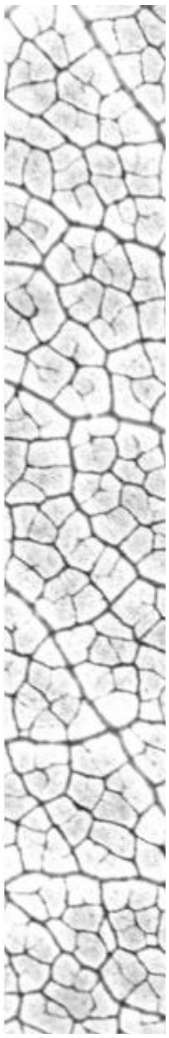

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Use of bioactive chitosan and *Lippia multiflora* essential oil as coatings for maize and sorghum seeds protection

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Abstract
Essential oil (EO) extracted from leaves of *Lippia multiflora* were used alone or combined with chitosan at 0.25% and 0.5% as coating solutions for maize and sorghum seeds. Different coating formulations made were tested on seeds to determine their antifungal activity against *Rhizopus sp* and *A. Flavus* respectively isolated from sorghum and maize seeds. Coating solutions impact on seeds germination rate and plant growth in the laboratory condition were also evaluated. Results revealed that chitosan and *L. Multiflora* EO coating used separately exhibited fungicidal effect against *Rhizopus sp* and fungistatic effect against *A. Flavus.* When there were associated, the coating formulation demonstrated a strong inhibition against *A. flavus* and became ineffective against *Rhizopus ssp.* Chitosan solution (0.25% and 0.5%) without EO significantly increased seeds germination percentage and height maize and sorghum plant. On the other hand, EO coating alone displayed a total inhibition of seeds germination. When EO was mixed with chitosan solution, a decrease in the height of plants was observed.

Keywords: Chitosan, *Lippia multiflora*, coating, antifungal, germination

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INTRODUCTION

Cereal products such as sorghum, maize and rice represent the basis of the diets of most sub-Saharan African populations (Guèy et al. 2011). Maize (*Zea mays*) and sorghum (Sorghum bicolor) are the most important cereals (Abdel-Sater et al. 2017) since they are the staple food in many underdeveloped countries. These two cereals are usually grown in the northern region of Côte d'Ivoire and occupy an important place in the diet of Ivorian people. However, their yields remain constantly low due to unfavorable climatic conditions, poor quality of the cultivated soils and the presence of a variety of parasites (fungi, bacteria, viruses, nematodes and insects). In addition, the germinability of a seed depends closely on its sanitary quality. Regular analyses conducted on cereal seeds have revealed the presence of saprophytic fungi that may either come from a poor control of the drying process used by the producers or the storage conditions. Several kinds of fungi can contaminate maize and sorghum seeds during storage. These fungi could either deteriorate the seeds or simply remain viable to infect germinating seedling. Fungi typically found in stored grains of maize and sorghum are Aspergillus, Penicillium, Fusarium and Rhizopus (Mohamed et al. 2013). Uzma and Shahida (2007) ranked fungi as the second leading cause, after insects, of cereal deterioration and loss. The constant use of chemical products as phytosanitary treatment to maintain healthy seeds gave rise to a greater awareness on their impacts on environment and consumers health. An alternative to these chemicals could be the use of natural products in the treatment of cereal seeds and plants. Many studies on the use of natural products (Lizárraga-Paulín et al. 2013, Mancini and Romanazzi 2014, Orzali et al. 2014) for crop protection led to the development of seed coating technology as a pesticide against crop pests and diseases. Among these natural products, chitosan represents a potential candidate since it can protect both the seeds and the plant from pests by improving the quality of the seeds, its germination potential and seedling growth. Chitosan is a carbohydrate biopolymer derived from deacetylation of chitin, which is found in the crustacean's shells, insect's cuticle, and cell wall of fungi. The positive charge of chitosan confers to this polymer numerous and unique physiological and biological properties which could potentially be exploited in a wide range of industries such as pharmacology, medicine, and agriculture. Chitosan has been used to coat corn, tomato, rice and

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wheat seeds and has been associated to several beneficial effects that include better physiological quality, increased vigor, higher germination rates, induction of plant defenses and good harvests yields (Ziani et al. 2009, Zeng et al. 2012). Yuan et al. (2016) reported a gradual release of drugs and agrochemicals through the application of chitosan. Essential oil of *L. multiflora* can be incorporated in chitosan solution in order to strengthen the coating formulation. Indeed, essential oil of *L. multiflora* has been reported to exhibit a fungicidal (Bassolé and Juliani 2012, Goly et al. 2015), a bactericidal and an insecticidal activity (Ilboudo et al. 2015). It has also been used to protect seed against pest and fungi (Boonlertnirun et al. 2008, Zeng et al. 2012). The purpose of this study was to assess the effect of chitosan and *L. multiflora* essential oil coating on maize and sorghum seeds fungi, seeds germination along with their impact on the growth of the plants.

MATERIALS AND METHODS

Maize and sorghum seeds were provided by a cooperative located in Korhogo (Côte d'Ivoire). Chitosan (>90% DDA viscosity 500–2000 cps) was obtained from France Chitin (Marseille, France). Essential oil of *Lippia multiflora* was extracted from a local plant.

Isolation and Identification of Fungi from Maize and Sorghum Seeds

Seeds were incubated in blotter plate under specific environmental conditions to allow pathogens growth on the seeds. Hundred seeds from maize and sorghum were used in this test. Seeds from each type of cereal were divided into 4 replicates containing 25 seeds each. Seeds were aseptically placed in separate blotter plate moistened with sterile distilled water and incubated at room temperature under alternating cycles of 12h light/darkness for 7 days. After incubation, each petri dish (blotter plate) was examined under stero-binocular microscope for fungi isolation based on identification key. Each fungal colony was examined to identify the form, length and arrangement of conidiophores as well as the size, septation and chain formation of conidia.

Extraction of *Lippia Multiflora* Essential Oil (EO)

Leaves of *Lippia multiflora* were collected in Dikodougou located in the northern region of Côte d'Ivoire. Leaves were dried, away from the sun, for 7 days. Then kg of dried leaves were used to extract the essential oil by steam distillation using a hydro-distillation system. After 3 hours of extraction, the EO obtained was kept in a glass bottle equipped with a screw lid cover. Bottle was hermetically sealed and stored at 4°C.

Chemical Analysis of Essential Oil of *Lippia Multiflora*

Analysis of the essential oil was carried out with the GC / FID and the coupling GC / MS. The analysis was carried out on a FOCUSGC equipped with a CP Wax52 CB capillary column of 15 m × 0.25 mm dimensions with an internal diameter of 0.25 mm (J & W Scientific Column of Agilent Technologies, No. US1670726A, USA). Samples injection was done in splitless mode (injected volume: 1 ml, inlet temperature: 260 °C, split flow: 10 ml / min, splitless time: 0.80 min). Oven temperature was programmed as follows: initial temperature 50 °C; final temperature: 250 °C; temperature gradient: 6°C / min; Isothermal bearing at 250 °C for 5 min. Helium was used as a carrier gas at a constant rate of 1.2 ml / min. The temperature of the FID detector was set at 260 °C. Data was collected and processed with the Chrom Card software. Quantification was done by calculating the areas under the peaks (GC / FID, by the normalization process) and compounds identification was done by comparison of the retention indices with that of the reference.

Preparation of Chitosan Coating Solutions

Chitosan solutions were prepared by dissolving 0.25 or 0.5g of chitosan flakes in distilled water (80 mL) containing 0.7 mL of acetic acid. To allow complete dissolution of the chitosan, solution was stirred overnight at room temperature using a magnetic stirrer. After pH adjustment to 5.5 with NaOH (10%), the final volume was brought to 100 mL by addition of distilled water.

Preparation of the Chitosan-*Lippia multiflora* Coating Emulsion

Chitosan coating solutions prepared were used to make the emulsion. To each chitosan solution (0.25 and 0.5%) a specific amount of *L. multiflora* EO was incorporated to achieve a concentration of 1% (v/v) in the final volume. The mixture was homogenized for 5 minutes using a mixer to obtain the emulsion. Two control solutions composed of distilled water alone and distilled water containing *L. multiflora* EO at a final concentration of 1% (v/v) were prepared and homogenized under the same conditions. All the prepared emulsions were kept out of the light. The different coating solutions involved in this study were: distilled water alone (W), 1% essential oil (EO), 0.5% Chitosan (0.5% CH), 0.25% Chitosan (0.25% CH), 0.5% Chitosan + essential oil (0.5% CH + EO), 0.25% Chitosan + essential oil (0.25% CH + EO).

Assessment of Chitosan and *L. multiflora* EO Antifungal Effect on Maize and Sorghum Seeds

Four replicates of 25 seeds of each of the two cereals (maize and sorghum) were surface disinfected by soaking into 2% Sodium Hypochlorite solution for 2 min and then rinsed five times with sterile distilled water. Soaked seeds were transferred respectively into a 1.10^6 CFU/mL *Rhyzopus spp* inoculum (isolated from

sorghum seeds) and *Aspergillus flavus* inoculum (isolated from maize seeds) for 1 min. Seeds were then aseptically dried for 24 h before soaking them in the different coating solutions for 16 h. Soaked seeds were put on blotting paper slightly moistened with distilled water and placed in a petri dish (9 cm). Incubation was done at room temperature with a photoperiod of 12 hours light and 12 hours darkness. Visual observation of the seeds was carried out every 24h to detect any seeds contamination through the apparition of spores. Reading was performed when 50% of the seeds of a box were contaminated by molds. The contamination index after treatment was evaluated as follows:

Percent inhibition (PI) = (Gc/Gt) *100

where Gc= number of contaminated seeds and Gt = number of total seeds in the box.

Evaluation of Solution Coating Effect on Maize and Sorghum Seeds Germination

In the laboratory, seed germination test was performed by applying the rules for seed testing of the filter paper method. After 24h of soaking into the coating solution, 100 seeds taken from each group were arranged on wet filter paper in Petri dishes. Each Petri dish contained 25 seeds, and each treatment was replicated 4 times. All Petri dishes were incubated in a laboratory ambient temperature. Germination Percentage (GP) was determined when at least 50% of seed germination was observed in a group. The calculation was done as follows:

GP = Sg / St

where Sg is the number of germinated seeds and St is the number of total seeds investigated.

Effect of Solution Coating on Plant Height

Maize and sorghum seeds were individually mixed for 24h with each of the seed-coating agents. After 24 h of air-drying at the laboratory temperature, 10 of the maize and sorghum seeds were planted in pot containing compost soil. After 45 days of growth, plant height (cm) was determined. For each treatment, five measurements were done.

Statistical Analysis

All data collected from the experimental design used in this study were subjected to a non-parametric test and Kruskal-Wallis was used to compare the means of the different treatments. Xlstat 2014 were used.

RESULTS

Isolation and Identification of Fungi from Maize and Sorghum Seeds

In blotter test, predominance fungi isolated were *Aspergillus sp* (maize and sorghum), *Fusarium sp* (maize) and *Rhyzopus sp* (Sorghum). *Aspegillus sp* represented the highest fungi with 65% on maize and 51 % on sorghum followed by *Rhyzopus sp* (30%) from sorghum and fusarium sp from maize (**Fig. 1**). Others sp (no indentified) represented about 5% on maize and sorghum seeds.

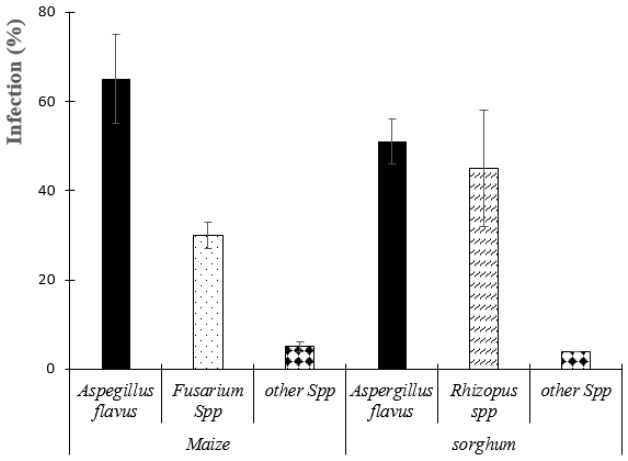

Fig. 1. Main fungi isolated from Maize and Sorghum seeds

Table 1. Chemical composition of *Lippia multiflora* essential oil

Compound	%	Compound	%
alpha-pinene	**9,20**	Caryophyllene	2,13
Sabinene	1,64	4-terpineol	0,02
α-phellandrene	**42,63**	rose furan epoxide	0,19
alpha-terpinene	0,05	1-terpineol	0,10
L-limonene	0,56	alloaromadendrene	0,05
β-phellandrene	**11,05**	alpha-humulene	0,53
β-ocimene Y	2,93	trans-beta-farnesene	1,39
p-cymene	**11,21**	z-citral	3,35
α-terpinolene	0,37	(-)-alpha-terpineol	0,08
6-methyl-5-hepten-2-one	1,30	germacrene-D	1,41
Linalool	0,41	α-muurolene	0,03
2-cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	0,24	**e-citral**	**7,64**

Chemical Analysis of Essential oil of *Lippia Multiflora*

Analysis and identification of the volatile constituents of *L. Multiflora* essential oil was done through GC-MS analysis. Main constituent of EO are listed in **Table 1**. Monoterpenes were the most abundant volatiles detected in this oil. It was characterized by its richness in L-phellandrene (42.63%), P-cymene (11.21%), beta-phellandrene (11.05%), alpha-pinene (9.20%) and e-citral (7.64%).

Evaluation of Antifungal Effect of Chitosan and *L. multiflora* EO on Maize and Sorghum Seeds

The antifungal effects of the different treatments applied on maize and sorghum seeds are respectively summarized in **Table 2** and **3**. In **Table 2**, we observed a decrease of the inhibition percentage of the different coating during storage. Significant difference between treatments effects on *Aspergillus flavus* inhibition was observed after 3 days of storage. Chitosan and EO coating as well as their emulsion efficiently inhibited *A. Flavus*. But, after 6 days of storage, all inhibitions against *A. Flavus* were lowed. Nevertheless, chitosan mixed with EO presented a high percentage of inhibition.

Table 2. Antifungal activity of the coating solutions on maize seed

	3 days	6 days	9 days
W	22.67^{b} ± 12	0^{c} ± 00	0^{c} ± 00
EO	100^{a} ± 00	26.67^{bc} ± 00	0^{c} ± 00
0.25% CH	92^{a} ± 10	56^{ab} ± 4	34.67^{ab} ± 18.20
0.25% CH+EO	72^{a} ± 20	59.33^{bc} ±12.2	54^{a} ± 10.58
0.5% CH	92^{a} ± 4	53.33^{ab} ± 18	49.33^{b} ± 12
0.5% CH+EO	100^{a} ± 00	69.33^{a} ± 6.11	57.33^{a} ± 4.61

Means are averaged values of three trials. Each trial contained three replicates per treatment. Values within a column with the same letter are not significantly different (p > 0.05)

Table 3. Antifungal activity of the coating solutions on sorghum seed

	2 days	4 days	10 days
W	0^{b} ± 0	0^{C} ± 0	0^{b} ± 0
EO	98.67^{a} ± 2.3	96^{a} ± 6.93	89.33^{a}±15
0.25% CH	86.67^{a} ± 4.61	72^{b} ± 4	81.33^{a}± 6.10
0.25% CHI+EO	89.33^{a} ± 12.2	0^{c} ± 00	0^{b} ± 0
0.5% CH	88.67^{a} ± 2.3	76^{b} ± 4	73.33^{a} ± 2.31
0.5% CH+EO	78.67^{b}±16.65	0^{C} ± 0	0^{b} ± 0

Means are averaged values of three trials. Each trial contained three replicates per treatment. Values within a column with the same letter are not significantly different (p > 0.05)

A maximum level of inhibition was recorded (69.33%) for 0.5 % chitosan + EO emulsion. After 9 days of storage, EO lost all fungistatic activity leading to total destruction of the seeds. However, 57% of maize seeds have been preserved by 0.5% CH+EO coating followed by 0.25% CH+EO (54%) and coating made with 0.5% and 0.25% chitosan alone. The results revealed that the antifungal effect of *L. multiflora* on *Aspergillus flavus* lasted only for 3 days and became totally inefficient after 9 days. Instead, 0.25% and 0.5% chitosan exhibited an efficient protection of maize seeds against *A. flavus*. Moreover, chitosan protecting effect was better when associated with L. *multiflora* EO.

Table 3 points out significant difference in the effect of all the treatments applied on sorghum seeds against *Rhizopus sp.* growth. Results showed that after 2 days of storage time, all the seeds coated in water were infected while those coated either with EO, chitosan or their emulsion inhibited efficiently *Rhizopus sp.* Both the 0.25 and 0.5% CH emulsified with EO lost their antifungal activity after 4 days while the individual coating (EO, 0.25% CH and 0.5% CH) remained effective up10 days.

Impacts of Coating Solution on Maize and Sorghum Seeds Germination

The impacts of coating solution on maize and sorghum germination are presented in **Table 4**. Data are defined as percentage of germination at 3 days after incubation on blotter test. Significant difference among coating treatment was observed at the 5% level. Highest seeds germination was recorded for chitosan at 0.25% (96% of maize and 98% of sorghum seeds) and chitosan at 0.5% (90% of maize and 96% of sorghum seeds). No germination was observed for seeds soaked in *L. Multiflora* essential oil. Moreover, a reduction of the percent of maize seeds germination was observed when

Table 4. Effect of the coating solutions on the percentage of germination

	Maize (%)	Sorghum (%)
W	81.33^{c} ± 2.31	65.33^{c} ±0
Uncoated	84^{c} ±4	88^{b} ±10
EO	0 ^{d} ± 0	0^{e} ±0
0.25% CH	96^{a} ±4	98^{a} ±6
0.5% CH	90 ^{ab} ±4	96^{a} ±4
0.25% CH + EO	88^{bc} ±3	18.67^{d} ± 10
0.5% CH + EO	85.33^{c} ±6.1	17.33^{d} ±13

Means are averaged values of three trials. Each trial contained three replicates per treatment. Values within a column with the same letter are not significantly different (p > 0.0)

EO was added to the chitosan solution. Furthermore, the percent germination of uncoated maize seeds (84%) was not statistically different to that of seeds coated with water (81.33%) and with 0.25 or 0.5% CH emulsified with EO (88% and 85.33% respectively). Sorghum seeds coated with 0.25 or 0.5% chitosan solution exhibited highest percent of sorghum seeds germination (98% and 96% respectively), followed by the uncoated ones (88%). Here too, no germination was observed for seeds coated with *L. multiflora* EO. A light decrease in the seed germination was found when chitosan coating was mixed with the *L. multiflora* EO thus reflecting a slowing down of chitosan coating effect in presence of EO. Reduction of the percent germination of seeds coated with chitosan emulsified with EO was more noticeable with sorghum seeds than maize.

Impacts of Coating Solutions on the Height of the Plant

Figs. 2 and **3** exhibit the impacts of seeds coating solutions on the height of maize and sorghum plants during growth. As compared to the plant derived from the uncoated seeds, an enhancement in the plant height was obtained for seeds (maize and sorghum) coated with all the coating solutions except with EO. Highest enhancement of plants height was noticed when seeds (maize and sorghum) were coated with chitosan solutions only. Moreover, an increase in plant height was remarked when concentration of chitosan solution was increased from 0.25% to 0.5%. In all cases, addition of EO to chitosan coating solution resulted in a decrease of the plant height. This decrease caused by the presence of EO was more perceptible with sorghum plants than maize. Results from this study have revealed the growth stimulation effect of chitosan on maize and sorghum seeds. However, this stimulation effect was reduced, especially with sorghum seed, when *L. multiflora* EO was added to the chitosan coating solution.

DISCUSSION

Blotter test revealed the great impact fungal pathogens have on maize and sorghum seeds during storage. High rate of infestation of seeds was observed with *A. Flavus*. Indeed, *A. Flavus* has been reported by Tsedaley and Adugna (2016) as weel as by Boukaew et al. (2017) to be one of the main fungal strain infesting

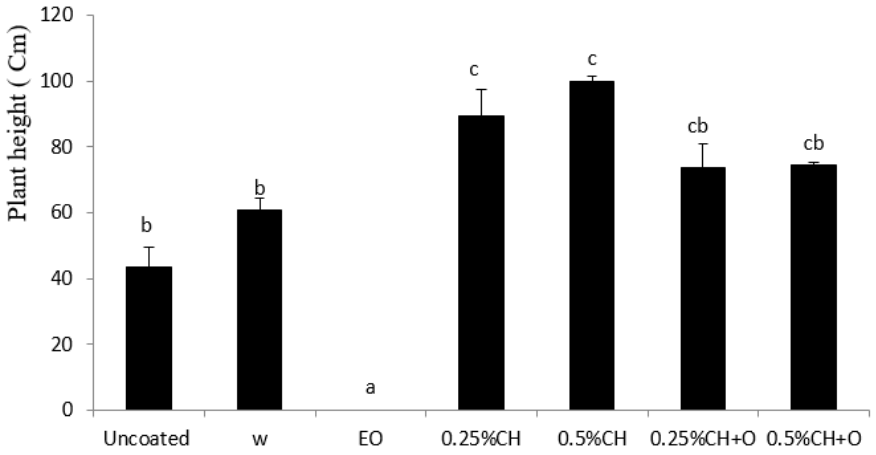

Fig. 2. Effect of the coating solutions on maize plant height

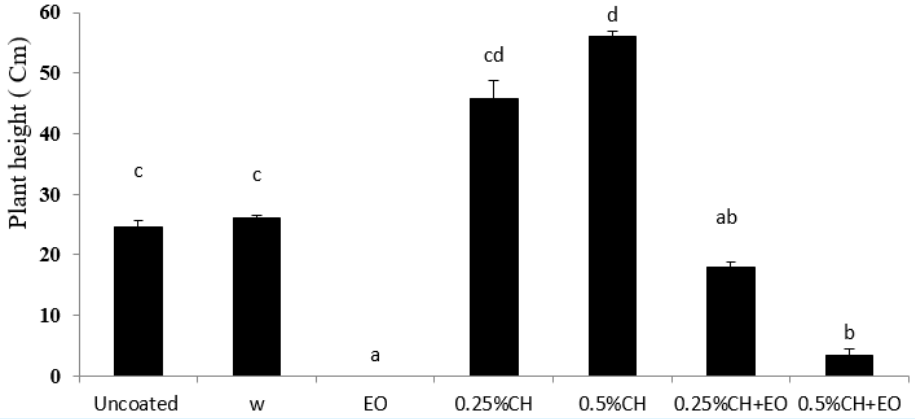

Fig. 3. Effect of the coating solutions on maize plant height

cereal seeds. This study revealed that the second fungal pathogen of sorghum seed was *Rhizopus Spp.* and fungal activities implicating this strain had recently been demonstrated (Abdel-Sater et al. 2017, Adebolu et al. 2018, Ologunde et al. 2018). According to Anjorin et al. (2008), the prominent fungi isolated from sorghum seeds were *Rhizopus nigrican* followed by *Aspergillus Flavus*. *A. Flavus* and *Rhizopus ssp* were reported to be responsible for losses of seed occurring during seed development, storage and may result affect seed viability or from seedling infection following germination.

According ours results, *L. multiflora* essential oil to contained high amount of α-phellandrene, P-cymene, beta-phellandrene, α-pinene and e-citral. This chemical composition was different to that reported by Owolabi et al. (2009) who indicated the presence of high levels of 1,8-cineole, sabinene, terpineol and pinene in *L. Multiflora* essential oil. This observed variability in the composition could probably be due to a number of interacting factors such as ecological origin, environmental stress (soil type, humidity, mechanical damage and cultures) and genetic factors (Kunle and Egharevba 2012). The high levels of P-cymene and α-phellandrene in *L. multiflora* essential oil had been respectively indicated by Bassolé et al (2003) and Avlessi et al (2005). Moreover, these two chemicals of *L. multiflora* essential oil were reported by Badawy and Abdelgaleil (2014) to exhibit antifungal activities.

Concerning the effectiveness of chitosan and *L. multiflora* essential, these two products have mainly been known to possess antifungal activities. Recently, the beneficial effect of chitosan on seed conservation before planting has been revealed. This present study was conducted to assess not only the antifungal activity of chitosan associated with *L. Multiflora* EO but also the impact of these coating solutions on maize and sorghum seeds germination and plants growth. Our research indicated that chitosan and *L. Multiflora* EO acted as seed coating agents able to control *A. flavus* and *Rhizopus sp* development. Besides, it was noticed that coating formulations made with chitosan maintained seed functionality and exhibited a fungicide effect on the seed surface. Antifungal activities of chitosan have previously been reported. Indeed, it has been used to coat many seeds and demonstrated an efficient activity to protect seeds against *Aspergillus ssp*. (Abdelbasset et al. 2010, Pichyangkura and Chadchawan 2015, Praveen et al. 2019, Won et al. 2018). Chitosan, as a novel seed disease inhibitor, could favor and improve seed resistance to disease by displaying a fungicide

effect on the mold in the soil. Additionally, it could increase soluble sugar content and enhance protease activity hence leading to an increasing level of free amino acids which have demonstrated obvious inhibiting effect for many plant pathogenic fungi. The use of *L. Multiflora* essential oil to preserve seed against fungi strains has also been stated. Indeed, *L. Multiflora* essential oil was reported to efficiently protect maize (Ezoua et al. 2017), Tomato (Soad and Elwagia, 2015), Sorghum (Bonzi et al. 2013) seeds from fungi growth. In our study, coating formulation made from chitosan and *L. Multiflora* essential oil demonstrated an efficient protection on maize seeds but not on sorghum. The strengthening of the antifungal activity of chitosan by addition of essential oil has been reported (Noshirvani et al. 2017, Perdones et al. 2016). Protection of strawberries fruits from *A. niger, B. cinerea and R. stolonifera* growth was achieved using chitosan emulsified with lemon essential oil (Noshirvani et al. 2017). Similar results were reported for chitosan + Mexican oregano essential oil against *A. niger* and *Penicillium sp*p (Avila-Sosa et al. 2012) and also on chitosan + *Eucalyptus globulus* against *C. albicans* and *C. parapsilosis* (Hafsa et al. 2016). Emulsion made from chitosan and EO displayed significant improvement in mycelial inhibition as compared to pure EO or chitosan. Mechanisms involved in the synergistic effect of chitosan and EO could be could be explained by the fact that EO alters the fungal cell wall surface and structure while chitosan acts as a potentiator by reducing cell wall synthesis thus facilitating the leakage of fungal cytoplasm (Mohammadi et al. 2016). I should also be noted that EO nature could affect the antifungal activity of chitosan coatings. Perdones et al. (2012) noticed a decrease in chitosan antifungal action against *Botrytis cinerea* when chitosan coating was mixed with lemon EO.

In this study, soaking the seeds in chitosan coating solution (0.25% and 0.5%) resulted in a great increase in the percentage of seed germination and plant height.

As regards to the impact of chitosan on seed germination, various effects have been outlined. An inductive effect on germination and plant height for some species, such as Sorghum or Egyptian anise has been described. However, an inhibitory effect on germination was reported for lettuce seeds. Other studies reported that chitosan may or may not affect seed germination rates and plant height as compared to control treatments. These variable effects could be attributed to the chitosan nature, its molecular size, crop characteristics and growth conditions (Lizárraga-Paulín et al. 2013, Mahdavi and Batool 2013, Pichyangkura and Chadchawan 2015). Chitosan positive effects on seed germination and plant height have been demonstrated in this study. Results showed that percentage of germination and plant height increased with increasing chitosan concentration; similar result was also reported by Mahdavi and Batool (2013).

Effect of EO on seed germination and plant height has also been discussed herein. Results have revealed a negative impact of EO on seed germination and plant height. Similar observations have been outlined by Pauldel and Gupta (2008) as well as by Liu et al. (2006) on the hinibitory effect of *L. Multiflora* essential oil on seeds germination and plant height. But, study from Montes-Belmont and Carvajal (1998) displayed no inhibitory effect of EO on maize germination. Report from Bonzy et al. (2013) study on *L. multiflora* essential oil coating showed no inhibition effect on sorghum seed germination but a significant lowering effect on germination rate. This discrepancy in EO coating effects on seed germination could be ascribed to the soaking time, EO concentration and EO constituents.

Maize and sorghum seeds germination was differently affected by the coating emulsion made from EO and chitosan. When compared to maize a sharp decrease in sorghum seed germination was perceived with the use of chitosan emulsified with EO hence showing a greater sensitivity of sorghum seed to the presence of EO.

CONCLUSION

This study revealed the presence of several fungi mainly *Aspergillus flavus* and *Rhizopus sp.* on maize and sorghum seeds. Among all the treatments used to control fungi growth, chitosan displayed a fungicidal effect against *Rhyzopus ssp* and a fungistatic effect against *A. Flavus.* Using chitosan solution as seed coating enhanced the percentage of seed germination and seedling size. When seeds were coated with *L. Multiflora* EO, an inhibitory effect on *Rhyzopus sp* growth and a fungistatic effect against *A. Flavus* were noticed. However, using EO as coating totally inhibited maize and sorghum seeds germination. Furthermore, using EO and chitosan emulsion as coating inhibited the antifungal activity of chitosan against *Rhizopus sp,* decreased germination percentage and delayed seedlings growth. Therefore, the combination of *L. multiflora* EO with chitosan could be used to conserve maize and sorghum seeds.

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