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Research Article

Effect of Essential Oil of *Callistemon viminalis* on Germination, Seed Mycoflora and Seedling Vigour of Three Groundnut Varieties

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ABSTRACT

In vitro evaluation of seed germination, seed infection and seedling vigour of groundnut seeds when tested at 30 mn; 1hr and 1hr 30mn of duration at 0.05; 0.1 and 0.15 g/ml concentration of essential oil of *Callistemon viminalis* was assessed in Petri dishes. Varieties JL24, ICGV and Mechicha (local variety) collected from IRAD of Foumbot were used. At 1 hour, ICGV variety showed 100 % germination and high vigour index (1732.) for seeds treated with 0.05 g/ml of essential oil. At the same time significant activity was also observed for JL24 variety with 100% of seed germination, very low seeds infected and vigour index around of 1375 for seeds treated with 0.15 g/ml of essential oil. At 1 hr 30 min of treatement, 0.15 g/ml showed high effect on seed germination and vigour index for Mechicha variety compared to positive and negative controls. Moderate activities were observed in 30 min of duration of treatment. It can be suggested that essential oil of *Callistemon viminalis* have antifungal effect and can be used to treat seed against seed borne pathogens of groundnut seeds, increase seed germination and vigour index.

Key words: Groundnut seeds, Essential oil, Fungal infection, Seed germination, Plant vigour

INTRODUCTION

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). The use of plants by humans dates back thousands of years and relies on chance discoveries that are frequently proven by science. They were used to cure diseases, for food preservation and insect control. Many natural compounds present in plants, herbs and spices have shown biological activity and serve as a source of antimicrobial agents against various pathogens.Many African plants are potential sources of pesticides and have been shown to exhibit biological activities, especially antimicrobial, since ancient time (Amvam *et al*., 1998).Among these products, the essential oils receive emphasis. They are also called volatile, ethereal oils or essences because they have an oily appearance at room temperature, are important in phytosanitary control and in the reduction of the negative effects of oxidants, free radicals and microorganisms that cause damage in the food industry (Simões *et al*., 2007; Pereira *et al*., 2007; Amvam *et al*., 1998.

Groundnut (*Arachis hypogaea* L.) belongs to the Fabacaea family and is grown in most tropical and sub-tropical countries including Cameroon (FAOSTAT, 2013). It provides income and food for a lot of peoplein Cameroon and many countries. Yields of groundnut have generally been reported to be low in developing countries compared to developed countries due to many factors including diseases (Janila *et al*., 2013a). Among the diseases, leaf spots have the greatest impact on groundnut yield which may cause more than 50% yield losses (Leal-Bertioli *et al*., 2009; Waliyar, 1991).

Seed borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure and/or causing disease to the newly emerged seedlings or growing plants, but also contaminate the soil by establishing its inocula permanently. It was therefore necessary to search for control measures that cheap, ecologically sound and

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environmentally safe to eliminate or reduce the incidence of these economic important pathogens, so as to increase seed germination and to obtain healthy and vigorous plant as well as better yield of groundnuts.

Callistemon viminalis, popularly known as escova-de-garrafa (bottle brush), belongs to the Myrtaceae family. It is a medium-sized tree with persistent foliage. It is found throughout the world, being distributed mainly in the humid tropical regions. Many species of this family are important sources of edible fruits, spices, and ornamental and medicinal plants (Oyedeji *et al*., 2009). Some species of *Callistemon*, especially *Callistemon viminalis*, are widely used as environmental bioindicators, being an important source of chemical compounds with insecticidal, fungicidal and antimicrobial properties (Silva *et al*, 2010). In the present study, the essential oil from *Callistemon viminalis* leaves was studied, and its antifungal activity against Groundnut (*Arachis hypogaea* L.) seeds infection, seeds germination and seedling vigour were evaluated.

MATERIALS AND METHODS

Plant material

The *Callistemon viminalis* (Myrtaceae) blossoms were collected in July 2015 in the morning on a day with mild temperatures and no precipitation, from the locality of Dschang, West region of Cameroon. The identification was confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang.

Groundnut seed samples were collected from IRAD of Foumbot in West region of Cameroon, and include: ICGV, JL24 and MECHICHA.

Extraction of the essential oil

The leaves were hydro-distilled for about 5 hours using a Clevenger apparatus in the laboratory of Microbiology and antimicrobial substances. Oils recovered were dried over anhydrous sodium sulfate and stored at 4°C until they used. Together with the extraction, the moisture content was determined according to the method of Pimentel *et al*., 2006. The yield of the essential oil was calculated and expressed in weight of oil per unit weight of plant material on a Moisture-Free Basis (% w/w MFB).

Seed treatment

A total of 100 seeds were used for each groundnut variety. Seeds were first washed with distilled water then surface sterilized with 2% sodium hypochlorite for ten minutes and rinsed thoroughly with distilled water and kept on blotting paper to remove excess moisture from seed surface. Afterwards seeds were treated by soaking them separately in different concentrations of essential oil for 30 min, 1hr and 1hr30 min duration. The concentrations used were 0.05g/ml; 0.1g/ml and 0.15g/ml, prepared by using Tween 80 in a 1:1 (V/V) ratio and dissolved in distilled water. A 1.0% v/v solution of Tween 80 in water was used as negative control and mancozeb (1mg/ml as recommended) was used as positive control. The excess extract was drained off by kepting in blotting paper to remove excess moisture from seed surface and dry in the open air. The seeds were then plated on moist blotters Petri dishes (Five seeds/dish) and incubated at 22±1°C for seven days using a 12 h photoperiod.

Seed germination, seed infection and seedling vigour

Percentage of seed germination (%G) was monitored for seven days, and seedling vigour were evaluated 15 days after sowing. Thirty seedlings from each tray were randomly selected for measurement of shoot or root length. The seedling vigour was determined following the formula of Varadarajan and Rao (2002) as shown below:

Vigour index (VI) = Percent germination of seed × (Root length + Shoot length).

Percentage of seed infection (%I) was evaluated following the formula:

%I = Number of seed infected x 100/Total seed used

In both experiment, Seeds soaked in distilled water and Mancozeb (1g/ml) for the same period served as negative and positive control. Complete randomized design (CRD) was used in the experiments and each treatment was repeated thrice.

Statistical analysis

The data collected on different parameters were subjected to Analysis of variance (ANOVA) using STAT Graphic. 5 and means for all treatments were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

RESULTS

Results on the effect of seed treatment with essential oil at 30 min on seed germination, infection and seedling vigour are presented in Table 1.

For JL24 and Mechicha Varieties, seed germination obtained with seed treated by essential oil (86.6 - 96.7%) are different from those of the positive and negative controls however no significant difference was observed. High germination was noted for seeds treated with 0.15 g/ml of EO. Concerning ICGV variety, germination for treated seed (90-93%) is lower than those of controls (100%) however no significant difference was observed. In general for all the varieties, seeds infection percentage varies with the concentration of essential oil, more the concentration is high, less the seeds are infected. At 30 min of seed soaking with essential oil, Mechicha variety presents higher vigour index than other varieties of soybean which varies from 747 to 928.

Results on the effect of seed treatment with essential oil at 1 hour on seed germination, infection and seedling vigour are presented in Table 2. It is generally an increase in germination, the seedling vigour and a decrease of seeds infection compared to results of seeds treated for 30 min.

Seed germination obtained with seed treated by essential oil for all varieties (88.3-100%) are not statistically different to those of positive and negative control (93.3-100%).Once more high germination was noted for seeds treated with 0.15 g/ml of EO according to JL24 and Mechicha varieties. Concerning ICGV variety, 100 % germination and high vigour index (1732.) were noted for seeds treated with 0.05 g/ml of essential oil. In general, percentage of seed infection varies with the concentration of essential oil, more the concentration is high, less seeds are infected.

Table 1: Effect of *Callistemon viminalis* essential oil on seed germination, seed infection and seedling vigour of groundnut – 30 mn

	JL24			MECHICHA			ICGV		
Con	%G	%I	VI	%G	%I	VI	%G	%I	VI
$T_{0.15}$	96.7^{a}	5.3^{c}	953.3^{a}	96.7^{a}	6.7^{cd}	1093.1^{a}	93.3^{a}	1.8^{c}	747.1^{ab}
$T_{0.1}$	86.6^{ab}	4.7^{c}	779.8^{a}	96.0^{a}	12.0^{bc}	1094.0^{a}	90.0^{a}	8.3^{c}	771.3^{ab}
$T_{0.05}$	78.3^{b}	19.7^{b}	772.5^{a}	93.3^{a}	14.7^{b}	912.1^{a}	90.0^{a}	19.0^{b}	928.9^{a}
T_W	90.0^{a}	19.3^{b}	871.7^{a}	94.3^{a}	4.7^{d}	1038.9^{a}	100^{a}	4.0^{c}	641.8^{ab}
T_M	91.7^{a}	30.0^{a}	781.2^{a}	93.3^{a}	35.3^{a}	900.8^{a}	100^{a}	33.3^{a}	579.8^{b}
CV (%)	7.85	6.2	19.9	5.02	7.9	16.5	7,8	9.02	2.16

Means in a column for each cultivars followed by the same letter(s) are not significantly different according to Duncan New Multiple range Test (P = 0.05).$T_{0.15}$ = 0.15g/ml; $T_{0.1}$ = 0.1g/ml; $T_{0.05}$ = 0.05 g/ml; T_W = Water + Tween 80 ; T_M = Mancozeb

Table 2: Effect of *Callistemon viminalis* essential oil on seed germination, seed infection and seedling vigour of groundnut – 1hour

	JL24			MECHICHA			ICGV		
Con	%G	%I	VI	%G	%I	VI	%G	%I	VI
$T_{0.15}$	100^{a}	1.2^{c}	1375.0^{a}	99.3^{a}	10.0^{b}	827.2^{a}	99.3^{a}	0.5^{d}	887.1^{b}
$T_{0.1}$	96.7^{a}	1.2^{c}	1301.7^{ab}	99.3^{a}	20.0^{a}	697.3^{a}	99.3^{a}	6.0^{c}	1155.4^{b}
$T_{0.05}$	88.3^{a}	5.3^{c}	864.5^{c}	100^{a}	8.0^{c}	516.3^{a}	100^{a}	10.3^{a}	1732.7^{a}
T_W	96.7^{a}	14.7^{b}	1030.3^{ab}	96.6^{a}	3.0c	817.5^{a}	99.7^{a}	2.7^{d}	1014.0^{b}
T_M	96.7^{a}	27.3^{a}	459.7^{d}	93.3^{a}	20.3^{a}	873.8^{a}	100^{a}	26.8^{a}	601.8^{b}
CV (%)	6.7	6.6	3.9	5.6	5.7	3.9	0.07	1.6	3.9

Means in a column for each cultivars followed by the same letter(s) are not significantly different according to Duncan New Multiple range Test (P = 0.05).T0.15 = 0.15g/ml; T0.1 = 0.1g/ml; T0.05 = 0.05 g/ml; TW = Water + Tween 80 ; TM = Mancozeb

Table 3: Effect of *Callistemon viminalis* essential oil on seed germination, seed infection and seedling vigour of groundnut – 1h30 min

	JL24			MECHICHA			ICGV		
Con	%G	%I	VI	%G	%I	VI	%G	%I	VI
$T_{0.15}$	100^{a}	0^{c}	806.7^{a}	93.3^{ab}	4.7^{b}	1096.2^{a}	100^{a}	0.5^{c}	1098.2^{a}
$T_{0.1}$	98.3^{a}	0.7^{c}	1007.4^{a}	100^{a}	3.7^{b}	1037.9^{a}	96.3^{a}	3.0^{bc}	573.0^{bc}
$T_{0.05}$	95.7^{a}	1.0^{c}	949.7^{a}	99.3^{a}	7.0^{b}	808.5^{a}	100^{a}	4.7^{b}	1276.4^{a}
T_W	100^{a}	4.0^{b}	1069.2^{a}	100^{a}	2.0^{b}	636.2^{a}	100^{a}	1.3^{c}	742.2^{b}
T_M	96.7^{a}	21.3^{a}	830.5^{a}	99.3^{a}	13.3^{a}	887.9^{a}	100^{a}	21.5^{a}	320.5^{c}
CV (%)	2.9	1.6	2.8	3.5	6.6	3.0	1.5	1.3	4.8

Means in a column for each cultivars followed by the same letter(s) are not significantly different according to Duncan New Multiple range Test (P = 0.05).$T_{0.15}$ = 0.15g/ml; $T_{0.1}$ = 0.1g/ml; $T_{0.05}$ = 0.05 g/ml; TW = Water + Tween 80 ; T_M = Mancozeb

Table 3 presents results on the effect of seed treatment with essential oil at 1 hour on seed germination, infection and seedling vigour. At 1h30min period of soaking, in general, seed germination are 100% or close to 100% for all varieties of soybeans. The %I are 0 for seed treated with 0.15 g/ml of essential oil for JL24 and ICGV varieties. Vigour index for treated and untreated seeds are considerably lower than those obtained after treatment for 1 hour period of soaking. Seeds treated with 0.05 g/ml of essential oil during 1 hour gave 100% germination and 1732 of IV but at 1 h30min period of soaking there is certainly 100% germination but a significant decrease in seedling vigour which drops to 1276. It is also noted that all seeds treated with essential oil showed lower %I compared to those of negative and positive controls, this demonstrates the protective effect of essential oil.

DISCUSSION

In the present study, an attempt was made to investigate the effect of essential oil of *Callistemon viminalis*at different concentrations on seed germination, seed infection and vigour index on three groundnut seed varieties.

The results showed that the essential oil significantly ($P \leq 0.05$) reduced the incidence of seed infection and improved seed germination when compared with untreated control seeds. Percent seed infection was highest with lower concentration of essential oil.

Maria de Oliveira *et al*. (2014) detected a high toxicity of the essential oil from *C. viminalis* which presented all elopathic activity at intensities that were proportional to the concentration of the essential oil and caused a reduction in the germination speed index (GSI) of lettuce seeds and in the dry mass and length of shoots and roots of lettuce seedlings.

Singh *et al*. (2005) detected a high toxicity of the essential oil from eucalyptus species (*Eucalyptus citriodora*), which belong to the same family as *C. viminalis* (Myrtaceae), against the weed Parthenium hysterophorus. The authors observed that the seedling length decreased with increasing concentration of the Eucalyptus oils (0.2 - 5.0 µL·mL−1).

In this study, slight improvement of germination was noted for treated seeds, also the essential oil affected the growth of soybean seedlings and caused a reduction in the length of shoots and the root system, when increasing concentrations.

Verdeguer *et al.,* (2009), observed a decrease in the rate of germination and growth of *Amaranthus hybridus* and *Portulaca oleracea* with increasing concentrations of0.125; 0.5 and 1 µL/mL when essential oils from Eucalyptus (*Eucalyptus camaldulensis*) were used.

According to Rodrigues, Rodrigues and Reis (1999), a 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 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*. Also, Hamim *et al*. (2014) reported a highly positive relationship between germination failure and prevalence of seed borne fungal infection on several vegetable seeds.

Conclusion

This study has shown that the essential oil of *C. viminalis* have antifungal effects and can be used as fungicidal seed treatments for the control of ground nut seed infection and seedling emergence. Highly significant increase in vegetative growth parameters have been observed in seeds treated with 0.05 and 0.15 g/ml concentration of the essential oil for 1hr duration respectively for ICGV and JL24 varieties. For the local variety (Mechicha), significant activity was observed at 1hr30 duration of treatment at 0.15g/ml concentration. This suggesting that treatment of groundnut seeds with 0.05 and 0.15 g/ml concentration of the essential oil for 1hr is the most ideal treatment for growth promotion, germination improvement and significant decrease of seed mycoflora respectively for ICGV and JL24.However, further studies should be carried out under field's conditions.

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