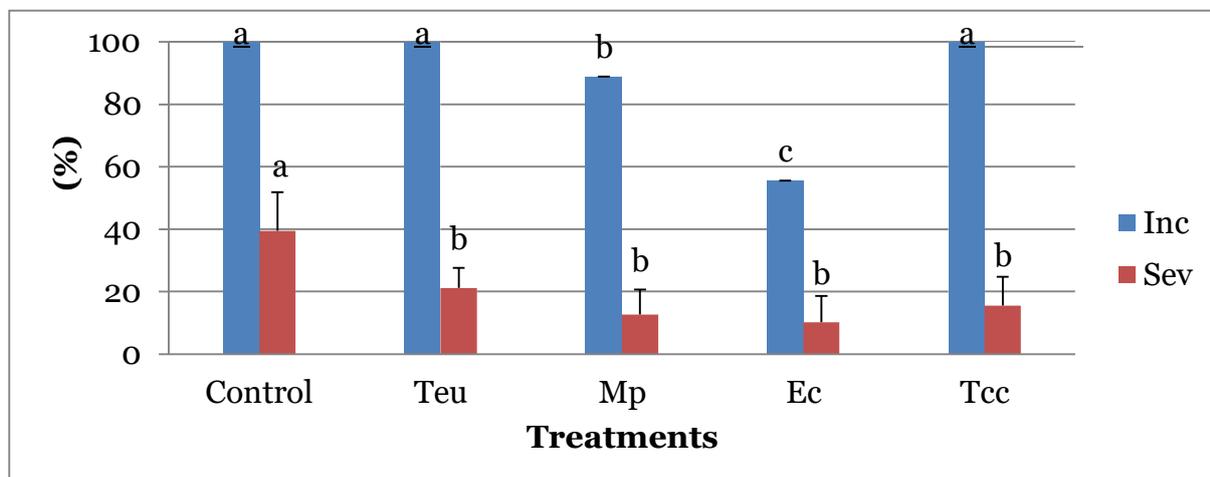


Figure 3 : Incidence and severity of mango anthracnose on inoculated fruits (n=45, Pinc=2e-16, Psev=1.49e-07), Inc = Incidence, Sev = Severity, Control = Distilled water + Tween 80 (0.1%), Teu = Eucalyptus camaldulensis, Ec = Eugenia caryophyllata, Mp = Mentha piperita, Tcc = Cymbopogon citratus.

IV. DISCUSSION

The test of essential oils on *Colletotrichum gloeosporioides* responsible for mango anthracnose showed good efficacy with a variability related to the essential oil and the concentration. The essential oil of *E. caryophyllata* demonstrated the greatest efficiency with a complete inhibition of the in vitro mycelial growth of *Colletotrichum gloeosporioides* at 800ppm. Kocić-Tanackov and Dimić (2013) obtained with this same oil a total inhibition of *Aspergillus flavus* at 500ppm. The essential oil extracted from clove is mainly composed of eugenol (81.13-84.44%) (Sohilait., 2015). The in vitro toxicity of eugenol to fungi has already been reported by Hamini-Kadar et al. (2014) on phytopathogenic fungi of the genus *Penicillium*, *Fusarium*, *Aspergillus* and *Alternaria*. Average severities of 5% at 5000ppm and 10.22% at 500ppm, respectively, were obtained on mangoes naturally field infested and those inoculated with *Colletotrichum gloeosporioides*. He et al. (2007) have shown good in vitro and in vivo efficacy of eugenol against *Candida albicans*. With lemongrass, a total inhibition of mycelial growth is obtained at 2300ppm. Traoré (2018) noted a total reduction of mycelia of *Fusarium oxysporum* at 1200ppm while Stangarlin et al. (2011) reported that the use of 1, 20, 40, and 60µL of this essential oil has an inhibitory effect on the mycelial growth of *Colletotrichum martinii*, responsible agent of pepper anthracnose.

The inoculated mangoes exhibited an average severity of $15.55 \pm 9.16\%$ when treated with *C. citratus* essential oil compared to control mangoes that displayed a severity of $39.44 \pm 12.36\%$. According to Traoré (2018), the essential oil of *C. citratus* exerts only partial control of post-harvest fungal diseases in mango. For *Mentha piperita* essential oil, complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* was obtained at 3000ppm. These results are similar to those of Cissé et al. (2020). In the same way, Padman et al. (2012) obtained an inhibition of the mycelial growth of *C. gloeosporioides* responsible of leaf spot diseases of *Murraya koenigii* at 2500ppm. The chemical composition of essential oil of *Mentha piperita* is essentially menthol (28 to 42%) in association with menthone, limonene, β -terpinene and peritone oxide (Bouhdid et al., 2012; Yadegarinia et al., 2006). These compounds considerably reduce the mycelial diameter of fungi leading to an alteration in their metabolism until their total destruction (Zani et al. (1991)). Mangoes infested in the field and treated with the essential oil of *M. piperita* presented an average severity of $8.95 \pm 17.69\%$ compared to the control ($19.16 \pm 26.44\%$). Sellamuthu et al. (2013) obtained a reduction in lesion size in avocado and *Thymus vulgaris* respectively at the concentration of 1060ppm and 667ppm after their inoculation with *C. gloeosporioides*.

Similar observations were made by Cissé (2016), who obtained a reduction of lesion diameters from $7.47 \pm 2.91\text{cm}$ down to $3.04 \pm 2.43\text{cm}$ respectively for control and treated mangoes with this same essential oil at a concentration of 3000ppm. In the present work, the essential oil of *Eucalyptus camaldulensis* was the least effective both in vitro and in vivo. When it was applied at 10000 ppm, the observed reduction of mycelial growth was only $58.03 \pm 9.50\%$. With this same concentration, Cissé et al. (2020) and Gakuubi et al. (2017) respectively obtained 84.07% inhibition of mycelial growth of *C. gloeosporioides* and 100% inhibition of mycelial growth of *Fusarium sporotrichioides* and *Fusarium graminearum*. It is known that, the essential oil of *E. camaldulensis* is mainly composed of eucalyptol, 1,8-cineole, α -pinene and γ -terpinene, all substances with antifungal activity (Bamayi et al., 2004). In vivo, treatment with the essential oil of *E. camaldulensis* led to a reduction of the severity of anthracnose from $19.16 \pm 26.44\%$ for absolute control to $13.41 \pm 21.82\%$ for fruits from the field. For the inoculated mangoes, the severity of the disease decreased from $39.44 \pm 12.36\%$ (absolute control) to $21.11 \pm 6.50\%$ for the treated mangoes. According to Somda et al., (2007), this essential oil is able to inhibit seed-transmitted fungi such as *Colletotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme*. Abd-Alla et al. (2013) suggested that the hydrophobic nature of essential oils and their components allow them to penetrate the lipids of the cell membrane and mitochondria of fungi by disrupting their structure. Mangoes inoculated with *Colletotrichum gloeosporioides* showed a delayed onset of symptoms. This is put in connection with the ability of essential oils to slow down the secretion of enzymes produced by the fungus that are responsible for the stains noted on the surface of the mango. According to Kramer-Haimovich et al (2006), the secretion of a significant amount of pectinase lyase, an enzyme capable of inducing maceration of the pectocellulosic wall of the host, would be the cause of the onset of symptoms of anthracnose.

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