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International Journal of Current Research
Vol. 9, Issue, 05, pp.50900-50905, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF ESSENTIAL OIL OF *CYMBOPOGON CITRATUS* ON SEED-BORN FUNGI AND SOYBEAN SEEDS PERFORMANCE

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ARTICLE INFO

Article History:
Received 09th February, 2017
Received in revised form
20th March, 2017
Accepted 04th April, 2017
Published online 31st May, 2017

Key words:

Cymbopogon citratus,
Essential oil,
Antifungal activity,
Soybean, Seeds,
Vigour index.

ABSTRACT

Antifungal activity of essential oil of *Cymbopogon citratus* against seed-born fungi, the possible effect on seed germination and seedlings vigour were investigated. Five soybean seeds varieties naturally infected and collected from IRAD of Dschang (MAGBA and TGX-1835-10E), and three from IRAD of Foumbot (TGX-1910-14F, HOULA 1, ENGOPA 316) were used. Soybean seeds were distributed among storage plastic, treated with essential oil and stored for 40 days in storage conditions as defined by IRAD. Seed germination rates, fungal seed infection and seedlings vigour were evaluated each 10 days of storage. About 18 fungal species were isolated on all the seeds varieties by using blotter and agar technique respectively. According to both methods, fungi which were frequently isolated were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum Fusarium moniliforme, F. solani, Cercospora kikuchii*. Soybean seed treatment with essential oil of *C. citratus* significantly reduce seed infection by fungi and also improves soybean seed germination for MAGBA, TGX-1835-10E and TGX-1910-14F varieties. ENGOPA 316 and HOULA 1 varieties treated with essential oil showed a non-significant reduction in germination. Soybean plants obtained from seeds treated with essential oil of *C. citratus* grow very well than those coming from untreated seeds. Essential oil of *Cymbopogon citratus* for seed treatments may become a bio-fungicide for soybean crop protection.

Citation: YAOUBA Aoudou and ESSOLA ETOA Louis Childeric, 2017. "Effect of essential oil of *Cymbopogon citrates* on seed-born fungi and soybean seeds performance", *International Journal of Current Research*, 9, (05), 50900-50905.

INTRODUCTION

Soybean is a primary source of vegetable oil, protein concentrates and starch (Anonymous, 2013; Venugopal *et al.*, 2015). It is an excellent source of major nutrients, about 40% of dry matter is protein and 20% fat (CaldWell, 1973). Due to these nutritional qualities, it is highly used in human and animals nutrition. In Cameroon, production is mainly carried out in the north and west regions despite the great potential of the country. However, its production is very low in Cameroon because of some constraints such as foliar diseases in which the most recent is the rust, the attacks of pests, and lack of access to improved seeds of good quality (IRAD, 2013). Seed pathogens have various methods of infection. Some pathogens are soilborne, such as *Pythium*, and are present in the soil. Seed pathogens can be seedborne, seed transmitted, or both. Seedborne pathogens are found on the surface or inside the seed (McGee, 1994). The environmental conditions that promote seed germination usually are favourable for pathogen growth and seed infection.

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Soybean seeds are thus infected with various seed borne microorganisms, including fungi, bacteria and viruses. Many researchers have reported that *Aspergillus flavus, A. niger, Cercospora kikuchi, Macrophomina phaseolina, Fusarium oxysporum* are the most associated soybean fungi (Krishnamurthy *et al.,* 2006 and 2008; Impullitti *et al.,* 2013; Lakshmeesha *et al.,* 2013 and 2014; Venugopal *et al.*, 2015). These fungi are responsible for the loss of germination potential, loss of seedling and rot of seed. Disease free quality seed production in soybean is of utmost importance to sustain productivity and maintain the quality of the crop. The conservation of seeds in Cameroon especially those of soybeans requires much attention. Seeds conservations technologies are very specifics because the embryo have to be kept in good condition to prevent decreasing of germination percentage. The purpose of any seed treatment is to improve seed performance in one or more of the following ways: eradicate seed borne pathogens or protect from soil-borne pathogens ; optimize ease of handling and accuracy of planting (reduce gaps in stand or the need for thinning of seedlings, particularly when mechanical planters are used), and improve germination percentages and rates" (Gatch, 2014). Unfortunately, there are very few organic seed treatments that encourage seedling growth and inhibit fungal growth.

Traditionally, chemical treatments are used widely to protect the germinating seedling, during vegetative and reproductive growth and after harvest from pathogenic fungi infection (Aleieri *et al.,* 1984). Currently, the use and expectations of chemical treatments are greatly concerned due to the impact of the chemical to the environment, which can be primary or secondary influences on human or other living organisms (Baruah *et al*., 1996). So new strategies for fungicide use and disease management must be developed and identified. The essential oils of some aromatic plants have antifungal properties. When these essential oils are applied to the seed, they eliminate the soilborne and seedborne pathogens. Velluti *et al*. (2004) showed that the essential oils from oregano, clove, cinnamon, lemon grass, and palmarosa oils reduced the levels of *Fusarium verticillioides* infection in maize seeds. These essential oils also reduced the amount of fumonisins produced by the fungi. Essential oil of *Cymbopogon citratus* (Lemon grass) have shown their efficiency in controlling *Aspergillus flavus, Penicillium expansum, A. ochraceus, P. verrucosum, Listeria monocytogenes, S. aureus, E. coli, S. typhimurium* (Burt, 2004; Nguefack *et al.,* 2009, 2012). Lemon grass powder and essential oil, have showed great efficiency to protect cowpea seed against physical deterioration and mouldiness. Furthermore, it inhibited the growth of some fungi like *A. flavus* and *Penicillium chrysogenum* Thom since it does not affect germination (Adegoke and Odesola, 1996). The objectives of this study are to evaluate the efficacy of the essential oil of lemon grass at controlling seedborne pathogen in some soybean varieties and to determine its effect on seedling vigour.

MATERIALS AND METHODS

Plant material and extraction

Fresh leaves of lemon grass have been collected at Dschang locality in West Region of Cameroon at April 2016, and dried naturally at room temperature for five days. Extraction of essential oil was done in Laboratory of Biochemistry of University of Dschang by hydro distillation process for about 5 hours, using a Clevenger apparatus. Oil recovered in a dark sterile glass was dried over anhydrous sodium sulphate and stored at +4 °C until it was used (Sessou *et al.,* 2012).

Soybean seeds varieties

Five varieties of soybean seeds have been used: MAGBA, TGX-1835-10E (collected from IRAD of Dschang), HOULA 1, ENGOPA 316, and TGX-1910-14F (collected from IRAD of Foumbot). Seeds where collected in store conditions as recommended by IRAD. They were naturally infected after harvesting.

Seeds treatment

Treatment have been made using the method of Yaouba *et al*. (2012) modified and adapted to seed storage conditions as defined by IRAD. Therefore, four small bundles of 15g have been made for all varieties and each of them have been treated with one of the three concentrations of essential oil (0ppm, 2000 ppm, 4000 ppm, 6000 ppm) and then stored for 40 days at room (laboratory) temperature (22±2°C) (figure 1). Data were collected after every 10 days using Blotter paper and Agar plate methods for identification of seed-borne fungi and to evaluate the effect of essential oil on seedling vigour. Plastic tray were used to evaluate percentage of seeds germination and seedling vigour. Vigour index have been calculated using the formula of Varadarajan & Rao (2002) as shown below:

Vigour index (VI) = Percent germination of seed × (Root length + Shoot length). In both experiment, untreated seeds for each variety and stored at the same periods served as control. Complete randomized design (CRD) was used in the experiments and each treatment was repeated thrice.

Figure 1. Treated seeds and stored in storage plastic

Statistical analysis

Statistical analysis of data of three independent replicate trials were done using R version 3.2.3 and SPSS 21 (IBM). Differences between means were tested using Duncan test at $p< 0.05$, and principal components analysis (PCA) has been carried out in order to show relations between experimental factors.

RESULTS AND DISCUSSION

Prevalence of seed-born fungi of soybean varieties

A total of 18 fungal species have been identified on all the soybean seeds varieties. Variety TGX-1835-10E was mostly infected by *A. flavus* (6.2%) and *A. Niger* (10.2%). *Fusarium moniliforme, F. solani, F. oxy*sp*orum* and *Cerco*sp*ora kikuchii* (11.1% on ENGOPA 316) were present on all varieties with high infection on all varieties collected from Foumbot locality. *F. oxy*sp*orum* was present on ENGOPA 316 (5.4%) and absent on both MAGBA and TGX-1835-10E collected from IRAD of Dschang. *Phomopsis* sp., *Rhizopus stolonifer,* and *Colletotrichum demaintium* causative agents of high losses of germination and seedling losses have been identified on the seeds collected from Foumbot. Other fungi like *Botrytis* sp, *Chaetomium* sp, *Cladosporium cladosporioides, Melanospora zamiae, Mucor* sp*, Nigrospora* sp, *Penicillium* sp, *Peronospora* sp, *Phoma* sp, and *Trichoderma* sp were also isolated.

Effect of essential oil on seeds germination

Table 1 shows results of effect of essential oil on germination of soybean seeds after 0; 10 and 40 days of storage. Sometime after soybean seed treatment with essential oil of *C. citratus*, the control (0 ppm) present percentages of germination ranging from 55 (TGX-1910-14F) to 95 for TGX-1835-10E.

Table 1. Germination percentage of treated soybean seed

	Germination percentage			
Varieties	0 ppm	2000 ppm	4000 ppm	6000 ppm
		Storage days : 0		
ENGOPA 316	71.7 ± 22.6a	72.3 ± 10.8a	68.1 ± 3.5a	78.3 ± 22.9a
MAGBA C.C	91.8 ± 2.7a	91.8 ± 5.9a	90 ± 1.3a	91 ± 1a
TGX-1835-10E	95.1 ± 1.4a	92.5 ± 3a	90.2 ± 3.7ab	87.3 ± 3.7b
HOULA 1	63.4 ± 12.6ab	73.2 ± 3a	71.3 ± 7.5a	50.5 ± 0.5b
TGX-1910-14F	55.2± 4.2b	92.7 ± 3.2a	79.6 ± 7.4a	72.4 ± 2.2a
		Storage days : 10		
ENGOPA 316	71.7 ± 22.6a	78.7 ± 5.8a	70.7 ± 4.2a	61.2 ± 5.8a
MAGBA C.C	97.5 ± 5a	95.3 ± 5a	91.7 ± 1.6a	93.6 ± 3.3a
TGX-1835-10E	98.9± 0.1a	89.4 ± 4.2b	92.9 ± 6.6ab	91.2 ± 5.8ab
HOULA 1	64.5 ± 9.2a	46.4 ± 3.4b	67 ± 7.5a	55.2 ± 9.2ab
TGX-1910-14F	60.7± 12.4a	92.3 ± 2.5b	77.1 ± 6.6bc	85.9 ± 0.8c
		Storage days : 40		
ENGOPA 316	71.7 ± 22.6a	55.9 ± 0.8a	60 ± 0.0a	58.7 ± 5.8a
MAGBA	97.5 ± 5a	90 ± 4.1a	76.6 ± 10.9a	91.7 ± 1.6a
TGX-1835-10E	98.9± 0.1a	88.2 ± 1.6b	87.7 ± 2.5b	98.2 ± 1.6a
HOULA 1	64.5 ± 9.2a	67.1 ± 6.7a	71.1 ± 8.4a	55.9 ± 0.8a
TGX-1910-14F	60.7± 12.4a	90.5 ± 4.1b	78.2 ± 1.6b	85.9 ± 0.8b

Values with the same letter on the same line are not significantly different at P ≤ 0.05 according to Duncan test

Table 2. Fungal infection percentage of treated soybean seed during storage

	Infection percentage by concentrations			
Varieties	0 ppm	2000 ppm	4000 ppm	6000 ppm
		Storage days : 10		
ENGOPA 316	53.7± 15.3a	21.1 ± 6.6b	35.7± 13.4b	11.7 ± 1.6b
MAGBA	42 ± 19a	14.7 ± 9.2ab	3.5 ± 3.3b	7.1 ± 6.6b
TGX-1835-10E	67.2 ± 4.9a	14.1 ± 0.8b	7.1 ± 6.7b	4.1 ± 0.8b
HOULA 1	62 ± 30.1a	10.5 ± 4.1bc	5.9 ± 0.8c	21.7 ± 1.6b
TGX-1910-14F	67.8± 32.3a	44.7 ± 5ab	15.9 ± 0.8c	14.7 ± 9.2c
		Storage days: 40		
ENGOPA 316	53.7± 15.3a	27.40 ± 4.1ab	26.4 ± 3.4b	8.9 ± 8.4c
MAGBA	42 ± 19a	17.6± 2.5 bc	11 ± 1.6c	33.6 ± 3.3ab
TGX-1835-10E	67.2 ± 4.9a	24.7 ± 5b	5.3 ± 5c	29.4 ± 4.1b
HOULA 1	62 ± 30.1a	14.7 ± 9.2b	10.7 ± 10b	1.7 ± 1.6b
TGX-1910-14F	67.8± 32.3a	19.9± 14.2b	51.3 ± 10ab	42.3 ± 2.5ab

Values with the same letter on the same line are not significantly different at P ≤ 0.05 according to Duncan test

Table 3. Vigour index of treated soybean seeds after 40 days of storage

	Vigour index by concentration			
Varieties	0 ppm	2000 ppm	4000 ppm	6000 ppm
		Storage days : 0		
ENGOPA 316	743.8± 154.6a	1259 ± 144.5b	1081.7 ± 163.2b	1115.3 ± 238.9b
MAGBA	1069.6± 154.5a	1188.3 ± 103.7a	1196.7 ± 27.7a	1180 ± 141.1a
TGX-1835-10E	803.3 ± 259.3a	931.7 ± 52a	1036.7 ± 81.4a	813.3 ± 168.5a
HOULA 1	1344 ± 49.6a	901.3 ± 217.2b	1136.3 ± 7.5ab	955.7 ± 125.7b
TGX-1910-14F	955.7 ± 212.7a	1097.3 ± 121.2a	650 ± 52.9b	1039 ± 129.2a
		Storage days: 10		
ENGOPA 316	764± 154.6b	1597.5 ± 77.5a	1366.7 ± 2.8a	1415 ± 130a
MAGBA	1194.3± 134ab	1320 ± 20a	1617.5 ± 12.5a	1580 ± 15.1a
TGX-1835-10E	822.8 ± 376.4b	1200 ± 140ab	1280 ± 30ab	1400 ± 65a
HOULA 1	1539 ± 289.1a	1182 ± 42.5b	1507.5 ± 2.5a	1601,5 ± 94.3a
TGX-1910-14F	1283.7 ± 243.9b	1677.5 ± 172.5ab	2200 ± 40a	1719.3 ± 114.8a
		Storage days: 40		
ENGOPA 316	764± 154.6c	1237.5 ± 49.5b	1142.95 ± 14.5b	1760.5 ± 122.5a
MAGBA	1194.3± 134b	2053.5 ± 5.5a	1861.5 ± 94.5a	1069 ± 214b
TGX-1835-10E	822.8 ± 376.4b	931.5 ± 53.5b	887.5 ± 52ab	1333.5 ± 195.5a
HOULA 1	1539 ± 289.1a	1223 ± 99ab	1723.5 ± 105.5a	1383 ± 108b
TGX-1910-14F	1283.7 ± 243.9b	1666.5 ± 75.5ab	1693 ± 105ab	1994.5 ± 124.5a

Values with the same letter on the same line are not significantly different at P ≤ 0.05 according to Duncan test

It was a significant effect of essential oil on the germination rate. This is for example the case of TGX-1910-14F variety whose germination was increased from 55% to 92, 79 and 72% respectively for 2000, 4000 and 6000 ppm concentrations. At 2000 ppm, HOULA1 variety produced a better germination of 73% compared to the 6000 ppm dose that was 50%. 10 days after conservation of seeds treated with essential oil, HOULA 1 variety showed a significant reduction of germination rate which fell from 64.5 to 46.4 and 55 percent, respectively, for 2000 and 6000 ppm. After 40 days of storage, there is a reduction in germination of ENGOPA 316 and HOULA 1 which rose 71 to 55% and 64 to 56% respectively. However these reductions are not statistically significant. Similarly TGX-1835 had a reduction in germination at 2000 and

4000ppm concentrations. TGX-1910-14F however presented a significant increase of its germination rate which rose from 60 (0 ppm) to 90; 78 and 85% respectively for 2000, 4000 and 6000 ppm concentrations.

Effect of essential oil on seed-born fungi

Table 2 presents seeds infection percentage after 10 and 40 days of storage. It is clear that all concentrations of EO have significantly reduced the infection rate in all soybean varieties compared to the control where the infections percentage ranged from 42 (MAGBA) to 67% (TGX-1910-14F). Ten days after conservation, the infection percentages recorded on different varieties of soybeans seed treated with essential oil are significantly lower than those of the untreated control. The infection rates vary from 14 to 44% for 2000 ppm; 3.5 (MAGBA) to 35% (ENGOPA316) for 4000 ppm and 4.1% to 21% (HOULA 1) for 6000 ppm. After 40 days of conservation, the percentages of infection of seeds treated with essential oil are significantly lower than those of the control. Concentration 4000 ppm provided better conservation of varieties such as MAGBA and TGX-1835-14F with percentages infection of 11 and 5.3 respectively. As for ENGOPA 316 and HOULA 1 they presented infection percentages of 8.9 and 1.7 respectively at 6000 ppm. Low infection rate have been registered at 2000 ppm on TGX-1910-14F variety compared to those of 4000 and 6000 ppm concentrations.

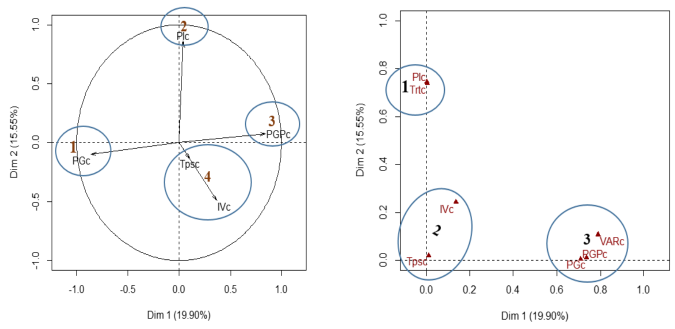

Figure 2. Relation between quantitatives and qualitatives variables

Effect of essential oil on seedling vigour

Table 3 presents vigour index (VI) of seedlings from seeds treated with essential oil and stored up to 40 days. In general vigour index of seeds treated with essential oil are higher than those of control. After treatment of seeds and before their storage, VI obtained are certainly higher than those of the control, but there is no significant differences for MAGBA, TGX-1835-14E and 14F-1910-TGX varieties. After 10 days of conservation, the VI of seedlings treated with essential oil are significantly higher than those of control. ENGOPA 316 presents the highest VI (1597) at 2000 ppm concentration. At 4000 ppm, MAGBA and TGX-1910-14F present their highest VI which were 1617 and 2200 respectively. At 6000 ppm, HOULA 1 and TGX-1835-14E presented their high VI which are 1601 and 1400 respectively. After 40 days of conservation, ENGOPA 316; TGX-1910-14F and TGX-1835-14E presented their best VI 1760, 1994 and 1333 respectively at the 6000 ppm dose. Moreover, MAGBA variety presented its best VI (2053) at 2000 ppm. Finally the HOULA 1 variety showed its best VI (1723) at 4000 ppm.

Correlations between seeds germination percentage, seeds infection and seedling vigour

This study has assessed the antifungal activity of essential oil (EO) of *Cymbopogon citratus* against seed-borne fungi of some soybean varieties present in West Cameroon and the effect on seedlings vigour. PCA (principal component analysis) reveals four groups of components in 2 dimensions. Germination percentage (GP) contribute mainly to the formation of Dimension 1 while infection percentage (IP) contribute to the formation of the second Dimension. Ever since, the graph of quantitative variables (Figure 2) shows a high negatives correlations between GP and percentage of rotten seeds (PRS). Also a low negative correlation is established between vigour index (VI), time of storage and IP. Correlations between variables are represented in figure 2. Therefore it is clearly shown that, treatments are highly correlated to IP, VI has low correlation with treatments and IP. The representation in figure 3 reveals that, all treatment except Treatment 1 (0ppm) have significantly negative correlation with IP; meaning that Treatment 2 (2000 ppm), Treatment 3 (4000 ppm) and Treatment 4 (6000 ppm) reduce significantly IP, also all treatments have an effect on VI at all periods of data collection (0 days of storage, 10 days after treatment, 20 days after treatment, 30 days after treatment, and 40 days after treatment).

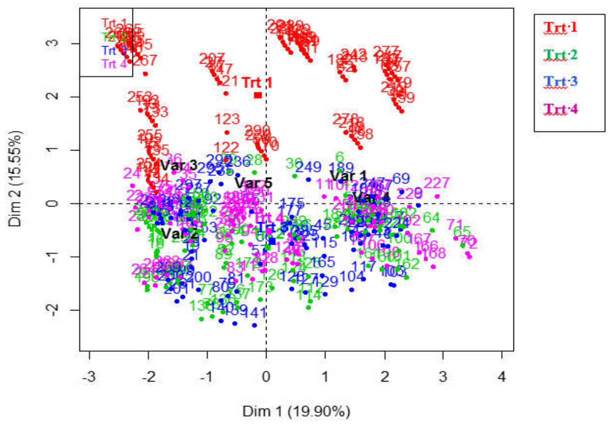

Figure 3. Correlations between concentrations and others variables

DISCUSSION

In the present study, an attempt was made to investigate the effect of essential oil of *Cymbopogon citratus* at different concentrations on seed germination, seed infection and seedling vigour on five soybean varieties. The results showed that the essential oil significantly ($P \leq 0.05$) reduced the incidence of natural seed infection and improved seed germination of some variety when compared with untreated control seeds. Percent seed infection was highest with lower concentration of essential oil. The essential oil of *C. citratus* was found to be most effective against seed-borne fungi. The inhibitory effect of essential oil of *C. citratus* against these fungi may be due to the presence of Citral and others phenolic compound. The GC analysis identified citral as major component with a percentage of 76%. Citral as main components of *C. citratus* an oxygenated terpenoid, which has been identified as a compound exhibiting antifungal properties (Paranagama *et al.*, 2003). This monoterpene has proved effective in controlling mycelial growth and conidial germination of *C. gloeosporioides* (Palhano *et al*., 2004). Similarly it is reported that the anti-fungal activity of lemon grass oil may be due to the presence of its aldehyde containing the active constituent citral (Gupta *et al*., 2011). Paranagama *et al*. (2003) reported that *C. citratus is* of West Indian origin and yields an essential oil with high content of citral (>70%). According to effect of *C. citratus* on seed germination noted in this study, there are similarity with some previous works. In this study it was observed that at 6000 ppm, *C. citratus* reduce the germination rates of HOULA 1 and ENGOPA 316 varieties. Similarly Bonzi *et al.* (2013) showed that at 8%, essential oil of *C. citratus* at 8% was effective in the control of *P. sorghina* in naturally infected sorghum seed. At this proportion, essential oil of *C. citratus* lowered sorghum seed germination. Somda *et al.* (2007) showed that essential oil of *C. citratus* at 6% was effective against *C. graminicola* and *P. sorghina* and did reduce germination of treated seed. Furthermore it was also noted the cases of seeds germination improvement. TGX-1910-14F presented a significant increase of its germination rate which rose from 60 (control) to 90; 78 and 85% respectively for 2000, 4000 and 6000 ppm concentrations. Tagne *et al.* (2008) repported that the germination of treated maize seed with the essential oil of *C. citratus*, shows important improvements between the treated seed and the untreated control. According to Rodrigues, Rodrigues and Reis (1999), an essential oil compound inhibit germination and growth by interfering with cell division, membrane permeability and the activation of enzymes. This effect presented by the essential oil from *C. viminalis* may be due to the presence of the principal constituents 1,8 cineole, *α*-pinene and limonene or to chemical synergism between the compounds in the essential oil(Maria de Oliveira *et al*. 2014). According to the improvement of germination, the ability of some plant extracts to increase seed germination could be attributed to the suppression of seed borne fungi that could have consider to kill the embryo of the seeds leading to germination failure. Parimelazhagan and Francis (1999) established an increase in germination rates and an improvement in seedling development of rice seeds with leaf extract of *Clerodendrum viscosum*. An improvement of vigour of soybean seedlings was also recorded during this study. This can be illustrated by VI presented by ENGOPA 316; TGX-1910-14F and TGX-1835-14E which are 1760, 1994 and 1333 respectively at the 6000 ppm after 40 days of conservation. These indices are significantly higher than those of the untreated control which are 764, 1283 and 822 respectively. Similarly MAGBA and HOULA 1 varieties presented their best VI (2053 and 1723) at 2000 ppm and 4000 ppm respectively while their controls indices are 1194 and 1539.

Conclusion

Soybean seed treatment with essential oil of *C. citratus* significantly reduce seed infection by fungi and also improves soybean seed germination for some varieties. Other soybean (ENGOPA 316 and HOULA 1) varieties treated with essential oil showed a non-significant reduction in germination. Soybean plants obtained from seeds treated with essential oil of *C. citratus* grow very well than those coming from untreated seeds. Essential oil of *Cymbopogon citratus* for seed treatments may become a bio-fungicide for soybean crop protection. However, further investigations and product developments are needed as well as field experiments.

Acknowledgements

The authors express their gratitude to all the members of the Phytopathology Laboratory of the Faculty of Agronomy and Agricultural Sciences and those of Foumbot IRAD station, for their kind collaboration.

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International Journal of Biosciences (IJB) ISSN: 2220-6655 (Print) 2222-5234 (Online), 2(6) : 41-48, 2012.

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