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Full Length Research Paper

Antifungal activities of essential oils from Southern African medicinal plants against five *Fusarium* species

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In the present study, essential oils from 9 plants traditionally used to treat infectious diseases were tested against five *Fusarium* spp. using the agar diffusion method. The minimum inhibitory concentrations (MIC) of the oils were determined by the microdilution technique. The killing kinetics of the oils was further evaluated against the fungal organisms. Antifungal activity was exhibited by essential oils from *Conyza scabrida*, *Eriocephallus panculatus*, *Artemesia afra, Pelargonium graveolons* and *Mentha peripeta* with 100% growth inhibition against all the five *Fusarium spp* with the zone of inhibition ranging from 10 to 18 mm. The essential oils of *C. scabrida* and *M. piperita* were fungicidal to four of the five fungal species tested with minimum fungicidal concentration (MFC) values ranging from 0.95 to 7.50 mg/ml. All the essential oils tested were able to kill the cells at different rates varying from 48 to 100% after 2 days of experimentation. The results of the present study indicate that essential oils tested are promising sources of natural products with which could be used for the control of *Fusarium* infections. These results will guide the selection of some plant species for further pharmacological and phytochemical analysis. These results also support the traditional use of these essential oils.

Key words: *Fusarium* spp, anti-fungal activity, essential oils, fungi, Southern Africa, medicinal plants.

INTRODUCTION

Fusarium is a group of filamentous fungal organisms widely distributed in nature. They are responsible for a broad spectrum of infections in humans animals and plants and are therefore of public health and economic importance (Sampietro et al., 2011; Vascellari et al., 2011; Kalkanci and Ozdek, 2011). Examples of fusarial infections include superficial, locally invasive (such as keratitis and onychomycosis) and disseminated infections among humans and animals (Carneiro et al., 2011). *Fusarium* species may cause allergic diseases (sinusitis) in immunocompetent individuals (Wickerns, 1993) and mycotoxicosis in humans and animals following ingestion of food contaminated by toxin-producing *Fusarium* spp. (Nucci and Anaissie, 2007; Girgis et al., 2010). These organisms are also important plant pathogens causing various diseases such as crown rot, head blight and scab on cereal grains (Nelson et al., 1994) and they may occasionally cause infection in animals (Evans et al., 2004). *Fusarium* infections can be treated with different antifungal drugs. For example, Keratitis is usually treated with topical antifungal agents and natamycin is the drug of choice although voriconazole treatment has been reported. With the increased number of immunocompromised individuals it is likely that the treatment of *Fusarium* infections will become more problematic. Therefore, there is need to search for alternative treatment options. Essential oils have been used for centuries by cultures all over the world. Despite the development of antibiotics, bacterial and fungal infections are still major issue in medicine and are the most common pathogens infecting man and beasts and

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probably plants as well. Several studies have shown that essential oils have antimicrobial activities including African essential oils. However, less than 10% of the biodiversity in Africa is said to have been evaluated.

Essential oils have been used as biological agents for their therapeutic activity and toxicity against insects and plant pathogenic fungi as well as human fungal pathogens. They are needed for reduction in the use of chemical in agriculture to increase interesting possibilities of application of essential oils to control plant pathogens. For example, essential oil from *Thymus Vulgaris* has been reported to inhibit the fungal growth (Pinto et al., 2006). Their fungistatic activity has been attributed to the presence thymol which constitutes 50% in the oil tested. This natural product obtained from many plants has been attracting scientific interest in traditional medicine (Christine et al, 2008). Although several studies have been conducted on the antifungal activities of essential oils in Sub-Saharan Africa, most have targeted a small group of organisms mainly *Candida* spp, *Aspergillus* spp and *Penicillium* spp (Gundidza, 1993; Magwa et al., 2006; Viljoen et al., 2005; Van Vuuren et al., 2009).

Recently, there has been a resurgence of emerging infections of which many are opportunistic particularly to immunocompromised patients such as Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) patients. With the increase in the number of HIV infected patients, now standing at more than 5.5 million in South Africa alone (DOH, 2008), the importance of the development of new and efficient control methods cannot be emphasized enough. The knowledge of the population combined to the rich biodiversity provides the perfect circumstance for the development of new drugs for the control of infectious diseases that plagues the lives of many citizens of the region. Moreover, out of the 3000 essential oils currently described, only about 10% is used in the Industry and a very negligible fraction is from the Sub-Saharan Africa. Therefore, the present study was designed to determine the antimicrobial activities of essential oils from Southern African plants traditionally used as medicine by local population.

The study determined the activity of essential oils from medicinal plants found in Southern Africa against five *Fusarium* spp responsible for plants, human and animal diseases.

MATERIALS AND METHODS

Preparation of essential oil

Medicinal plants commonly used by the population in Southern Africa were selected to be used in the present study from the literature. A total of 9 plants were selected (Table 1). The plants were all collected in Zimbabwe around the city of Harare. Essential oils were prepared by hydro-distillation method. Briefly, 200 g of fresh plant parts were submitted to hydro-distillation with a Clevenger-type apparatus and according to the European Pharmacopoeia, and extracted with two liters of water for 3 h. Nine essential oils were prepared from the leaves of the plants and were collected and dried under anhydrous sodium sulphate and stored at 4°C until used.

Preparation of microorganisms

In order to evaluate the antimicrobial activity of essential oil, identified pure cultures of five species of *Fusarium* were collected from University of Pretoria (UP). These included *Fusarium verticillioides* (Sacc.) Nirenberg 1976, *Fusarium nygamai* L.W. Burgess and Trimboli 1986, *Fusarium oxysporum* Schlecht., *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach and Nirenberg, (1976) and *Fusarium graminearum* also known as *Gibberella zeae* (Schwein.) Petch (1936). Broth cultures for the *Fusarium* spp. were prepared by using sterile brain heart infusion broth (BHIB) by cutting small piece of pure culture and place it in 500 ml of BHIB. The mixture was incubated at 30°C for 24 h. Then 1 ml of the broth was added into 9 ml fresh sterile media BHIB to dilute the organisms during susceptibility testing and adjust to 1McFarland standard. The same procedure was also applied to prepare the yeasts cultures.

Antifungal activities

Hole plate diffusion method

The antimicrobial activity of the essential oils was assayed by a modification of the agar diffusion method (Kirby‑Bauer). The experiments were conducted on Sabouraud Dextrose agar (SDA) (Oxoid, England) plates supplemented with 0.5% Tween 20. Briefly, SDA plates were inoculated with 1000 ul of a 1 Mac Farland standard of the organisms grown in brain heart infusion broth. Afterward, 6 wells of approximately 5 mm in diameters and 2.5 mm deep were made on the surface of the solid medium using the tip of a sterile plastic pipette.

Each well was then filled with 20 µl of the test oil or controls. Sterile dimethylsulfoxide (DMSO) was used as negative control and Nystatin was used as positive control. The plates were then incubated at 30°C for 2 to 3 days. After 3 days the radial zone of inhibition was measured by using a ruler and the diameters of inhibition zone was determined in millimeters. Essential oils with zone of inhibition greater or equal to 6 mm diameter were regarded as active (Apak and Olila, 2006).

Determination of minimum fungicidal concentration (MFC)

The microtitre plates previously used to determine the MIC of the oils were used to determine the MFC (Yaya et al., 2008). The concentrations tested included: 7.5, 3.75, 1.87, 0.93, 0.46, 0.23, 0.11 and 0.05 mg/ml. The wells in the plates showing no visible growth were inoculated onto a potato dextrose agar (PDA) plates as described above. The Petri dishes were marked according to the number of wells and EO as appearing on the microtitre plates. The plates were incubated at 30°C for 2 days. The smallest

concentration that did not show any growth on the agar plates so was regarded as MFC.

Colony growth inhibition assay

Twenty eight medicinal plant extracts that showed activity using the above described hole plate diffusion method and microdilution method, were tested against the five *Fusarium* species to determine the rate of growth inhibition using the agar dilution methods. Briefly, PDA (Oxoid, England) was prepared and left to cool down to 50°C and was mixed with the plant extracts to give a predetermined concentration. Then 10 ml of the mixture was poured in the Petri dishes and left to solidify.

A piece of the *Fusarium* culture on the agar (with dimensions 4 × 4 mm) was cut and placed in the middle of the plate containing the extract. Then the plates were incubated at 25°C for 7 days. Radial growth of the fungal organisms was recorded everyday for 7 days or until the plates were overgrown. Negative and positive controls were run along each fungal isolate and crude extract. The percentages of inhibition were calculated as previously described (Khalil et al., 2005).

Killing curve determination

Sterile microtitre plates, of 96 wells were used with fresh brain heart infusion broth for the determination of the killing curve (Samie et al., 2009). Briefly, 200 µl of sterile fresh brain heart (Oxoid, England) infusion broth was added to the wells together with 100 µl of the fungal culture. About 20 µl of extracts was added to the wells and the plates were incubated at 30°C. Ten microlitres of the mixture from the first plate was transfer to a new microtitre plate with 200 µl of sterile distilled water and the optical density (OD) was read using ELISA reader every day for six days. All the experiments were repeated twice.

Statistical analysis

All the tests were conducted in duplicates. The data were analyzed using the Statistical Package for Social Sciences (SPSS) program. The Chi square was used and the p values were determined. The difference between two variables was considered significant when the p value was less than 0.05.

RESULTS

Antifungal activities of the essential oils against the *Fusarium* spp. as determined by the hole plate diffusion

In the present study the activity of the essential oils on the molds (*Fusarium* spp.) was first tested in an agar medium. Of all the oils, the essential oil of *A. afra* gave the largest inhibition zone against *F. proliferatum* with zone of growth inhibition of 25 mm.

However this plant was not active against all the fungal organisms (Table 2). *M. piperita* was active against all the five *Fusarium* spp with zone of growth inhibition ranging from 10 to 18 mm. *C. scabrida* was active against 4 out of the 5 molds tested, with different zones of inhibition ranging from 7 to 13 mm. The largest zone of inhibition (13 mm) was observed against *F. oxysporum* where as the lowest was against *F. graminearum* (7 mm). *F. verticillioides* was the organism which was resistant to the oil since there was no inhibition observed. *Helinchyrysum foetidum* was found to have inhibition against *F. oxysporum, F. nygamai* and *F. graminearum* with zones of inhibition ranging from (10 and 15 mm).

MIC of the essential oils against *Fusarium* spp.

Essential oil *C. scabrida, Adansonia digitata, E. punculatas, A. afra, Pelargonium graveolens* and *M. piperita* had reveal excellent fungicidal activity against all fungal strains tested in this study with MIC values ranging from 0.24 to 7.50 mg/ml (Table 3). The essential oil of *C. scabrida* was the most active with MIC against 4 fungal isolates less than 1 mg/ml except for *F. verticillioides* with an MIC of 3.75 mg/ml. *M. elissa officinalis* was not active against any of the fungal organisms at the concentration tested (7.5 mg/ml). *Leucosidea sericea* essential oil was active against two fungal organisms including *F. proliferatum* (MIC = 7.5 mg/ml) and *Fusarium verticilioides* (MIC = 3.75 mg/ml). *F. graminearum* was the most susceptible to the essential oils tested in a liquid media with the highest number of the smallest MIC (0.24 mg/ml). This smallest MIC was obtained with essential oils from *Eriocephallus punculatus*, *P. graveolens* and *A. afra*.

Fungicidal activity of the essential oils against five *Fusarium* spp.

Essential oils *A. digitata, E. punculatus, P. graveolens and A. afra* were fungicidal to all the fungal strains tested in this study with MFC values ranging from 0.24 to 7.50 mg/ml (Table 4). However essential oils from *M. officinalis* and *L. cericiae* were not fungicidal to any of the fungal organisms tested. The essential oils of *C. scabrida* and *M. piperita* were both bactericidal to four or five fungal strains tested with MFC values ranging from 0.95 to 7.50 mg/ml, whereas the oil from *H. foetidum was* fungicidal against only two organisms out of five tested with MFC values ranging from 0.95 to 7.50 mg/ml.

Colony growth inhibition of the *Fusarium* spp. by the essential oils

The essential oils were tested for their inhibition activity

Table 1. Ethnobotanical information on the plants used for the preparation of essential oils.

Scientific name	Common name	Source	Traditional applications
Adansonia digitata L	Boabab oil	Seeds	Traditionally used for prophylaxis and cure of dry and thin skin (Esterhuyse et al., 2001)
Artemesia afra Jacq. ex Willd.	Wormwood	Leaves	For the treatment of cough, croup, whooping cough, influenza, fever, diabetes, gastro-intestinal disorders and intestinal worms (Van Wyk et al., 1997)
Conyza scabrida DC	Gozo-plant	leaves	Help in the production of pharmaceutical products such as antibiotics
Eriocephallus punctulatus	Cape chamomile	Stem and leaves	The leaves of *E. punctulatus* are used in baths for its relaxing and invigorating scent. Used in pillows, the scent encourages pleasant dreams. The fumes of the burning fresh plant are used to disinfect the house and clear evil spirits after a death has occurred
Helichyrysum foetidum (L.) Moench	Strawflower and everlasting	leaves	*Helichrysum* species are used as food plants by the larvae of some Lepidoptera species (Van Wyk et al., 1997)
Melissa officinalis	Lemon balm	fruits	It is used as a medicinal plant and as a seasoning herb
Mentha piperita	peppermint	leaves	Peppermint oil is used mainly for flavouring toothpaste, other oral hygiene products, and chewing gum. Smaller quantities are used for flavouring confectionaries
Pelargonium graveolens	Rose geranium	flower	Rose geranium oil has a balancing effect on the nervous system and relieves depression and anxiety, while lifting the spirits and making the world an easier place to live in. It has a balancing effect on the adrenal cortex and is great for relieving stress (Van Der Walt and Vorster, 1988)
Tagetes minuta	Tagetes	flower	Tagetes contain antihelminthic, antibacterial, and antifungal properties, and is also used in making herbaceous and floral perfumes (Thembo et al., 2010)

against *Fusarium* spp. As compared to the negative control, there was generally a significant reduction of the growth of the organisms by the essential oils (Figures 1, 3, 5 and 7). The percent inhibition was calculated using the mean inhibition for three days and are represented in Figures 2, 4, 6 and 8. Of all the essential oils tested against *F. verticillioides*, the oils of *C. scabrida, E. punculatus* and *P. graveolens* were the most active oils by giving 100% inhibition for all the five days of the experiment (Figure 1A). *Melissa officinalis* oil was not active against *F. verticilioides* since 0% inhibition was observed. *C. scabrida, E. punculatus* and *M. piperita* were the most active against *F. oxysporum* with 100% inhibition from the first to the last day of experiment (Figure 3A). Of all the nine essential oils tested against *F. graminearum* the most active essential oils were those of *C. scabrida, E. punculatus, A. afra* and *M. piperita* which gave 100% inhibition of the organism for all the days of the experiment. However the oils of *H. foetidum, A. digitata, M. officinalis* and *Leucosidea cericea* were not active giving 0% inhibition for all the days during the investigation (Figures 5 and 6). The essential oils of *C. scabrida, H. foetidum, A. digitata, E. punculatus, P. graveolens, M. piperita* and *L. cericea* all gave 100% inhibition against *F. nygamai* (Figures 9 and 10). Essential oil of *M. officinalis* was the only oil which was not inhibitory to *F. nygamai.* Of all the essential oils tested, the oils of *C. scabrida, H. foetidum, E. punculatus, P. graveolens* and *M. piperita* were the most active oils against *F. proliferatum* giving 100% inhibition followed by the oil of the *M. officinalis* with 90% inhibition. Both essential oils from *A. afra and L. cericea* gave 20%

Table 2. Antifungal activity of 10 essential oil using the hole plate agar diffusion method. The zones of inhibition are in mm.

Essential oil	*F. oxysporum*	*F. nygamai*	*F. proliferatum*	*F. verticilioides*	*F. graminearum*
Adansonia digitata	15	0	15	0	0
Artemesia afra	0	8	25	15	10
Conyza scabrida	13	8	12	0	7
Eriocephallus punculatus	13	0	0	0	0
Helinchyrysum foetidum	15	10	0	0	10
Leucosidea sericea	10	15	0	0	12
Melissa officinalis	5	0	0	20	0
Mentha piperita	18	13	15	10	10
Pelargonium graveolens	0	7	15	15	15

Table 3. Minimum inhibitory activity of the essential oils against the *Fusarium* isolates.

Fungi	MICs values for nine essential oils (mg/ml)								
	C. scabrida	*H. foetidum*	*A. digitata*	*E. punculatas*	*P. graveolens*	*A. afra*	*M. officinalis*	*M. piperita*	*L. cericea*
F. graminearum	0.48	0.95	0.95	0.24	0.24	0.24	>7.5	3.75	>7.50
F. nygamai'	0.95	>7.50	3.75	0.95	0.95	0.95	>7.50	3.75	>7.50
F. *oxysporum*	0.95	>7.50	7.50	7.50	3.75	3.75	>7.50	0.48	>7.50
F. proliferatum	0.48	0.48	0.48	0.24	0.48	0.48	>7.50	0.48	7.50
F. verticillioides	3.75	7.50	7.50	7.50	3.75	3.75	>7.50	1.90	3.75

Table 4. Minimum fungicidal activity of the essential oils against the F*usarium* isolates.

Fungi	MFCs values for nine essential oils (mg/ml)								
	C. scabrida	*H. foetidum*	*A. digitata*	*E. punculatus*	*P. graveolens*	*A. afra*	*M. officinalis*	*M. piperita*	*L. cericea*
F. graminearum	0.48	>7.5	3.75	0.48	0.24	0.24	>7.5	7.5	>7.5
F. nygamai'	0.95	>7.5	3.75	0.95	0.95	0.95	>7.5	3.75	>7.5
F. *oxysporum*	>7.5	>7.5	7.5	7.5	3.75	3.75	>7.5	3.75	>7.5
F. proliferatum	0.48	0.95	0.48	0.24	0.48	0.48	>7.5	7.5	>7.5
F. verticillioides	3.75	7.5	7.5	7.5	7.5	7.5	>7.5	>7.5	>7.5

inhibition of the fungal organism.

Killing activity of essential oils against the *Fusarium* spp.

Essential oils *C. scabrida, P. graveolens, M. peripeta, H. foetidum* and *A. digitata* were tested for fungicidal activity by curves against five molds based on previous fungicidal activity testing. All of the essential oils tested some were MFC positive during minimum fungicidal determination by agar diffusion method. All the essential oils tested were able to kill the cells at different rates and a killing rate of about 90 to 95% was observed for all the oils tested after 2 days of experimentation (Figure 11). Essential oils of *C. scabrida* and *P. graveolens* had similar killing profiles against *F. proliferatum.* They were able to kill about 50% of the *F. proliferatum* cells after the first day of the experimentation as indicated by the reduction of the OD values. The essential oil of *H. foetidum* had the highest experiment (Figure 11B). *A. digitata* was very active in killing *F. nygamai* as it was able to kill up to 80% of the cells after the first day of experimentation compared to *C. scabrida* which had killed only about 35% after the first day of incubation (Figure 11C).

DISCUSSION

Essential oils have been recognized for their therapeutic

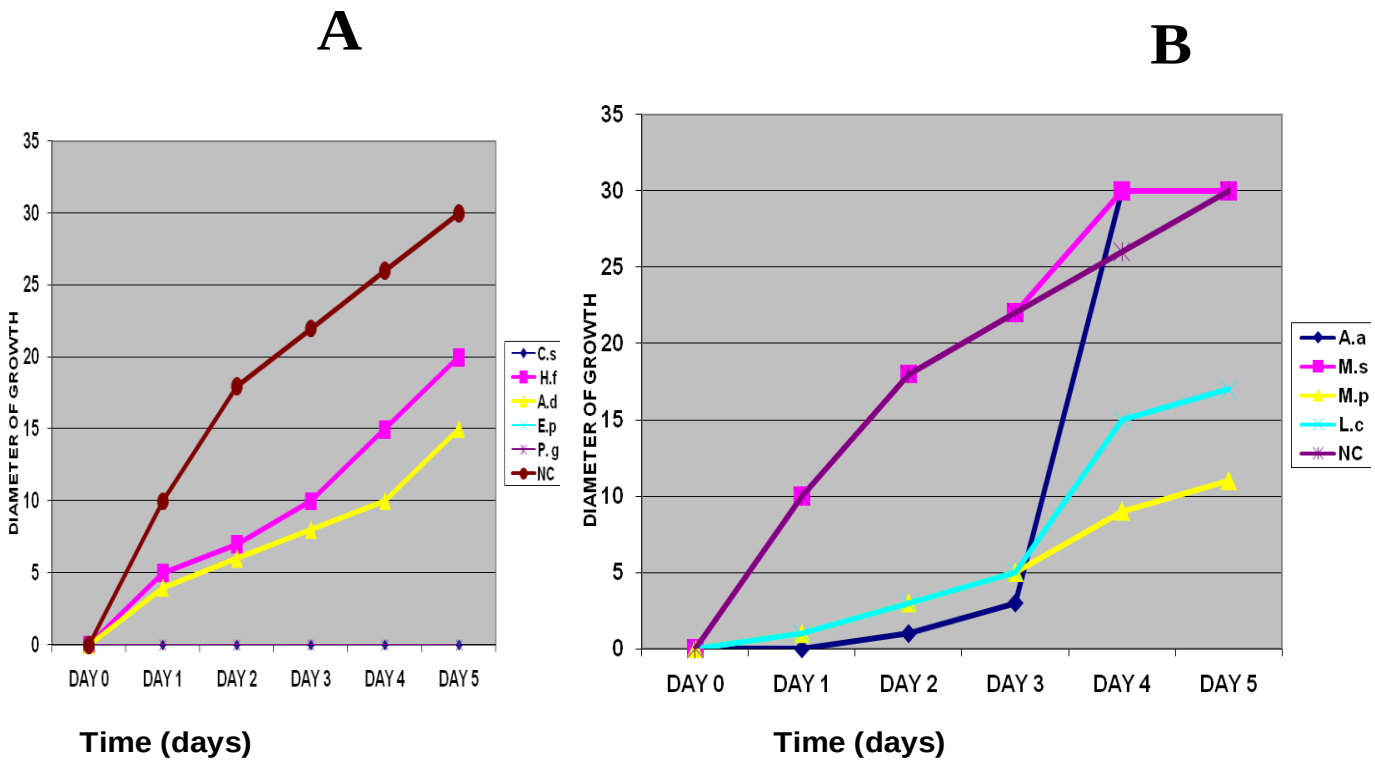

Figure 1. Colony growth inhibition curve of essential oils against *F. verticillioides* indicated by the reduction in the colony diameter compared to the negative control measured for five days; A: Activity of C.s = *C. scabrida*, H.f= *H. foetidum*, essential oils against *F. verticillioides;* B: Activity of A.a = *A. afra*, M.s = *M. officinalis*, M.p= *M. piperita* and L.c = *L. cerecea* essential oils against *F. verticillioides.* N.C = negative control.

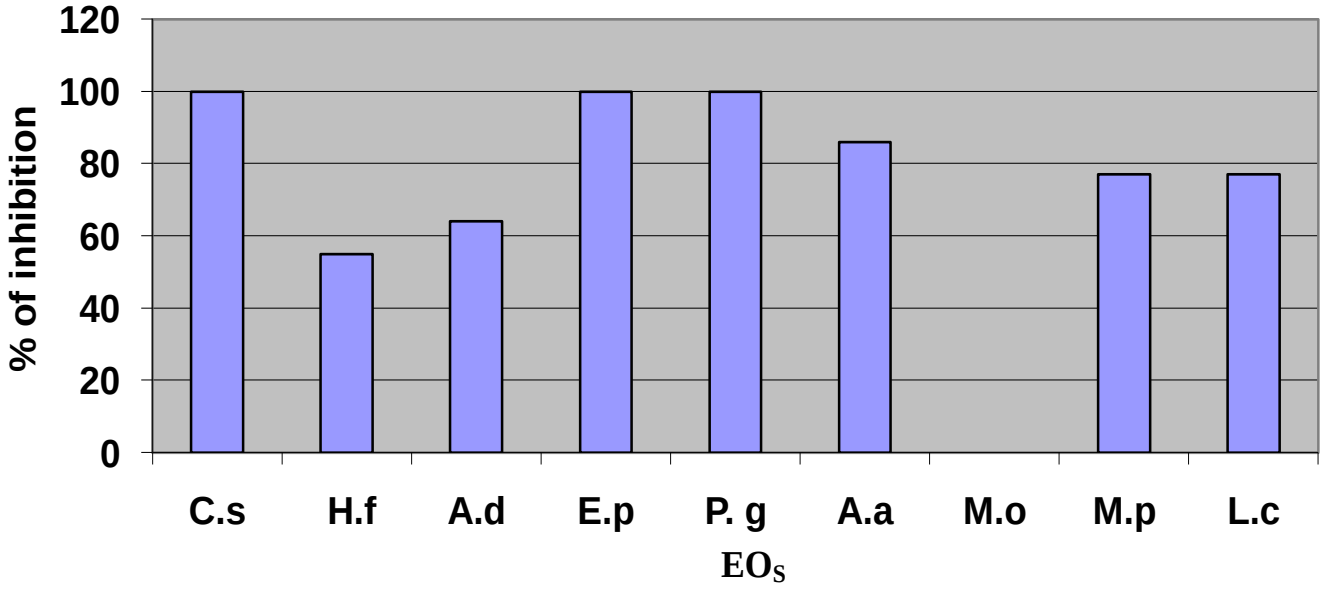

Figure 2. Percentage inhibition of different essential oils against *F. verticillioides* . C.s = C. *scabrida*; H.f = *H. foetidum*; A.d = *A digitata*; E.p = *E panculatus*; P.g = *P. Graveolens*; A.a = *A. afra*; M.o = *M. officinalis*; M.p = *M. piperita*; L.c = *L. cerecea.*

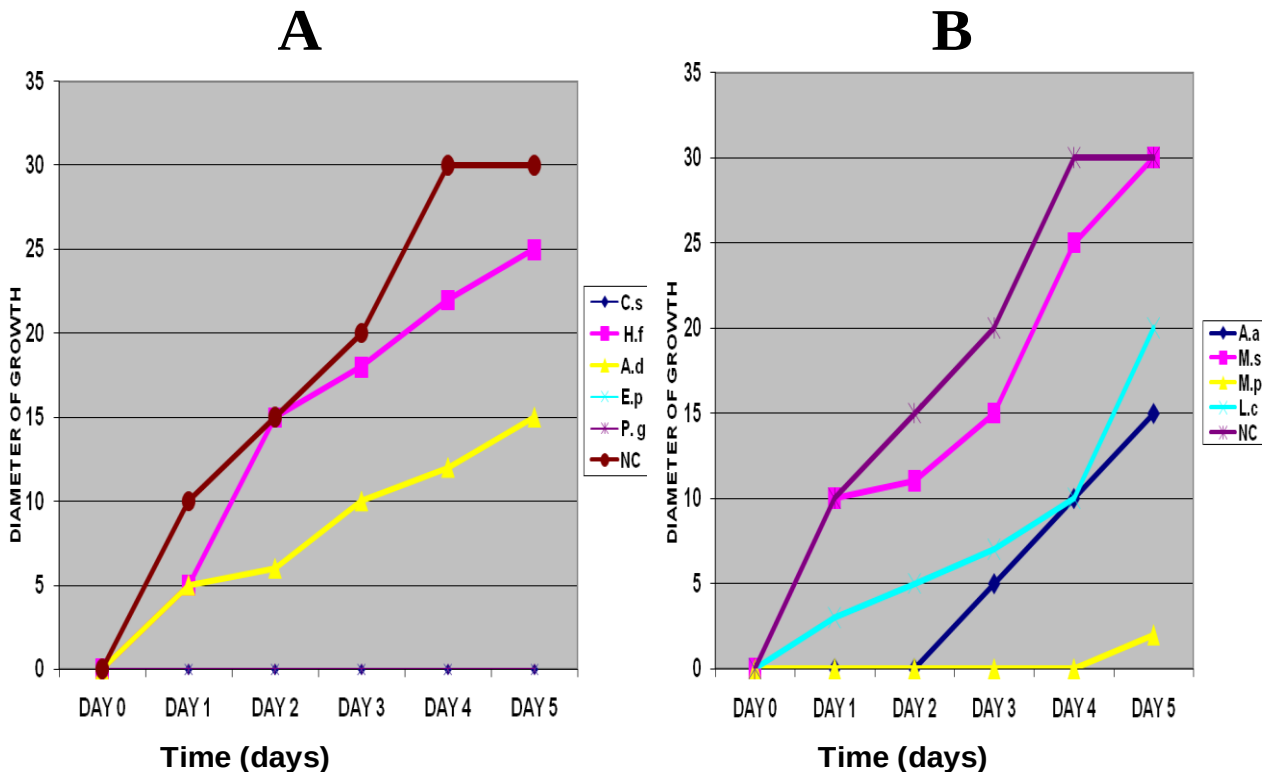

Figure 3. Colony growth inhibition curve activity of essential oil against *F. oxysporum.* A: activity of C.s = C. *scabrida*, H.f = *H. foetidum*, A.d = *A digitata*, E.p = *E. panculatus* and P.g = *P. graveolens* essential oils against *Fusarium oxysporum.* B: activity of A.a = *A. afra,* M.s = *M. officinalis*, M.p = *M. piperita* and L.c = *L. cerecea* essential oils against *Fusarium verticillioides*. N.C = negative control.

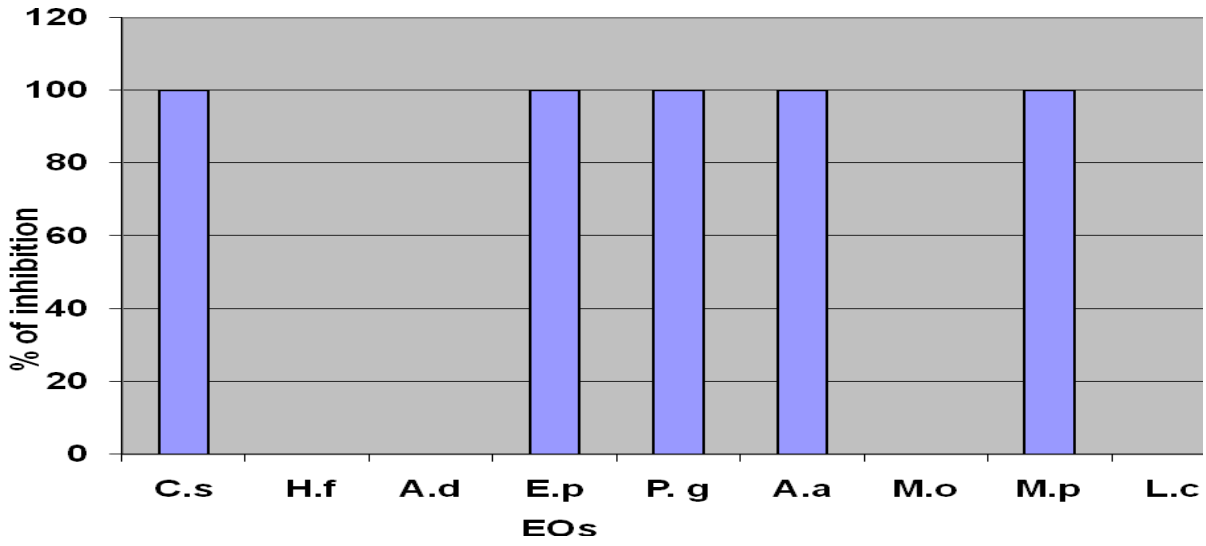

Figure 4. Percentage inhibition of different essential oils against *Fusarium oxysporum* C.s = *C. scabrida*; H.f = *H. foetidum*; A.d = *A digitata*; E.p = *E panculatus*; P.g = *P. Graveolens*; A.a = *A. afra*; M.o = *M. officinalis*; M.p = *M. piperita*; L.c = *L. cerecea.*

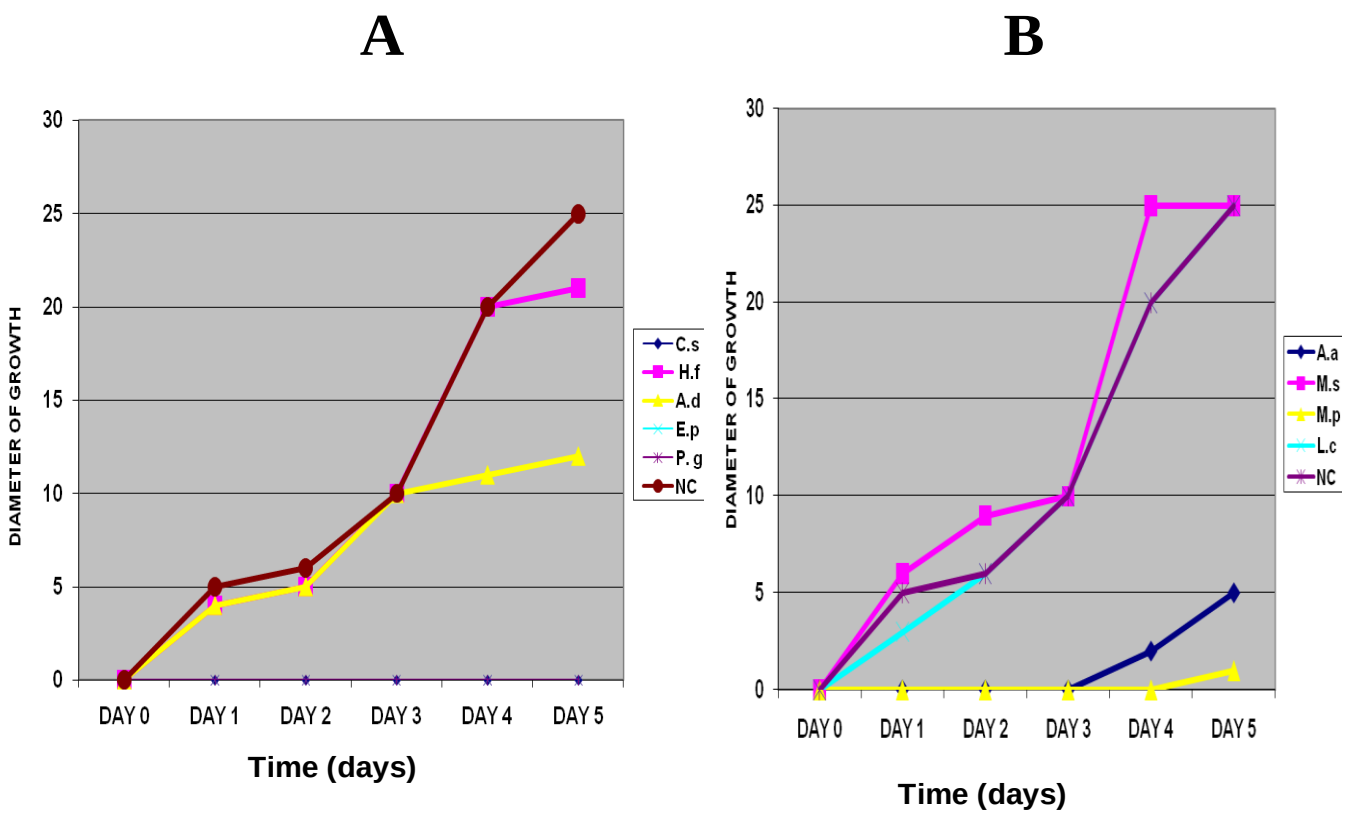

Figure 5. Colony growth inhibition curve of essential oils against *F. graminearum*. A: activity of C.s = C. *scabrida*, H.f = *H. foetidum*, A.d = *A digitata*, E.p = *E. panculatus* and P.g = *P. Graveolens* essential oils against *F. graminearum.* B: activity of A.a = *A. afra,* M.s= *M. officinalis*, M.p = *M. piperita* and L.c = *L. cerecea* essential oils against *F. graminearum.* N.C = negative control.

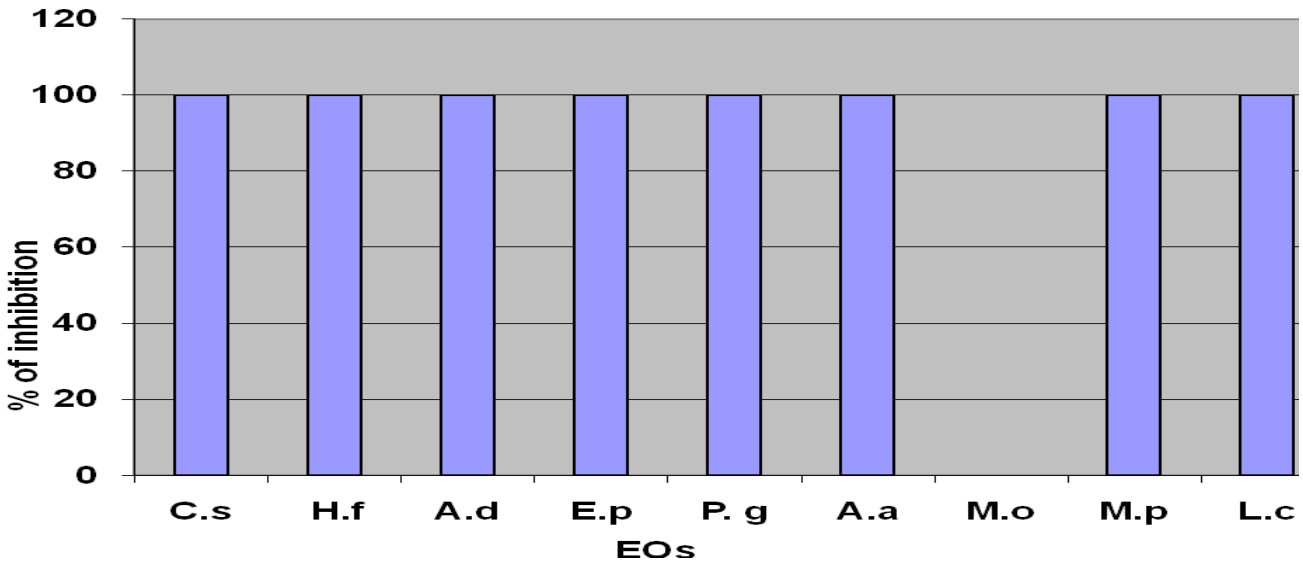

Figure 6. Percentage inhibition of different essential oils against *F. graminearum.* . C.s = *C. scabrida*; H.f = *H. foetidum*; A.d = *A. digitata*; E.p = *E. panculatus*; P.g = *P. Graveolens*; A.a = *A. afra*; M.o = *M. officinalis*; M.p = *M. piperita*; L.c = *L. cerecea.*

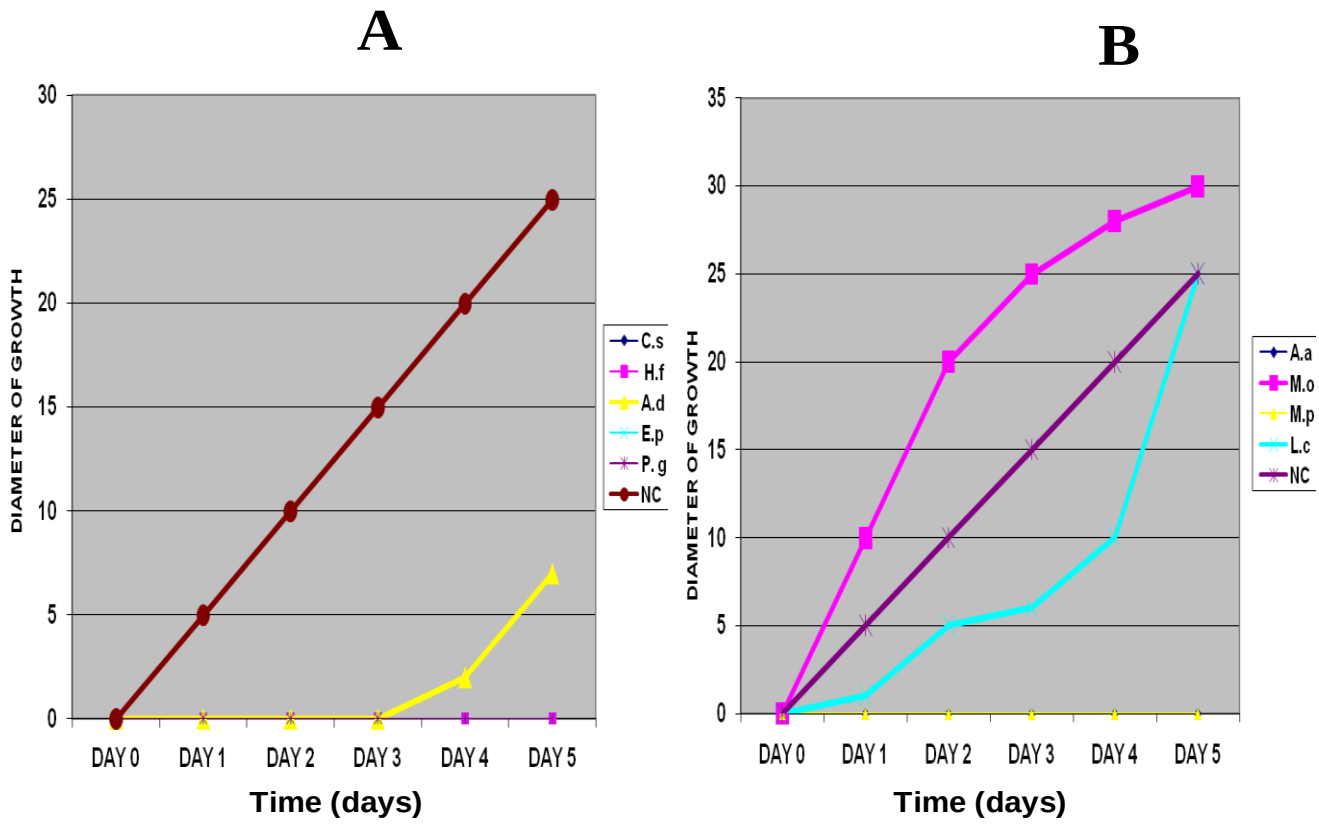

Figure 7. Colony growth inhibition curve of essential oils against *F. nygamai*. A: activity of C.s = C*. scabrida*, H.f = *H. foetidum*, A.d = *A digitata*, E.p = *E panculatus* and P.g = *P. Graveolens* essential oils against *F. Nygamai.* B: activity of A.a= *A. afra,* M.s = *M. officinalis*, M.p = *M. piperita* and L.c = *L. cerecea* essential oils against *F. nygamai*. N.C =negative control.

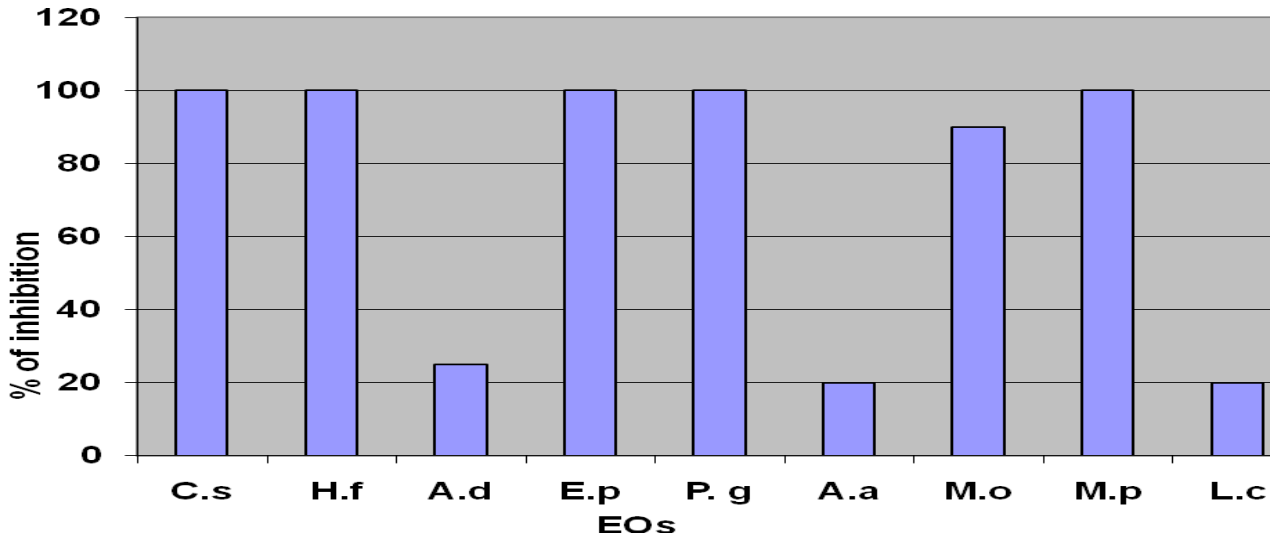

Figure 8. Percentage inhibition of different essential oils against *F. nygamai.* C.s = *C. scabrida*; H.f = *H. foetidum*; A.d = *A digitata*; E.p = *E panculatus*; P.g = *P. Graveolens*; A.a= C.s = A*. afra*; M.o = *M. officinalis*; M.p = *M. piperita*; L.c = *L. cerecea.*

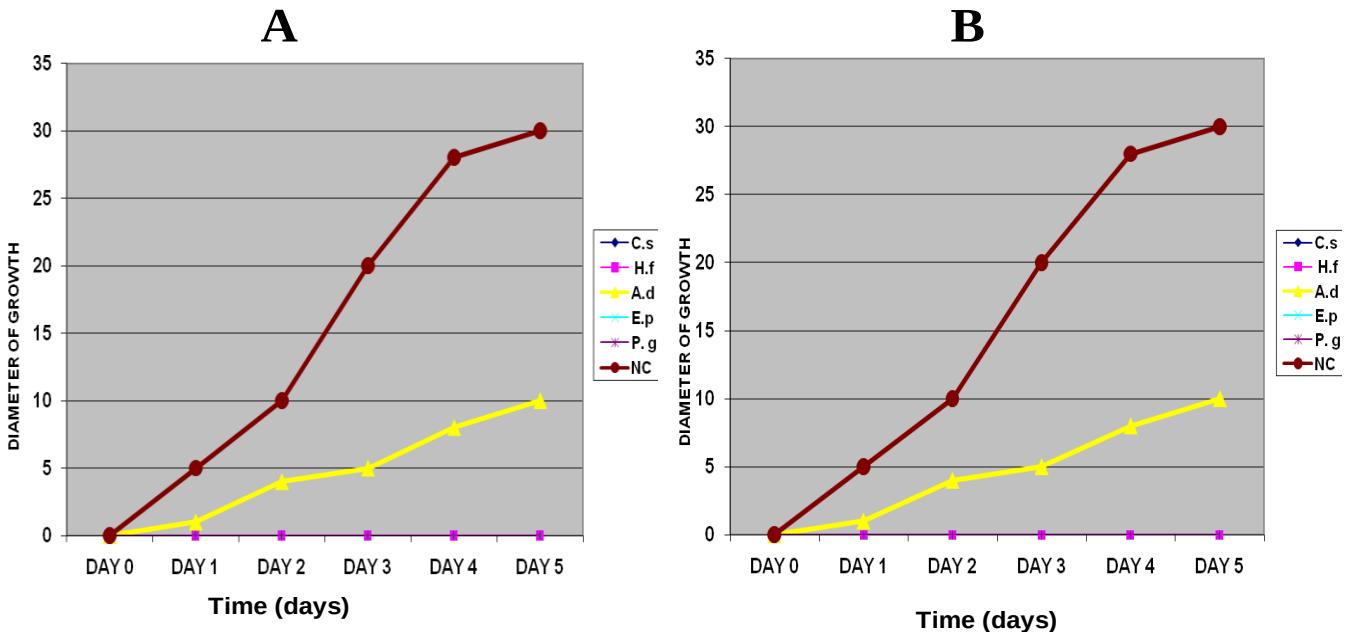

Figure 9. Colony growth inhibition curve of essential oils against *F. proliferatum.* A: activity of C.s = C*. scabrida*, H.f = *H. foetidum*, A.d = *A digitata*, E.p = *E panculatus* and P.g *P. graveolens* essential oils against *F. proliferatum*. B: activity of A.a = *A. afra*, M.o = *M. officinalis*, M.p = *M. piperita*, L.c = *L. cerecea* essential oils against *F. proliferatum.* NC = negative control.

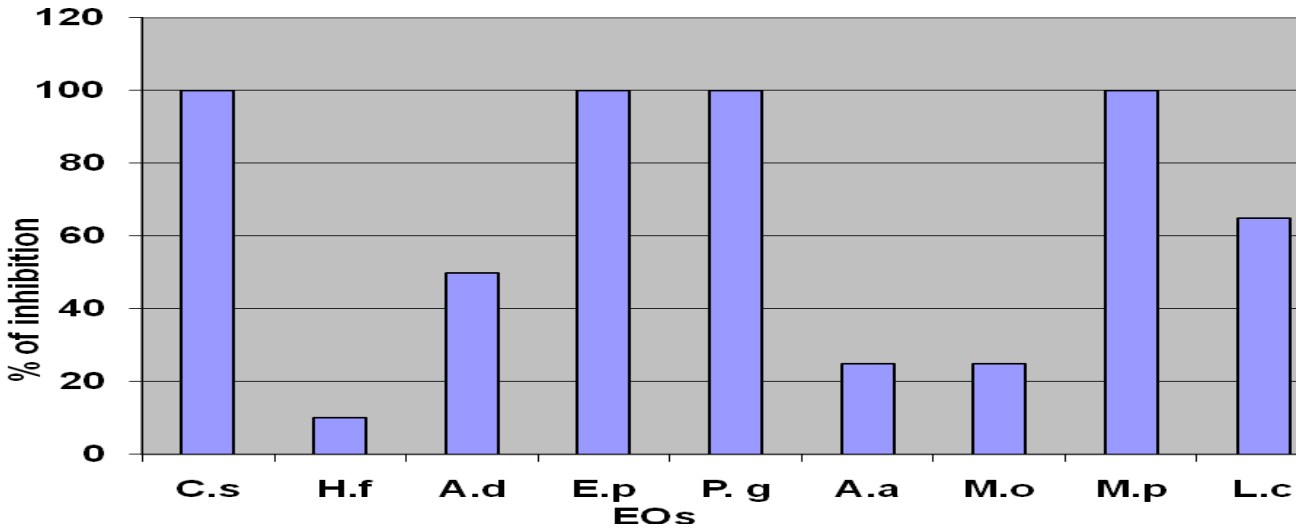

Figure 10. Percentage inhibition of different essential oils against *F. proliferatum.* C.s = *C. scabrida*; H.f = *H. foetidum*; A.d *A digitata*; E.p = *E panculatus*; P.g *P. graveolens*; A.a = *A. afra*; M.o = *M. officinalis*; M.p = *M. piperita*; L.c = *L. cerecea.*

activity against *F. oxysporum* since it was able to kill about 95% of the cells on the first day of the experimentation compared to *M. piperita* oil which had killed only about 50% of the cells after the first day of

properties for centuries, however, only few of them have been characterized for their antimicrobial activities (Halcon and Milkus, 2004). Anecdotal and traditional use of the plants as medicines provide the basis for indicating

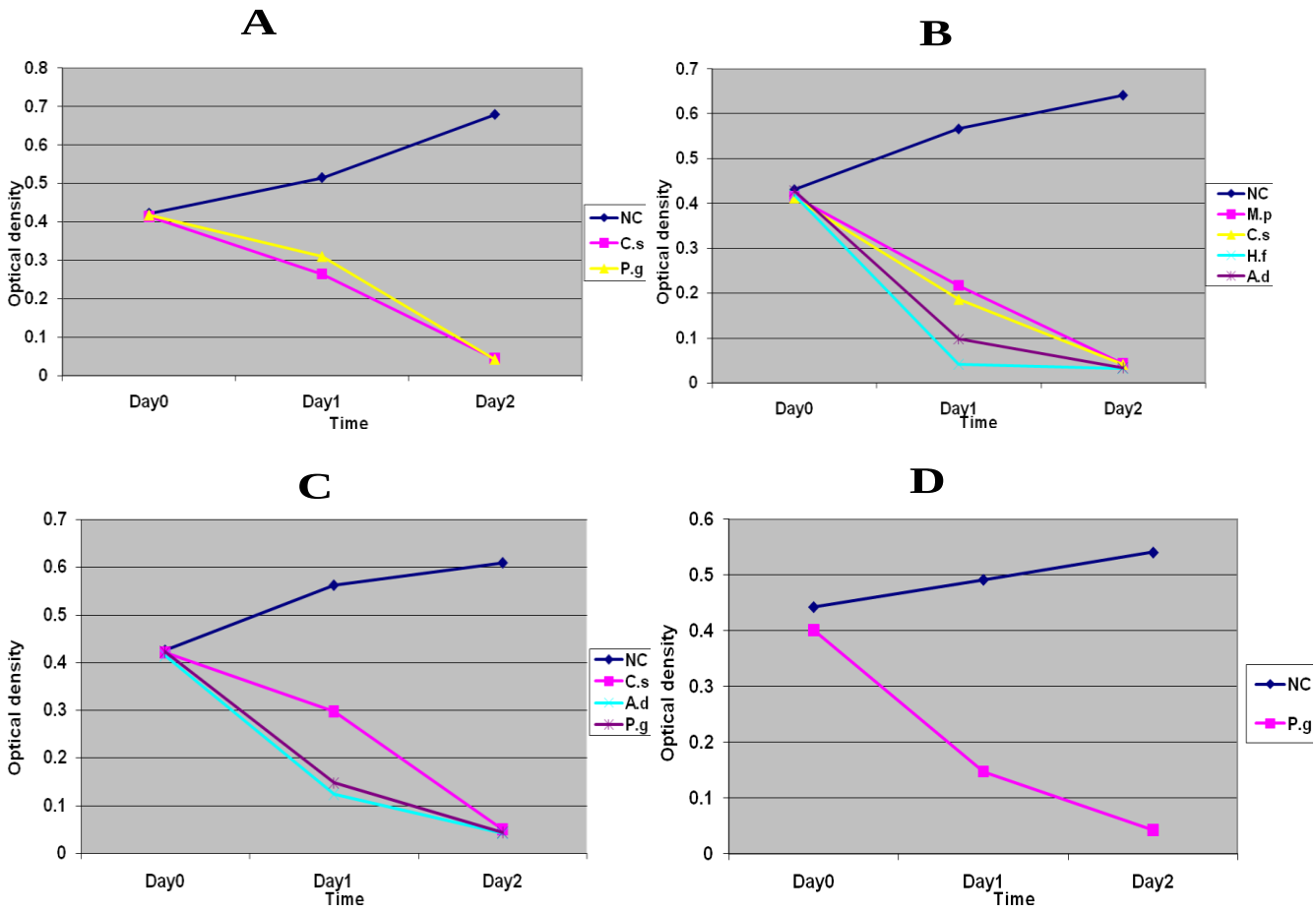

Figure 11. Killing curves of different essential oils against five *Fusarium* spp. indicating the rate at which the oil was killing the *Fusarium* strains showed by the variation of optical density at 590 nm at different time periods. A: killing activity of the oils of C.s = *C. scabrida* and P.g = *P. graveolens* against *F. proliferatum*. B: killing activity of oils M.p = *M. piperita*, H.f = *H. foetidum* and A.a = *A. digitata* against *Fusarium oxysporum*. C: Killing activity of P.g = *P. graveolens* against *F. nygamai*. D: killing activity of M.p = *M. piperita*, C.s = *C. scabrida*, H.f = *H. foetidum* and P.g = *P. graveolens* against *F. graminearum*. NC = negative control.

which essential oil may be useful for specific medical conditions. Historically, many plant oils such as, tea tree; myrrh and clove have been used as topical antiseptic or antimicrobial properties (Mondello et al., 1998; Haq et al., 2011). In spite of the rich biodiversity, very few studies have been conducted in Africa on the biological activity of essential oils and even fewer essential oils have entered the commercialization pipeline (Yirga, 2010). The present study showed that the essential oils from plants commonly used in the Southern African region are inhibitory and fungicidal against *Fusarium* pathogens. *A. digitata* commonly known as baobab is a very common plant found in the savannas throughout the African continent. It has been used for centuries by indigenous people in the continent and even from outside the continent and is associated with many beneficial effects.

In the present study, baobab oil was active against the *Fusarium* spp. indicating the possibility of using this plant in the control of infections by *Fusarium* spp among patients particularly those who are immunocompromised. Although previous studies have demonstrated the antibacterial activities of baobab extracts very few or no studies have reported the activity of the oil against *Fusarium* species (Motsei et al., 2003). The fatty acid profile of the oil showed that oleic and linoleic were the major unsaturated fatty acids, whereas palmitic was the major saturated acid (Osman, 2004). Previous studies by Masola et al. (2009) have indicated that the extracts of the stem bark of *A. digitata* contained chemical substances such as tannins, phlobatannins, terpenoids,

cardiac glycosides and saponins while the roots bark contained mostly terpenoids (Nkafamiya et al., 2007). These substances could be responsible for the activity of the oil against the organisms. Limited studies have been conducted on the activities of baobab oil on fungal organisms. Therefore the present study represents one of the rare studies to identify the activity of baobab oil on *Fusarium* spp. The essential oil from *A. afra* was found to be active against 4 out of 5 *Fusarium* spp. tested in the present study. These findings highlight the importance of this oil in treating some medically important fugal pathogens. In previous studies this oil was tested for antifungal activity against 10 fungal species.

The results obtained showed that the essential oil exhibited significant activity against *Aspergillus ochraceus*, *Candida albicans, Alternaria alternata, Geotrichum candidum, Aspergillus niger, Penicillium citrium and Aspergillus parasiticus* (Gundidza, 1993). However, they did not test the oil from this plant against *Fusarium* spp. In a study by Mangena and Muyima (1999) the essential oils of *A. afra* growing in Eastern Cape, South Africa showed broad antimicrobial activities and therefore may have preservative potential for the food and cosmetic industries. The activity of the essential oil could be dependent on geographical location of the plant. A recent study has demonstrated that the composition of *A. afra* essential oil varied between the three different provinces particularly in the levels of alpha-and beta-thujone, 1, 8-cineole and camphor (Oyedeji et al., 2009). This will explained the variation of activities of plants from different areas. Their study further indicated that fresh leaves had less alpha thujone than dried leaves indicating that the oil from the fresh leaves are safer. Further studies are needed to clarify the effect of the compositional variation of the antimicrobial potential. Essential oil from *C. scabrida* showed very good activities against all the microorganisms tested including bacteria and fungi.

Traditionally, this plant is used to treat influenza, chest and stomach afflictions, fever, diarrhea, sores and inflammation (Watt and Breyer-Brandwijk, 1962; Scott et al., 2004). Studies in the Western Cape, South Africa showed that the aqueous infusion of *C. scabrida* showed good inhibition against *C. albicans* with a MIC value of 0.625 mg/ml (Thring et al., 2007). The oil of *C. scabrida* gave 100% inhibition of all *Fusarium* spp. for the duration of the experiment. It has been reported that the chemical constituents of different essential oil support their microbial activity (Watt and Breyer-Brandwijk, 1962). *Helichrysum* species are used extensively in ethnomedicine in South Africa and many of the uses are associated with the treatment of infections, for example, it is used widely for treatment of respiratory diseases. Very few studies have been conducted on extracts or essential oils from *H. foetidum*. Steenkamp et al. (2004) did not find high antibacterial activity. In the present study, the essential oil of *H. foetidum* was found to be very active against the *Fusarium* spp. Although, no studies have been conducted on the phytochemistry of *H. foetidum* itself, studies on *H. Helichrysum pallasii* from Italy have indicated that the oil from this related *Helichrysum* spp. contained hexadecanoic acid (16.2%), (Z,Z)-9,12-octadecadienoic acid (6.8%), tetradecanoic acid (2.6%), and (Z)-caryophyllene (4.2%) as the main constituents of the oil from leaves (Formisano et al., 2009). Another study on related *Helichrysum* spp in Tanzania indicated that *Helichrysum cymosum* and *Helichrysum fulgidum* contained trans-caryophyllene, caryophyllene oxide, beta-pinene, p-cymene, spathulenol and beta-bourbonene as the main components (Bougatsos et al., 2004).

Similar compounds could be found in *Helichrysum foetidum* and might be responsible for the activities observed. However, more studies are needed to identify the potential active compounds and the safety of the oil from this plant for human consumption. Based on the activities observed against the *Fusarium* spp. the oil could be possible alternative to synthetic fungicides in the fight against phytopathogenic fungi. *M. piperita* essential oil exhibited weak activity against some of the *Fusarium* organisms tested and was not fungicidal to any of the five *Fusarium* spp. In a previous study by Mimica-Dukic et al. (2004) the essential oil from *M. piperita* was active against *Trichophyton tonsurans* and *C. albicans* with low MIC (8 µl/ml). A study on the plants from Iran indicated that Menthanol (36.24%) and menthone (32.42%) were the major compounds of the *M. piperita* essential oil (Behnam et al., 2006). Menthol has been reported to be responsible for the antimicrobial activity of *M. piperita* (Barrera-Necha et al., 2009).

Further studies are needed in order to identify the active compounds from this plant growing in the Southern African region and to associate the presence of these compounds to the biological effect observed.In the present study, the essential oils from *M. officinalis* and *L. sericea* were not active against most microorganisms tested. Although several studies have been conducted on *M. officinalis*, very few studies have been conducted on *L. sericea*. *M. officinalis* essential oil has been shown to have good antioxidant effect (Marongiu et al., 2004) as well as antiviral effect (Schnitzler et al., 2008). Cytotoxicity studies of essential oils *M. officinalis*, have shown that the oil from this plant was highly toxic to *Spodoptera littoralis* larvae with LD50 < or = 0.05 microl/larvae (Pavela, 2005). Mimica-Dukic et al. (2004) indicated that a significant rate of antifungal

activity was exhibited on *Trichophyton* species. We did not test the activity of the oil against this organism. Therefore it is necessary to further the antimicrobial activity of these essential oils to confirm their activities on a large variety of organisms. In the present study, *L. sericea* essential oil showed some activity against the *Fusarium* spp with inhibition activities up to 80% against *F. verticillioides*. Studies by Bosman et al. (2004) indicated that the petroleum extract of the leaves of this plant had antimicrobial activity against *C. albicans.* Bioassay-guided fractionation by the same authors of the leaves and flowers extracts yielded the phloroglucinol derivatives, aspidinol and desaspidinol.

Essential oil of *P. graveolens* exhibited absolute fungitoxicity against the toxigenic strains of *Aspergillus flavus* with MIC of 0.75 g L^{-1} and exhibited a fungistatic nature (Singh et al., 2008). The oil also showed excellent anti-aflatoxigenic efficacy as it completely inhibited aflatoxin B_1 production even at 0.50 g L^{-1}. Jeon et al. (2009) studied the acaricidal activities of compounds derived from the oil of *P. graveolens* leaves against *Tyrophagus putrescentiae* and discovered that the toxic compounds against the food mite were geraniol (1.95 microg/cm^3), followed by nerol (2.21 microg/cm^3), citral (9.65 microg/cm^3), benzyl benzoate (11.27 microg/cm^3), and beta-citronellol (15.86 microg/cm^3).

In the present study, the essential oil of *P. graveolens* showed excellent activities against all the *Fusarium* organisms tested. In a study by Rosato et al. (2008), *P. graveolens* essential oil was the most effective in combination with amphotericin B in inhibiting all the *Candida* species evaluated. This indicates that *P. graveolens* essential oil can be used for the control of fungal pathogens both alone or in combination with purified drugs such as amphotericin B. The activities observed could be due to the compounds previously identified such as geraniol or beta citronellol all identified in the essential oil of *P. graveolens* with anti mites activity.

Conclusion

This study reports for the first time the antifungal activities of these essential oils against *Fusarium* species *C. scabrida*, P*. graveolens* and *H. foetidum*. The present investigation together with previous studies provides support to the use of these essential oils as antibacterial and antifungal supplements in the developing countries towards the development of new therapeutic agents. The results of the present study indicate that essential oils are promising sources of natural products with potential antimicrobial activity and will guide the selection of some plant species for further pharmacological and phytochemical analysis. Additional studies both *in vitro* and *in vivo* and clinical trials would be needed to further characterize the active principles and evaluate the potential toxicity of these oils.

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