



## EFFICACY OF AROMATIC PLANT AND TWO FUNGICIDES ON FUNGI CAUSING ONION BULB ROT IN BURKINA FASO

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### ABSTRACT

This study is focus on research for solutions to fungal diseases involved in the bulb rot in the field. In all, seven fungal species including *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria porri*, *Acremonium strictum* and *Rhizopus stolonifer* have been isolated from rotten onion bulbs. Two conventional fungicides and a botanical extract have been tested dominant fungi such as *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger*, and *Aspergillus flavus*. The results revealed antifungal effects of both fungicides and botanical extract on mycelial growth. Thiophanate methyl has completely inhibited the mycelial growth of *Fusarium solani*, *Aspergillus niger* and *A. flavus*. Mancozèb induced 100% efficacy rate on *F. solani* and *A. niger*; 92.71% and 61.88% respectively on *F. oxysporum* and *A. flavus*. In contrast to fungicides, essential oil of *L. multiflora* at 0.1% was moderately reduced the mycelial growth of most fungi, showing a partial antifungal activity. Efficacy of essential oil of *L. multiflora* was 65.96 and 56.59% respectively for *A. flavus* and *F. oxysporum*; 45.32 and 43.66% respectively for *A. niger* and *F. solani*. Thiophanate methyl had fungicidal activity on *A. niger*, mancozèb used having a fungi static effect.

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## INTRODUCTION

Onion (*Allium cepa* L.), is one of the main vegetable crops introduced in Burkina Faso in 1920. Vegetable crops have increased significantly due to severe drought in 1970 that affected Africa. Nowadays, the development of vegetable crops is an alternative for achieving food self-sufficiency and additive source of income for farmers. In Burkina Faso, onion ranks first with 32.4% of the total vegetable production and 41.4% of the cultivated vegetable area (DPSAA, 2011). There is a trend to higher area planted onion from 16 281 ha in 2013-2014 to 19 496 ha in 2014-2015 with an increase of production between two campaigns from 385 251 tons to 445 568 tons (MARHASA, 2015).

In Burkina Faso, onion is affected by two main difficulties such as diseases and water insufficiency affecting respectively 57.8% and 53.1% of vegetable farmers (DPSAA, 2011). Fungal diseases affect the quality of onion bulbs and are responsible for yield losses. Important fungal diseases include basal rot caused by *Fusarium oxysporum*, dry rot of bulb

caused by *Fusarium solani* and black rot caused by *Aspergillus niger* and *Aspergillus flavus*. Onion seeds are a real storage tank for various pathogens including *Botrytis*, *Fusaria*, *Aspergillus* and *Rhizopus*, as well as bacteria (Currah et al., 1993). *Fusarium* basal rot is the most important onion disease in Burkina Faso. Primary symptom of the disease is yellowing of leaves and stunted growth of plant and later on, the leaves dry from tip downwards. In early stage of infection, the roots of the plants become pinkish in colour and rotting take place later. In advanced stage, the bulb starts decaying from lower ends and ultimately whole plant die (Mishra et al., 2014).

To fight against these devastating fungal diseases, many methods have been developed to reduce incidence of pathogens causing onion bulb rot. Thus, the use of fungicides for seed treatment reduces the rate of transmission of pathogens and reduces the spread of fungal species such as *Botrytis allii*, *Aspergillus niger* and *Fusaria* (Currah et al., 1993). Among these fungicides, benomyl, thiophanate methyl and mancozèb are the most active in seed treatment against the target fungi. Benomyl was removed from the list of pesticides because of its adverse effects on the environment and human health. Other alternative methods including the use of plant extracts have shown their effectiveness in reducing the radial

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growth of fungi. Kaboré *et al.*, (2007) showed that aqueous extracts of plants such as *Azadirachta indica*, *Securidaca longepedunculata* and *Portulaca oleracea* can inhibit the growth of *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme* and *Phoma sorghina*. These extracts also reduced the contamination of sorghum seeds by these fungi by 46.7-61.2% and improved their germination.

But the first step towards the development of more effective control system remains accurate identification of pathogens and parasites that cause complex damage from cultivation to harvesting and storage. This study aims to identify the main fungi of onion bulbs rot in field; and evaluate the efficacy rate of aromatic plant and two conventional fungicides on radial growth of *Fusarium solani*, *F. oxysporum*, *Aspergillus niger*, and *A. flavus*.

## MATERIAL AND METHODS

### Material

#### Plant material

Onion bulb samples used in this study were collected in twelve (12) sites in Central West region of Burkina Faso. Sample collection operations were conducted in 2015 from March to April and concerned two location Koudougou and Reo (Table 1). The different prospecting sites included vegetables production perimeters. Samples were stored carefully in a refrigerator before use. Cooler, envelopes and plastic were used to collected samples.

**Table 1** Sample collection site

Sites	Number of elementary plots	Locations (towns)
Poa yargo	4	Koudougou
Batondo	2	Koudougou
Tenado	2	Réo
Baloumnaba yiri	2	Koudougou
Kougsin	2	Koudougou
Total	12	2

#### Biological material

Four fungi species including *Fusarium oxysporum*, *F. solani*, *Aspergillus flavus* and *A. niger* frequently encountered in onion bulbs in Burkina Faso and causing onion rot were used. These fungi come from those isolated from infected onion bulbs.

#### Conventional fungicides and aromatic plant essential oil

Topsin-M is a systemic fungicide of the Carbamate family and Benzimidazole group. It contains 70% thiophanate-methyl. Biochemically, it inhibits the synthesis of nucleic acid and protein synthesis. Thiophanate-methyl is effective against a wide range of fungal pathogens of vegetable crops. Recommended dose is 1 kg / ha. Agrithane is a contact fungicide with the active ingredient mancozeb is owned by the family of dithiocarbamates. It is insoluble in water (15 to 20 mg / l at 20 ° C) and has excellent persistence. It acts on respiration, inhibits spore germination and mycelial growth of fungi. This fungicide also shows a frenetic action on the development of mites. It is used at 202.5 g / hl of water against fungi. The median lethal dose (LD50) for the rat by ingestion is greater than 8000 mg / kg. *Lippia multiflora* is an aromatic plant used for crop protection in rural area. It is found in the Sudano-Guinean region where it colonizes the undergrowth of woodlands and edges of trails (Fortin *et al.*, 2000). *Lippia multiflora* is widely used in traditional medicine. Fresh leaves

are used to treat malaria, cough, fever, liver disease, diarrhea and colds. The roots are involved in the treatment of gastrointestinal diseases, enteritis and rheumatism (Fortin *et al.*, 2000).

## METHODS

### Isolation and identification of fungi

Onion bulb rot samples are cut into several pieces at the rate of five pieces per sample and per Petri dish, each containing two (02) sterile blotter discs moistened with sterile distilled water. The pieces of onion bulb are placed in the dishes with the help of a forceps, which is each time sterilized with a flame between two samples. Petri dishes containing each sample are placed in the incubator at a temperature of 27 ° C. Thirty-nine (39) grams of dehydrated potato glucose agar, consisting of 4 g of potato infusate 20 g of D (+) glucose and 15 g of agar (PDA) were completely dissolved in one liter of distilled water. The mixture was sterilized by autoclaving at 121 ° C for 30 minutes. The culture medium was cooled in a water bath (approximately 40 ° C.) and then distributed in Petri dishes at the rate of 15 ml of culture medium per dish under a sterile laminar flow.

The growing fungi observed from onion pieces incubated on blotters above were transplanted on solid medium contained in Petri dishes. After three to five days of transplanting, each fungus mycelium is collected with Scotch tape for verification of the corresponding species. The identification of each was confirmed by examining the mycelium and or conidia under a compound microscope. Fungal species were identified based on fungal morphology (color of mycelium, conidia size, number of septa, ...) and compared with the published identification key of Mathur and Kongsdal (2003). After identification, each fungus is transplanted on a PDA medium, in order to obtain a pure colony.

### Preparation of culture media containing the essential oil of *L. multiflora* and two fungicides

Essential oil of *Lippia multiflora* used for this study was extracted by hydrodistillation at the Institute for Research in Applied and Technological Sciences of Burkina Faso. A quantity of 1 ml of *L. multiflora* is introduced into a flask containing 1000 ml of PDA medium and mixed thoroughly before to be distributed into Petri dishes. Five (05) g of thiophanate-methyl were added to 1000 ml of PDA medium and mixed, and the medium was distributed into Petri dishes. As for Mancozèb, a quantity of 3.33 g is introduced into a flask containing 1000 ml of PDA medium in cooling. This concentration of 0.33% is poured into petri dishes.

### Efficacy of aromatic plant and two fungicides against fungi of onion bulbs rot

The essential oil of *Lippia multiflora* at 0.1%, thiophanate-Methyl at 0.5%, and mancozeb at 0.33% per liter of PDA were used to evaluate antifungal activity of growth radial of *F. solani*, *F. oxysporum*, *A. flavus* and *A. niger*. Mycelial explants of 0.5 cm diameter of each fungus were placed in the center of Petri dishes containing the culture media prepared above. The dishes were incubated at temperature of 27 ° C in an incubator. The experiment was repeated four times.

Observations were made at 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 11<sup>th</sup>, 15<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days incubation and the radial growth of fungi was

measured in centimeters. Two diameters perpendicular to each other of the colony were measured. Determination of growth was made by subtracting 0.5 cm (corresponding to explant diameter) from the diameters means of fungal colony. Efficacy rate is determined by Greche *et al.*, (2000) which  $E (\%) = 100 [DMC - DMT] / DMT$ , DMC is mean diameter of control (no treatment) and DMT is mean diameter of treatment. Mean diameter is radial growth of fungus in centimeters. After 28 days of incubation, mycelial explants whose radial growth was zero were transferred aseptically from the tested media into freshly prepared PDA medium. New dishes were incubated under the same temperature conditions as before and were examined daily under a microscope for ten days to detect a possible start of mycelial growth. If a fungus restarts its growth after its transfer to PDA medium, the product used is considered as fungi static; and if a fungus does not develop, the product used is fungicidal.

**Data analysis**

Data were analyzed with software R, version 3.4.1. Post-hoc test of Tukey's multiple-comparison (multcomp package) of efficacy rate of all pairs of *L. multiflora* and two fungicides was used if the ANOVA showed significant differences between treatments at 5% level.

**RESULTS AND DISCUSSION**

**Results**

**Fungi isolated from rotted onion bulbs**

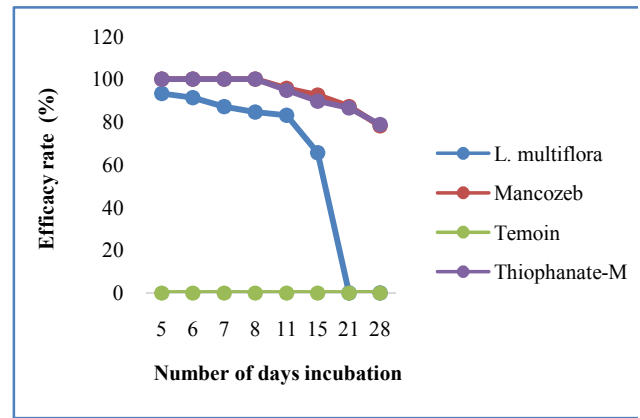
Seven fungal species including *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria porri*, *Acremonium strictum* and *Rhizopus stolonifer* were isolated from rotten onion bulbs (Table 2). The site with highest number of fungi is *Poa yargo*. These isolated fungi belong to four genus, the most important are *Aspergillus* and *Fusarium*, each containing two fungal species. They are also the most representative in the different sites of collection of rotten onion bulbs.

**Table 2** Fungi isolated from rotten onion bulbs in different locations.

Fungi	Locations				
	Poa yargo	Batondo	Tenado	Kougsin	Baloumnaba yiri
<i>A. niger</i>	4	2	0	1	2
<i>A. flavus</i>	4	2	0	1	0
<i>F. solani</i>	2	2	0	1	0
<i>F. oxysporum</i>	3	2	2	1	1
<i>Alternaria porri</i>	1	1	0	0	0
<i>Acremonium strictum</i>	0	1	0	0	0
<i>Rhizopus stolonifer</i>	1	0	0	0	0
Total	15	10	2	4	3

**Efficacy of essential oil of *L. multiflora* and two fungicides on *F. oxysporum***

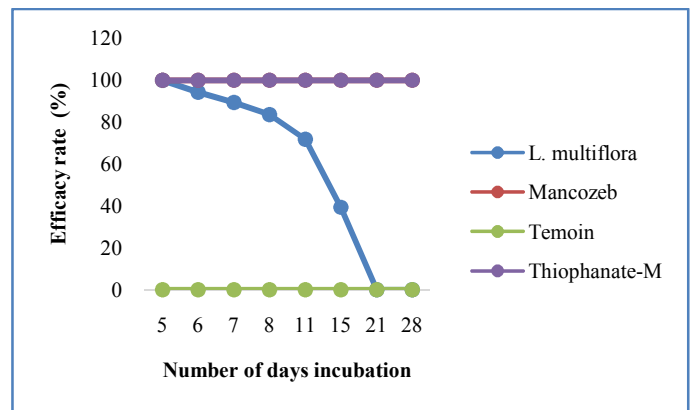
Thiophanate-methyl and mancozeb treatments induced a total inhibition effect on growth of *F. oxysporum* the 5<sup>th</sup> to 8<sup>th</sup> day while treatment with *L. multiflora* (0.1%) was efficacy at 93.21% at 5<sup>th</sup> day and 84.62% at 8<sup>th</sup> day (Figure 1). At 28<sup>th</sup> days, fungicide treatments show a similar efficacy around 79.88% while efficacy of essential oil of *L. multiflora* was zero in this time.



**Figure 1** Evolution of efficacy rate of essential oil of *L. multiflora* and two fungicides on radial growth of *F. oxysporum* from 5<sup>th</sup> to 8<sup>th</sup> day incubation.

**Efficacy of essential oil of *L. multiflora* and two fungicides on *F. solani***

Thiophanate-methyl and mancozeb treatments showed 100% efficacy on *F. solani* from 5<sup>th</sup> to 28<sup>th</sup> day (Figure 2). However, the essential oil treatment of *L. multiflora* showed 100% efficacy at 5<sup>th</sup> day and gradually decreases with time reaching 83.62% efficacy at 8<sup>th</sup> day and 39.37% at 15<sup>th</sup> day; and completely loses its efficacy 28<sup>th</sup> day of incubation.



**Figure 2** Evolution of efficacy rate of essential oil of *L. multiflora* and two fungicides on radial growth of *F. solani* from 5<sup>th</sup> to 8<sup>th</sup> day incubation.

**Efficacy of essential oil of *L. multiflora* and two fungicides on *A. flavus***

Thiophanate-methyl treatments showed 100% efficacy on mycelial growth of *Aspergillus flavus* from 5<sup>th</sup> to 28<sup>th</sup> day (Figure 3). Treatments with mancozeb and essential oil of *L. multiflora* revealed partial inhibition on *A. flavus*. Treatment with essential oil of *L. multiflora* was efficacy 100% during the first eight days of incubations and 96.87% on 11<sup>th</sup> day then the efficacy decreases progressively with 60.31% the 15<sup>th</sup> day and reached 6.56% on 28<sup>th</sup> day. Mancozeb induced 82.35% efficacy on 5<sup>th</sup> day incubation and 76.87% on 8<sup>th</sup> day, then a considerable decrease of efficacy where it stabilizes at 32.03% 28<sup>th</sup> day of incubation.

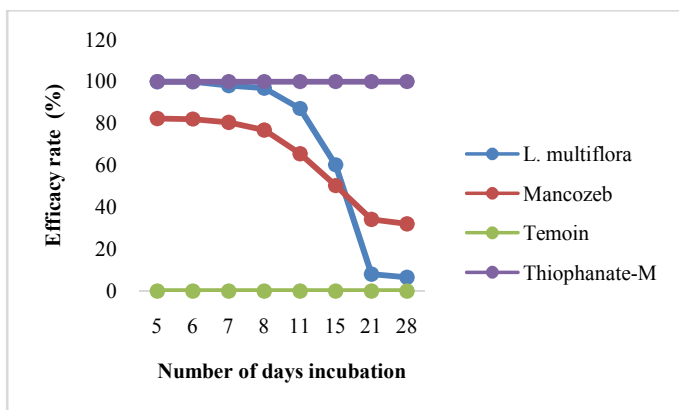


Figure 3 Evolution of efficacy rate of essential oil of *L. multiflora* and two fungicides on radial growth of *A. flavus* from 5<sup>th</sup> to 8<sup>th</sup> day incubation.

**Efficacy of essential oil of *L. multiflora* and two fungicides on *A. niger***

Fungicide treatments with thiophanate-methyl and mancozeb showed 100% efficacy on radial growth of *A. niger* (Figure 4). The essential oil of *L. multiflora* induced a partial inhibition on *A. niger*. Its efficacy rate decreases considerably over time. From 100% efficacy on 5<sup>th</sup> day after incubation, it reaches 76.56% on 8<sup>th</sup> day then registers a downward trend on the 11<sup>th</sup> day with 29.68% efficacy to finally completely lose its efficacy from 15<sup>th</sup> day to 28<sup>th</sup> day after incubation or the fungus reaches its maximum growth in the same way as control.

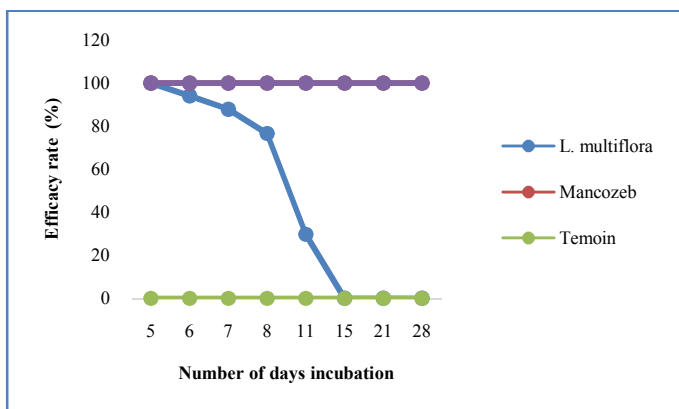


Figure 4 Evolution of efficacy rate of essential oil of *L. multiflora* and two fungicides on radial growth of *A. niger* from 5<sup>th</sup> to 8<sup>th</sup> day incubation.

**Comparison of efficacy rate of essential oil of *L. multiflora* and fungicides on fungal mycelial growth**

Efficacy rate varies with fungi and different fungicides and essential oil of *L. multiflora*. Thiophanate-methyl was induced a total inhibition on radial growth of *F. solani*, *Aspergillus niger* and *A. flavus*; and 92.25% efficacy on *F. oxysporum* (Figure 5). Mancozeb showed a 100% efficacy rate on radial growth of *F. solani* and *A. niger*; whereas it was 92.71% on *F. oxysporum* and 61.88% on *A. flavus*. However, the essential oil of *L. multiflora* was moderately effective compared to fungicide, it revealed an inhibition effect greater than 50% on *A. flavus* and *F. oxysporum* (respectively 65.96 and 56.59%) and less than 50% on *A. niger* and *F. solani* (45.32 and 43.66% respectively). Of these analyzes, Thiophanate methyl was the most efficacy on most fungi followed by mancozeb and finally essential oil of *L. multiflora* whose efficacy was average on growth of fungi studied.

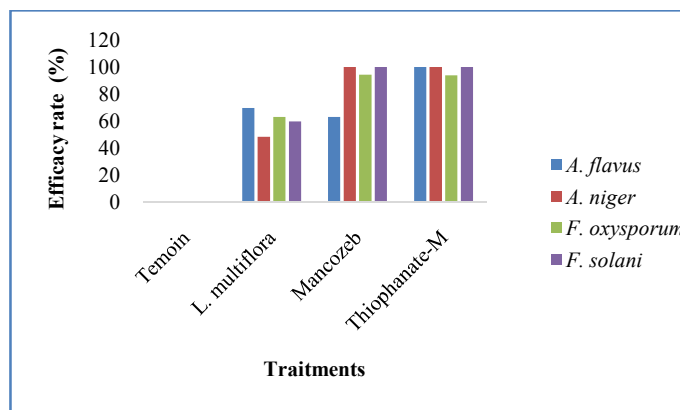


Figure 5 Comparison of efficacy rate of *L. multiflora* and fungicides on fungi radial growth.

**Demonstration of a fungicide or fungistatic**

Mycelial transfer from media treated with the fungicides and essential oil of *Lippia multiflora* into untreated PDA medium allowed some fungi to start growing and colonize the medium, while others failed to grow. Develop, as shown in Table 3. Thiophanate methyl, mancozeb revealed fungistatic activity on mycelial growth of *A. flavus*, *A. niger* and *F. solani* (Table 3). These fungi after their transfer into untreated PDA began to colonize the medium again. However, Thiophanate methyl is a fungicide on *A. niger*, this fungus being blocked after its transfer into the PDA has probably lost viability.

Table 3 viability of fungi after transfer into the PDA medium

Viability of fungi in PDA medium			
Treatments	<i>F. solani</i>	<i>A. flavus</i>	<i>A. niger</i>
Thiophanate méthyl	++	++	--
Mancozèb	++	++	++

++: start of growth, --: loss of viability

**DISCUSSIONS**

Inventory of fungal flora of rotten onion bulbs in Koudougou and Réo in vegetable crop in Burkina Faso have resulted in seven (7) species of fungus. They are *Fusarium solani*, *F. oxysporum*, *Aspergillus niger*, *A. flavus*, *Alternaria porri*, *Acremonium strictum* and *Rhizopus stolonifer*. The most common and involved in onion bulb rot are *usarium solani*, *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavus*. These results are similar to those studied by Shehu *et al.*, (2012) who listed seven (7) species of fungus rotten onion bulbs in five markets of Sokoto in Nigeria including *Aspergillus Niger*, *A. flavus*, *A. fumigatus*, *Alternaria porri*, *Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium citrinum*. Symptoms of most commonly found in vegetable crop prospected are basal and root rots bulbs that are continuing in storage with a bacterial rot. Among various species of *Fusarium* reported as agents of basal rot of onion in the world, included *F. oxysporum* and *F. solani* (Schwartz *et al.*, 2007 ; Zlata *et al.*, 2008). Molecular and pathogenic studies should allow us to specify species of *Fusarium* responsible for onion bulbs rot in Koudougou and Réo. But already our results are similar to some authors who have identified *A. niger*, *F. oxysporum*, *F. solani* in the seeds and rotten onion bulbs of vegetable crops area in Sourou, Dédougou and Ganzourgou in Burkina Faso (Dabire *et al.*, 2016).

Treatments with essential oil of *L. multiflora* and both fungicides helped to inhibit mycelial growth of main fungi involved in onion bulb rot, showing potential antifungal activity. Thiophanate methyl completely reduced mycelial growth of *F. solani*, *A. niger*, *A. flavus* and considerably *F. oxysporum*, thus showing greater antifungal activity and action spectrum wider than other evaluated products. These results are similar to those observed by Attrassi *et al.*, (2007) which showed that benzimidazole fungicides such as thiophanate methyl and benomyl are very efficacious against mycelial growth of *F. oxysporum*, *Aspergillus niger*, *A. fumigatus*, *Rhizopus stolonifer*, and *Trichothecium roseum*. Benzimidazoles and thiophanates have a curative action. Thiophanate methyl has probably interfered with the formation and functioning of microtubules blocking divisions, the elongation of mycelial hyphae and subsequently an irreversible loss of growth of this fungus. However the intensive use of these fungicides has led to development of new resistance (Toquin *et al.*, 2006). In addition to thiophanate methyl, mancozeb was also efficacious on mycelial growth of *F. solani*, *F. oxysporum* and *A. niger*. The efficacy of mancozeb has already been proven on *Fusarium oxysporum* f. sp. *Radicis lycopersici*, responsible for Fusarium wilt and root rot (Soro *et al.*, 2011).

In contrast to fungicides, essential oil of *L. multiflora* at 0.1% was moderately reduced the mycelial growth of most fungi, showing a partial antifungal activity. Efficacy of essential oil of *L. multiflora* was 65.96 and 56.59% respectively for *A. flavus* and *F. oxysporum*; 45.32 and 43.66% respectively for *A. niger* and *F. solani*. However, studies have revealed among oils of plant tested at 5% concentration, only essential oil of *L. multiflora* recorded a 100% inhibition on the spores of *Phaeoisariopsis personata* (Koïta, 2005). Treatments with aqueous extracts of *L. multiflora* at 40 g/L also showed antifungal activity with a 67% inhibition rate on *Pucciniaarachidis* isolate collected in the central region and 71% on western Burkina Faso (Koïta *et al.*, 2012). Kaboré *et al.*, (2007) also found that essential oils of *L. multiflora*, *Cymbopogon citratus*, *C. giganteus*, *Ocimum basilicum* could inhibit 100% mycelial growth of fungi affecting rice. Many factors could explain these differences in results. The concentration used may have been low for essential oil of *Lippia multiflora* l (0.1%). Studies should be continued with an increase in concentration to 1% or 1.5% to better appreciate efficacy rate of this essential oil on fungi tested. It would be necessary also to test fungicides and essential oil of *L. multiflora* in vivo and in real environment to better appreciate rate efficacy of these products on different fungi.

## CONCLUSION

At the end of study, seven (7) fungi were isolated from rotten onion bulbs. These are *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria porri*, *Acremonium strictum* and *Rhizopus stolonifer*. Thiophanate methyl has completely reduced mycelial growth of *F. solani*, *A. niger* and *A. flavus* with 100% efficacy rate. Mancozeb has significantly reduced radial growth of fungi with 100% efficacy on *F. solani* and *A. niger*; 92.71% for *F. oxysporum* and 61.88% on *A. flavus*. The essential oil of *L. multiflora* at 0.1% was moderately reduced mycelial growth of most fungi with 65.96 and 56.59% efficacy respectively for *A. flavus* and *F.oxysporum* , 32 and 43.66% efficacy respectively for *A. niger* and *F. solani*. Only Thiophanate methyl had fungicidal

activity on development of *A. niger*, other fungicides used being fungistatic. These conventional fungicides and botanical extract that showed good efficacy would be able to protect the seedling at transplanting against these fungi. It would be necessary to test fungicides and essential oil of *L. multiflora* in vivo and in real environment to better appreciate efficacy rate of these products on different fungi.

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