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Effectiveness of Biopesticides in Vitro Agains *Colletotrichum sp* Responsible Mango of Anthracnose (*Mangifera indica L*) in Burkina Faso

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Abstract

In Burkina Faso, Anthracnose is the main post-harvest disease which causes enormous economic losses due to the rejection of mangoes export. The present study aims to assess efficiency in vitro of biopesticides constituted of four essential oils of *Lippia multiflora, Ocimum graticimum, Eucalyptus camaldulensis, Cymbopogon citratus* at concentrations of 600 ppm, 800 ppm, 1000 ppm and 1200 ppm and aqueous extracts formulated based on *Carica papaya, Appichi and Cymbopogon giganteus* at concentrations of 15%, 30% and 60% against *Colletotrichum sp* responsible of mango anthracnose in Burkina Faso. The experimental device is a complete randomization of eight (8) treatments and five (5) repeatings for each biopesticide tested. The results exhibited an effectiveness of the antifungal activity of all the essential oils tested. The doses of 1000ppm and 1200ppm were the most effective with mycelium inhibition rates reaching 99%. The four oils have been effective with 1200 ppm as minimum inhibition concentration. The use of aqueous extracts at concentrations of 60% exhibited an inhibition rate of *Colletotrichum sp* mycelial growth from 80 % to 99 %. The most effective biopesticides were essential oil from *O. graticimum* with an inhibition rate of 93% at 600 ppm and *the Appichi* extract with an inhibition rate of 80% at concentrations of 15%. In vivo tests in orchards coupled with post-harvest treatments on fruit should allow to confirm the foreplay results in vitro.

Keywords: *Colletotrichum sp, anthracnose*, biopesticides, mango, Burkina Faso.

1. Introduction

In West Africa, the fruit and vegetable sector constitutes one of the agricultural sectors which are experiencing rapid growth. It contributes to the job creation and the fight against poverty. The mango ranks second in fruit production after pineapple [15]. In 2020, Burkina Faso exported 2,900 tonnes of dried mangoes and 8,000 tonnes of fresh mangoes. These exports generated more than 15 billion FCFA of incomes (fresh and dried). The mango value chain provides jobs. In 2020, the mango sector created more than 21,000 entrepreneurial jobs, for fresh export more than 7,000 jobs are generated, seasonal activities of fresh mango marketing on the local market also support nearly 10,000 retailers [1].

Commonly called “green gold” in Burkina Faso, the mango is the economic engine of the country’s horticultural sector. Indeed, it engages 62.50% of fruit production [11] and constitutes a source of income diversification for rural actors. The main export destinations are the countries of the West African sub-region, Europe, Asia and America. This interest for mango is essentially related to its nutritional quality [13].

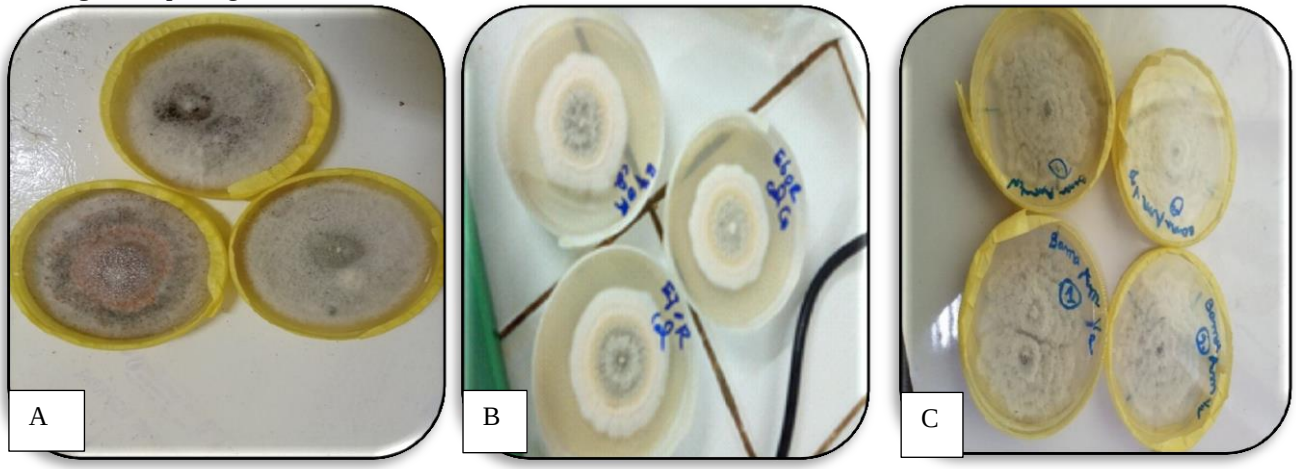
However, mango quality is constantly depreciated by pathogens. Among these pathogens, Anthracnose constitutes the main fungal disease which can reach an incidence more than 51.4 % [3]. Symptoms are often not visible when picking. Control strategies including the method of early elimination of the inoculum sources in the orchard, as well as phytosanitary treatments limited to periods of risk and possibly supplemented by post-harvest treatments, were put in place. These practices are rarely applied in a reasoned manner and then prove to be ineffective [9]. As part of the biological diversity protection and human health, the search for effective alternatives to phytosanitary products constitutes an important challenge both for agriculture conventional than organic. These are local products whose use in plant protection could be low-polluting, low-toxic and less hard, compared to synthetic fungicides. In Burkina Faso, several evaluations tests of plant extracts on fungi in particular *Pyricularia oryzae*, *Fusarium moniliforme, Bipolaris oryzae* and *Fusarium sp* were carried out by [12]. with satisfactory results. However, few study on the use of biopesticides on *Colletotrichum* has not yet been done, this is what has motivated the initiation of this study.

2. Material and Methods

2.1. Fungal material

Isolates *of Colletotrichum Sp* used come from the characterization made by Bougoum et al. [3]. White and reddish mycelial colonies appear two (02) to three (03) days after isolation on the PDA middle. It was characterized by aerial myceliums, dense, cottony in appearance whose coloring varies from white to pink to yellow. The isolates also presented colonies cottony mycelial covered which diffuse in the culture environment with black to pink conidia (Fig 1).

Fig-1.Morphological characteristics of the different isolates of *Colletotrichum* on used PDA medium culture



Legend: A: Cottony white colonies with pink spores; B: Yellowish s uniforms colonies in development; C: White colonies with black spores.

2.1. Biopesticides Used

Aqueous extracts from several plant species were prepared at Bioprotect-S/C ARFA in Bobo Dioulasso, specialized in production of biopesticides. Concerning essential oils, they were acquired at the Research Institute of Applied Sciences and Technologies (IRSAT).

2.2. Preparation of Essential Oils

Firstly, it has consisted to put in a tank (body of the still), the leaves of the different species plants to distill and some water. Secondly to heat the content in order to obtain water steam. Thirdly, to recover and cool this steam loaded with gasoline. Finally, recover essential oil contained in the steam.

2.3. Preparation of Aqueous Extracts

Fresh leaves of plant species were collected and then grind for the preparation of infusions. 1000 ml of distilled water has been next added in each plant species in order to obtain concentration of 100 %. The extracts obtained are filtered and then centrifuged in order to remove plant debris. The supernatant is recovered and then preserved at fridge.

Table-1. Different types of biopesticides and doses used

Kind of biopesticides	Plant species	Vernacular Name	Concentration used (%) and (ppm)	Composition
Essential oils	*c. citratus*	Citronnelle	600, 800, 1000, and 1200	Pure oil of lemongrass
	O. Gratissimum	False basi		Pure oil of basil
	L . multifora	Savannah the		Pure oil of lippia
	E . camaldulensis	Eucalyptus		Pure oils of Eucalyptus
Aqueous extracts	*Appichi*	Appachi	15 %, 30 % and 60 %	Infusion of chili pepper, Ginger and garlic
	C. Papaya	Papaya		Infusion fresh papaya leave
	C . Giganteus	Lemongrass		Infusion of fresh Lemongras leave

2.4. Sensitivity Evaluation in Vitro of Colletotrichum from Biopesticides Used

In this study, the poison bait technique was used to evaluate the different biopesticides . The culture environment PDA (Potato Dextrose Agar) due to 42g/l was used as a basic environment. After autoclaving the environments, quantities of each fungicide were taken and then incorporated in agar environment PDA on superfusion (45°C). So, four concentration levels are obtained (600, 800, 1000 and 1200 ppm) for essential oils and (15%, 30% and 60%) for the aqueous extracts. Once the environment prepared, mycelial discs of 1 cm diameter taken from pure cultures growth front of the Colletotrichum 10 days old, were deposited in the center of the

solidified environment incorporated by different concentrations. Solidified environments without fungicide were also used as witness. For each treatment, five Petri dishes were used. The experiment was repeated 5 times. Daily measurements of the mycelial diameter of the fungal colonies were made using a graduated ruler along two axes perpendicular to the back of the Petri box. for two weeks following the method of N'Guettia et al. [10]. Extracts efficiency was evaluated using the method of Kumar et al. [7] . For each concentration, the average of inhibition rate of mycelial growth was calculated according to the formula proposed by Vincent (1927).

I (%) =100 (D0-DT) / D0

Note: **I (%)**: Inhibition rate in percentage of mycelial diameter, **D0**: Mycelial diameter (cm) of colonies in the petri witnesses' dishes, **DT**.: Mycelial diameter (cm) of colonies in the environments of treated crops.

The level of sensitivity or resistance of the isolate to fungicides was determined according to the scale of Kumar et al. (2007) with slight modifications.

Table-2.Level of fungicidal effectiveness according to the scale of Kumar et al. (2007)

1	**I > 90%**	**Highly Sensitive**	**Very good efficiency**
2	75% < I ≤ 90%	Good Sensitive	Efficiency
3	3 60% < I ≤ 75%	Moderately Resistant	Average efficiency
4	40% ≤ I ≤ 60%	Weak Resistant	Efficiency
5	I < 40%	Very weak	Efficiency

2.5. Statistical Analysis of Data

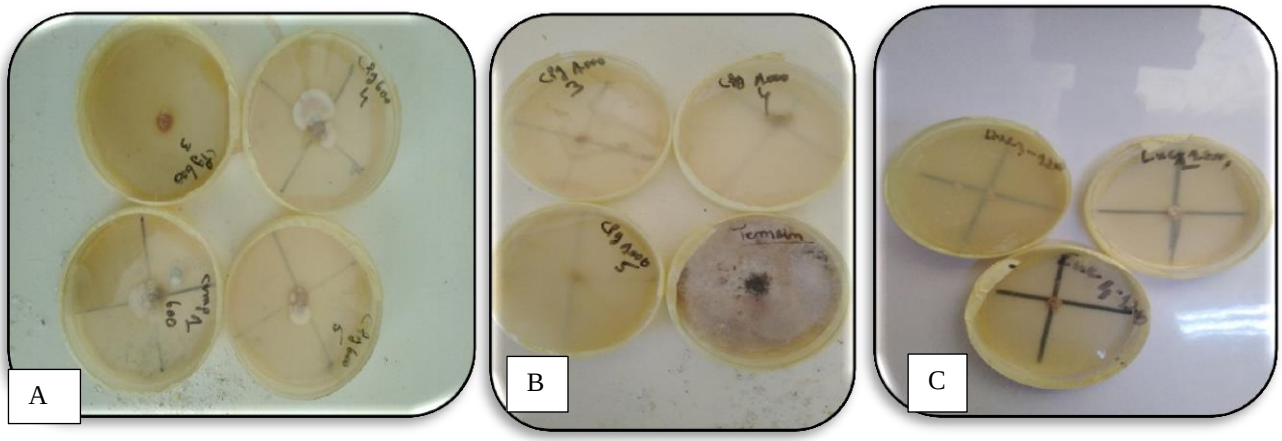
The data collected from effectiveness tests in vitro and colony diameters measured were seized and processed with Excel 2016 software. The effectiveness of essential oils and extracts has been apprehended through the analysis of the variance (ANOVA) and the comparison of the averages following Student Newman and Keuls test at the threshold for 5% with xlsat version 5.2 software.

3. Results

3.1. Antifungal activities of Essential Oils on *Colletotrichum SP* Mycelial Growth e of Colletotrichum Sp

All four doses of essential oils inhibited mycelial growth of the fungus (Figure 2). The inhibition rates was varied depending on the type of essential oils used and the different doses evaluated.

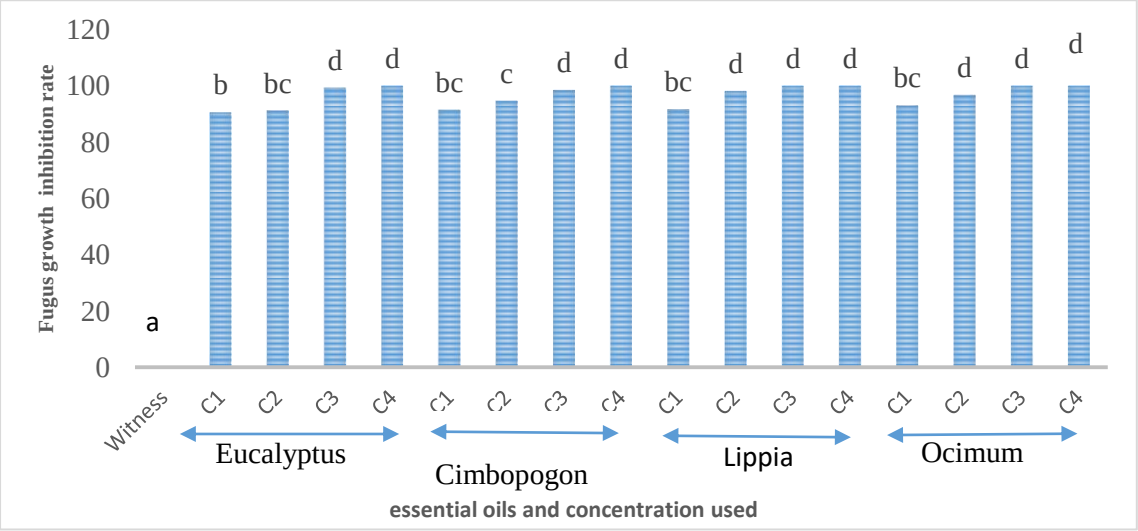
Fig-2. Inhibition of *Colletotrichum* mycelial growth by essential oils



A: *C citratus* at 600 ppm; B*: C. citratus* at 1000 ppm; C: *E. camaldulensis* at 1200 ppm

The figure 3 reflects the inhibition rate of the four essential oils at doses evaluated doses. Inhibition rates on mycelial growth are between 90 to 94% at concentration 600 ppm. *O. gratisimum* essential oil was the most effective at this dose with an inhibition rate of 93%, comparatively to *L. multifora* and E. camaldulensis. *C. giganteus* revealed itself less effective with inhibition percentages of 91%. The results obtained with doses of 800 ppm and 1000 ppm, show a greater inhibition rate of 91 to 99% of fungus development. The lowest inhibition rate at this dose is recorded for E. camaldulensis with a percentage of 91 % and the highest rate among L. multifora and O. gratissimum with inhibition percentage of 99%. Complete inhibition is achieved at concentrations of 1200ppm for all four essential oils evaluated. The variance analysis shows that there are very highly significant differences between the different doses of essential oils tested. The scale by Kumar et al. (2007) used in the sensitivity test allowed to classify the four essential oils studied according to their overall inhibition percentage of mycelial growth in the first efficiency level. So, for concentrations of 600 ppm, 800 ppm, 1000 ppm and 1200 ppm, the essential oils of *E. camaldulensis, L multifora, O. gratissimum, C. citratus* have trained an inhibition of the fungus greater than 90 %.

Fig-3. Inhibition rate of *Colletotrichum* mycelial growth depending on the product and concentration

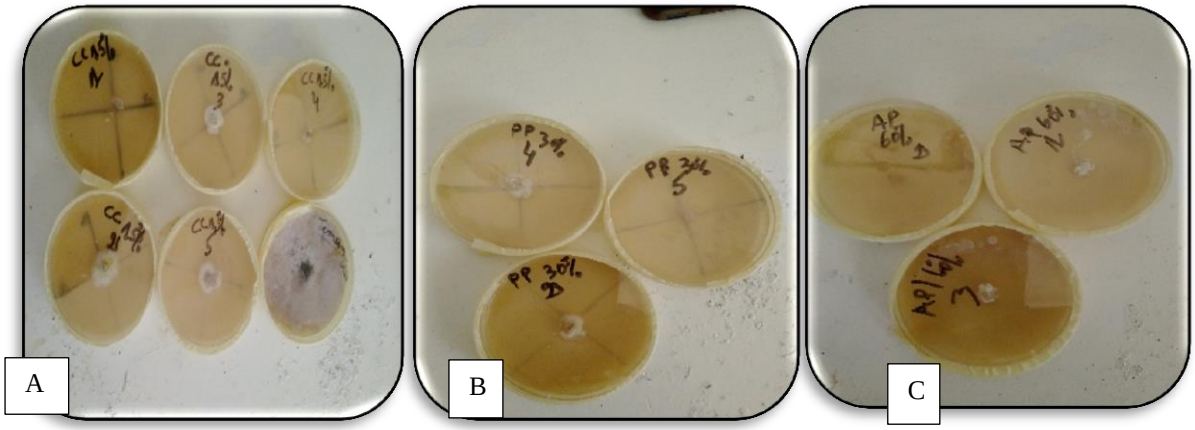


Note: Histograms assigned the same alphabetical letter do not differ significantly at the 5% threshold (Newman-Keuls test). C1: 600 ppm; C2: 800 ppm; C3: 1000 ppm; C4: 1200 ppm

3.2. Effectiveness of plant extracts on the mycelial growth of *Colletotrichum Sp*

Effectiveness evaluation tests in vitro of the plants aqueous extracts used against *Colletotrichum sp* by the poisoned bait technique overall show an inhibitor effect on mycelial growth (Fig 4).

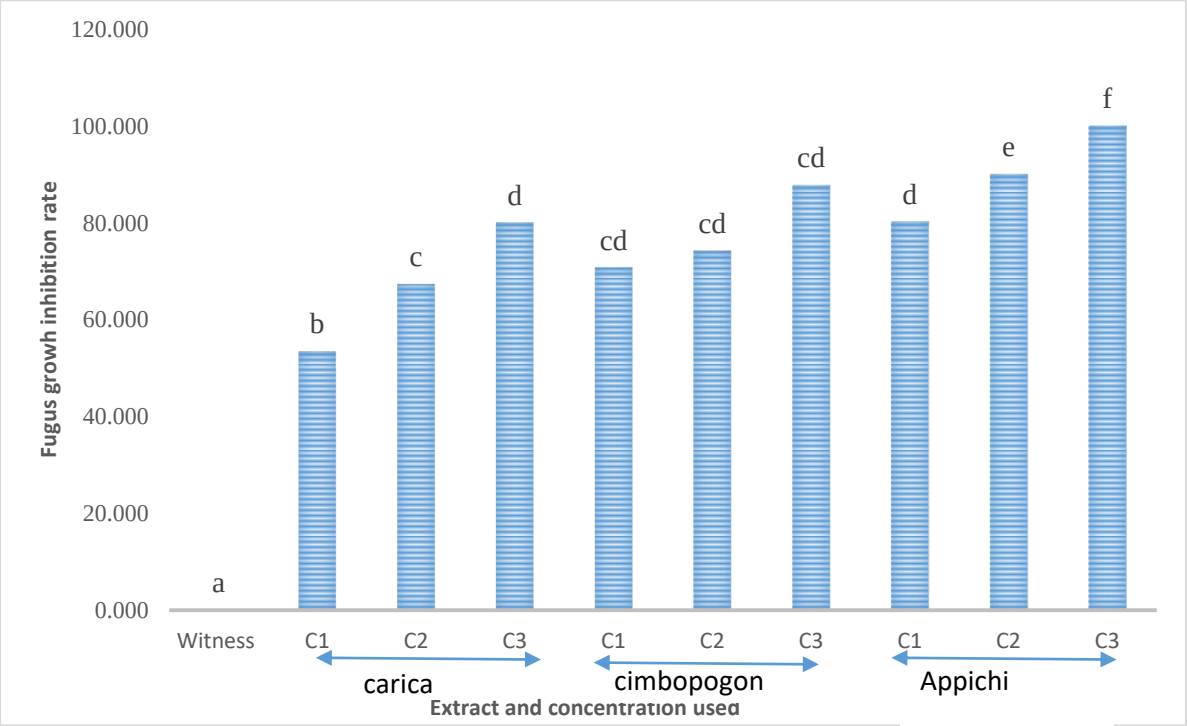
Fig-4. *Colletotrichum* mycelial inhibition growth by the extracts at 15%, 30% and 60%



Legend: A*: C. giganteus* and witness at 15%; B: *C. papaya* at 15%; C: *Appichi* at 60 %;

The results of the antifungal effect of three aqueous extracts at concentrations of 15% show a mycelial growth inhibition of isolates of 53 % to 80%. For concentrations at 30 %, the inhibition rate is between 65 % has 90 % and finally 80 % to 99 % of inhibition rate for concentrations at 60% for extracts used. *Appichi* aqueaous extracts revealed higher inhibitory effects at 99% in doses of 60% on the fungus. On the other hand, aqueous extracts of *C. Papaya* and *C. giganteus* did not have total inhibitory effect with respective inhibition percentages of 80 and 87% at concentrations of 60 %. Kumar et al. (2007) scale has allowed us to classify these extracts into four groups. So, the first group is constituted from *Appichi* extract at 30 % and 60 % whose inhibition rate is greater than 90 %. The second group is constituted of *Appichi* extract at 15, *C. giganteus* and *C. papaya* at 60 % whose inhibition rate is between 75 % at 9 0 %. The third group is constituted of *C. giganteus* at 15 % and 30 % whose inhibition rate is between 60 % and 75 %. Finally, the last group is constituted of *C. papaya* at 15 % whose inhibition rate is between 40 and 60 %. The average diameters of mycelial growth with the use of different treatment doses on *Colletotrichum Sp* are statistically different from the witness without fungicide (Fig 5).

Fig-5. Inhibition percentage of *Colletotrichum Sp* mycelial growth basis on extract concentrations used.



Note: The histograms affected of the same alphabetical letter do not differ significantly at the 5% threshold (Newman-Keuls test). C1: 15%; C2: 30%; C3: 60%.

3.3. Inhibition percentage of Mycelial Growth Basis on Days

The variance analysis results of the mycelial growth data showed that there are very highly significant differences (P<0.000) between essential oils doses and used extracts (Table 3).

Table-3. Biopesticides effect on the *Colletotrichum Sp* mycelium growth basis on days

	3rd day	6 rd day	9 rd day	12 rd day	15 rd day	d'inhibition Percentage
Witness	1,000 b	2,400 d	3,450 c	4,150 d	4,400 d	00
Eucalyptus camaldulensis 600	0,150 a	0,230 bc	0,250 b	0,400 c	0,420 c	90,571
Cymbopogon citratus 600	0,130 a	0,270 c	0,270 b	0,270 bc	0,290 bc	91,510
Lippia multifora 600	0,020 a	0,200 abc	0,290 b	0,360 bc	0,380 bc	91,714
0cimum gratissimum 600	0,100 a	0,100 abc	0,190 ab	0,250 bc	0,280 bc	93,142
Eucalyptus camaldulensis 800	0,000 a	0,140 abc	0,320 b	0,360 bc	0,360 bc	91,224
Cimbopogon citratus 800	0,040 a	0,160 abc	0,160 ab	0,220 b	0,220 b	94,693
Lippia multifora 800	0,020 a	0,020 a	0,020 a	0,080 a	0,100 a	98,204
0cimum gratissimum 800	0,000 a	0,000 a	0,000 a	0,000 a	0,020 a	96,755
Cimbopogon citratus 1000	0,000 a	0,060 ab	0,060 a	0,060 a	0,060 a	98,530
0cimum gratissimum 1000	0,000 a	0,000 a	0,000 a	0,000 a	0,000 a	100
Lippia multifora 1000	0,000 a	0,000 a	0,000 a	0,000 a	0,000 a	100
Eucalyptus camaldulensis 1000	0,000 a	0,000 a	0,000 a	0,000 a	0,050 a	99,387
Cimbopogon citratus 1200	0,000 a	0,000 a	0,000 a	0,000 a	0,000 a	100
Eucalyptus camaldulensis 1200	0,000 a	0,000 a	0,000 a	0,000 a	0,000 a	100
0cimum gratissimum 1200	0,000 a	0,000 a	0,000 a	0,000 a	0,000 a	100
Lippia multifora 1200	0,000 a	0,000 a	0,000 a	0,000 a	0,000 a	100
Carica papaya 15%	0,390 cd	1,020 c	1,430 d	1,640 d	1,700 f	53,506
Cimbopogon giganteus 15%	0,250 bc	0,590 b	0,810 c	0,980 bc	1,170 de	70,791
Appichi 15%	0,150 ab	0,320 ab	0,560 bc	0,700 b	0,710 bc	80,283
Carica papaya 30%	0,210 ab	0,570 b	0,920 c	1,130 c	1,350 e	67,391
Cymbopogon giganteus 30 %	0,210 ab	0,350 ab	0,730 c	0,860 bc	0,870 cd	74,286
Appichi 30%	0,080 ab	0,130 ab	0,260 ab	0,320 a	0,410 ab	90,059
Cymbopogon giganteus 60 %	0,150 ab	0,350 ab	0,590 bc	0,840 bc	0,900 cd	87.780
Carica papaya 60%	0,190 ab	0,450 ab	0,560 bc	0,730 b	0,760 bc	80,097
Appichi 60%	0,010 a	0,030 a	0,170 a	0,300 a	0,320 a	99,973
Pr > F	0,000	0,000	0,000	0,000	0,000	
Significatif	HS	HS	HS	HS	HS	

NB: The values of figures bearing the same letters in the same column are statistically equivalent at the 5% threshold (Newman Keuls test)

The witness without biopesticides using showed *Colletrichum* mycelial growth in the PDA environment contrary to different treatments at increasing doses. *Appichi* aqueous extract and *O gratisimum* oil were the most effective. Both products appear to have more repressive effect on the fungus growth. When compared to the witness, the difference is very clear. In addition, we notice that as the concentration of biopesticides increase, a fairly high inhibition rate of *Colletotrichum* is obtained. The fungus inhibition rate is therefore proportional to the aqueous extracts used.

4. Discussion

The in vitro evaluation of biopesticides allowed to obtain foreplay results on their effectiveness against *Colletotrichum*, fungal agent responsible for mango anthracnose. Considering the results obtained, all plants extracts at concentrations of 60 % have had a rate inhibition with efficiency ($75\% < I \leq 90$) according to Kumar et al [7]. The effectiveness of aqueous extracts on the *Colletotrichum* development varied depending to concentrations. The three aqueous extracts; *Cymbopogon giganteus*, *Carica papaya* and *Appichi* inhibit fungus development. However the *Appichi* extract by its composition, composed of several species plants has a more important inhibitory effect on the reduction of fungus mycelial growth compared to *Cymbopogon giganteus* and *Carica papaya*. The effectiveness of this extract could be explained by its antifungal properties, the synergy of active substances of different plant species which would allow it to stop or slow shooting the *Colletotrichum* mycelial production The three aqueous extracts at concentrations of 15 % have an average efficiency ($60\% < I \leq 75\%$) on the growth of *Colletotrichum* growth. The observed inefficiency could be explained by the weak concentrations used or by the resistance capacities of *Colletotrichum* concerned species. In addition to plant extracts used, other plants extracts have been effective against fungus studied. It is the case of *Portulaca oleracea* aqueous extracts and of *Cassia occidentalis* who effectively control the mycelial development of *Bipolaris oryzae*, *Curvularia sp*. and *Alternaria padwickii* Konaté al. [6]. Likewise, Kaboré et al. [5] showed the effectiveness of neem extracts, for *P. oleracea* and *Securidaca ongepe donculata* on *F. moniliforme*, *C. lunata and Phoma sorghina*.) Bonzi et al. [2] has revealed that the extract of *C. occidentalis* limit effectively mycelial development and sporulation of several fungus species. The inhibitory action of essential oils used was observed on the fungus tested at different doses. The results of in vitro tests have revealed that at 600 ppm and 800 ppm the oils *of Cymbopogon c itratus, 0cimum gratissimum, Lippia multifora, Eucalyptus camaldulensis* at concentrations of 600 ppm do not significantly affect the mycelial development of *Colletotrichum*. The minimal concentration having completely inhibited the *Colletotrichum* mycelial growth was 1200 ppm for essential oils. Indeed, the *Colletotrichum* is completely inhibited with oil of *Cymbopogon citratus*, *0cimum gratissimum, Lippia multifora, Eucalyptus camaldulensis* at 1200 ppm ($I > 90\%$), so a very good efficiency at this dose Kumar et al [7]. The different inhibition rates observed show that the different essential oils present interesting antifungal activities. The antifungal properties of *C. citratus* against F. *oxysporum* and *F. moniliiform* was confirmed by Dabiré.et al [4] during the evaluation of the antifungal activity of aqueous extracts of *Cymbopogon citratus, Eclipta alba* and *Portulaca oleracea* against the main fungi transmitted by onion seeds (*Allium cepa L*.) in Burkina Faso. The effectiveness of *L. multiflora* oil was highlighted by Tiendrebeogo et al [12]. against *Pyricularia oryzae*, *Fusarium moniliforme* and *Bipolaris oryzae*. All the essential oils at different concentrations have presented an inhibitory action on the radical growth radial of Colletotrichum compared to witnesses. These results confirm those of Kabore et al [5]. who also noted that the essential oils of the different plants such as *L. multiflora, C. citratus, C giganteus, Ocimum bassilicum* could inhibit at 100% mycelial growth of microscopic fungi. These different tests carried out in this work with the extracts and essential oils at different concentrations, allowed to verify their efficiency on the *Colletotrichum* associated with anthracnose of mango and determine appropriate doses.

5. Conclusion

Antifungal tests in vitro carried out with essential oils of *Eucalyptus camaldulensis, Lippia multifora, 0cimum gratissimum, Cimbopogon citratus* and the aqueous extracts of *Carica papaya, Cimbopogon giganteus* and *Appichi* show that they have significant inhibitory activity with respect to the radial growth of the *colletotrichum* responsible fungus of mango anthracnose. The results also show a very significant inhibition action of the aqueous extract *Appichi* on the *Colletotrichum* comparatively to *Carica papaya* and *Cimbopogon giganteus*. The essential oil of *O. gratissimum* was found to be the most effective.

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Competing interests

Authors have declared that no competing interests exist.

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