International Journal of Plant Science and Ecology
Vol. 1, No. 2, 2015, pp. 22-25
http://www.aiscience.org/journal/ijpse

The Effect of Alcoholic Extract of Proboscidea Parviflora on *Fusarium oxysporum and Alternaria spp*

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Abstract

The experiment was conducted during (2007/2008) at the experimental farm and plant pathology laboratory of the University of Khartoum, at Shambat, Sudan. The main objective of this experiment was to study the effect of the antimicrobial activities of *Proboscidea parviflora*. The experiment was laid out in randomize complete block design with four replications and five treatment to investigate the effect of the alcoholic extracts on some moulds (fungi). Fungicidal activity of alcoholic extract of wild plants *Proboscidea parviflora* was evaluated against the moulds species *Fusarium oxysporium* and *Alternaria spp.*. Alcoholic extracts 6% (w/v) were prepared using six grams of dried plant powders (leaves and stems) and alcohol (70% ethanol or 70% methanol). The two mould species were affected and controlled by the alcoholic extract of the leaves and stems of *Proboscidea parviflora.* Alcohol also had affected the mould by inhibiting their growth but lesser than leaves extract. The effect of stem extract was better than leaves extracts and alcohol against the two fungi.

Keywords

Alcoholic Extract, Mould, Fusarium, Alternaria

Received: February 1, 2015 / Accepted: March 7, 2015 / Published online: March 12, 2015

1. Introduction

Plants are able to produce different compounds which used to protect themselves against different types of pathogens (Cowam, 1999). Spices are mainly used in foods mainly because they give desirable flavors and aromas; in addition they show antimicrobial activity (Beuchat and Golden, 1989).

Proboscidea parviflora (*Martyniaceae)* belongs to a small New World family comprised of a small number of species from even fewer genera. It is often cited as belonging to the family Martyniaceae, so some sources have placed it in the same family as sesame (*Pedliaceae*).

Devil's claws are best characterized by their long, pointed claws. When dry, hooked claw readily attaches to passing furry-legged mammals, including the sock-wearing variety (Bertting, 1982). Devil's claw or cat's claw (English), Una de gato has been founded in Zalingei a few years ago and used traditionally by the practioners to remedy scorpion and snake bite, also in magic for preparing some medicine, and they called it Damin Asharah.

Tequida *et al*., (2002) found that the alcoholic extract of *Proboscidea parviflora* controlled two mould species. Brinker, (2004) stated that combinations of anti-inflammatory drugs with stinging nettle, willow, devil's claw' bromelain and thunder god vine products have shown improved therapeutic outcomes. The seeds of *Proboscidea parviflora* (cat's claw) have shown a great nutritional potential due to the good nutritional quality presented by its flour and oil presented good digestibility and high protein efficiency ratio (Ortega, 2003).

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2. Material and Methods

A laboratory experiment was conducted to study the effect of alcoholic extract from leaves and stems of *Proboscidea parviflora* on two of mold causes (*Fusarium oxysporium* and *Alternaria spp.)*

Alcoholic extracts 6% (w/v) were prepared using six grams of dried plant powders (leaves and stems) and 94ml alcohol used as solvent methanol or ethanol both at 70%. The extraction was done by soaking for 15 minutes with agitation machine in plate and a rest of 48 hr to 25±2°C. Extract obtained by filtered in two stages: first through fabric flax to remove larger plant particles, then followed by centrifugation at 7000 rpm-decanting by 10 min. and finally a second vacuum filtration in Whatman paper No 4 (Whatman, USA). The obtained extract were kept in plastic bottles amber and stored in refrigerator.

The species of fungi used were *Fusarium oxysporium* and *Alternaria spp.* isolated from grains of wheat and corn and to some extend brought from pure stand culture.

2.1. Preparation of Inoculums

Each of fungus species was leaning to grow in potato dextrose agar (PDAs) to 25±2°C for 10 days in test tubes (18x150). Later, spore suspension was prepared by adding to each test tube 10 ml saline solution (0.85%) with 0.1% tween 80 (Polysobrate 80, Sigma, USA) the last to prevent agglomeration and rapid precipitation of spores. The obtained suspension was transferred to another sterile test tube and determined its concentration, which adjusted to 1x106 CFU/ml with saline. The extracts were mixed with yeast agar (CYA) at 45-50 degrees C in 1:10 relation on Petri dishes. Triplicate Petri dishes of each treatment and for each mould were centrally inoculated and three Petri dishes were used without treatment as controls. The inoculated dishes and controls were incubated at 25 +/- 2 degrees C for eight days. The incubated dishes were examined each 24 hr and after the colony diameter (radial growth) was measured. The experiment was divided into four treatments (Leaves extract, stems extract, alcohol and control) and three replications.The inoculated dishes and controls were incubated at 25 +/- 2 degrees C for eight days. The incubated dishes were examined each 24 hr and after the colony diameter (radial growth) was measured.

The experiment was divided into four treatments (Leaves extract, stems extract, alcohol and control) and three replications.

2.2. Data Analysis

Analyses of variance appropriate to a randomized block design were performed for each mould. The least significant difference (LSD) method ($P < 0.05$) was used to evaluate differences among treatments when significant.

SAS statistical analysis system was used for the statistical analysis (SAS, USA, 2004).

3. Result

3.1. The Effect of Alcoholic Extract of *Proboscidea parviflora* on *Fusarium oxysporium*

Data presented in Table (1) showed that there were no significant differences among the treatments the first 24 hr. in term of diameter length of the spore.

After the first 24 hr the registered data indicated that there were significant differences between the treatments. The smallest diameter was found in the stem extract in all of the treatment period, while the control registered the highest ones (Figures 1-4). In the first 72 hr Alcohol (solvent) data was better that leaves extract. But this was changed after the fourth day to the end of the experiment.

Data in this experiment showed that stem extract was better than leaves extracts and so do Alcohol when compared with it.

3.2. The Effect of Alcoholic Extract of *Proboscidea parviflora* on *Alternaria spp.*

Data presented in Table (2) showed that there were highly significant differences (P= 0.05) among treatments in their effects on *Alternaria spp.* The smallest values of diameter length were recorded with the stem extract, while the greatest ones were recorded with the control.

This indicated that the ability of controlling mould growth was very good with the extract of the stems more than leaves alcoholic extract. Alcohol gave good results more than of the leaves. These results indicated that the concentration of the substance that inhibits or control the growth of the mould was to be greater in the stem and lesser in the leaves (Figures 5-8).

Table (1). The effect of alcoholic extract of *Proboscidea parviflora* on *Fusarium oxysporium*

Treatment	*24hr*	*48hr*	*72hr*	*96hr*	*120hr*	*144hr*	*168hr*
Leaves extract	29.97^{a}	35.1^{a}	40.2^{b}	42.56^{b}	44.53^{c}	49.33^{c}	54.67^{c}
Stem extract	27.57^{a}	31.5^{b}	33.9^{b}	37.33^{b}	42.87^{c}	49.43^{c}	50.53^{c}
Alcohol	27.63^{a}	32.3^{ab}	36.9^{b}	42.56^{b}	52.76^{b}	67.00^{b}	69.77^{b}
Control	27.23^{a}	34.8^{ab}	47.2^{a}	55.53^{a}	64.67^{a}	77.90^{a}	86.23^{a}
LSD $_{0.05}$	3.06	3.35	6.58	8.42	3.37	5.31	5.46

Table (2). The effect of alcoholic extract of *Proboscidea parviflora* on *Alternaria spp.*

Treatment	*24hr*	*48hr*	*72hr*	*96hr*
Leaves extract	58.10[a]	65.30[b]	69.20[b]	7657[b]
Stem extract	55.00[a]	60.53[d]	66.47[b]	73.00[b]
Alcohol	57.20[ab]	63.10[c]	69.57[b]	75.63[b]
Control	57.50[b]	67.67[a]	82.90[a]	89.80[a]
LSD $_{0.05}$	2.43	2.15	6.32	4.01

4. Discussion

The Effect of Alcoholic Extract of Proboscidea parviflora on The Inhibition Growth of Mould.

Data in Tables (1-2) indicated that the methanolic extracts of *Proboscidea parviflora* had significant effect on *Alternaia spp.* and *Fusarium oxysporium.* Khalil, (2001) reported that aqueous extracts of fruits and leaves of *Capsicum* fruitscens, *Capsicum* annum (Solanaceae) and *Nerium oleander* (Apocynaceae) inhibited the germination of *Alternaria solani* spores and decreased the mycelia dry weight of *Alternaria solani* and *Saprolengnia*. Similar observations was recorded by Tequida *et al.,* 2002 who evaluated the alcoholic extracts of leaves and stems of *Proboscidea parviflora* against the mould species *Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Penicillium expansum, Fusarium poae* and *Fusarium moniliforme*. They found that the *Fusarium spp.* and *Penicillium spp*. were controlled by *Proboscidea parviflora*.

Also this findings agrees with Shahidi *et.al.*, (2004) who studied the methanol plant-extracts of 221 species from 98 families which had documented uses in Iranian herbal-medicine with antibacterial and antifungal activity against 11 standard bacterial strains and 3 fungal species. They reported that *Proboscidea parviflora* one of the useful plant used in there experiment.

Similar observations agree with the findings of Okigbo and Ogbonnaya (2006). Who reported that the ethanolic extraction of *Ocimum gratissimum* inhibited more than that of *A. melegueta* the two plants extracts inhibited the spores of *Fusarium oxysporium* and *Aspergilus niger.* This observation could be attributed to the antifungal properties of *Proboscidea parviflora* acting against the growth of fungal species.

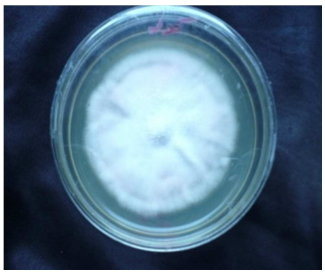
Hassan *et.al.,*(2005) stated that the alcoholic extracts of neem and garlic completely inhibited the presence of *Bipolaris sorokiniana*, *Fusarium spp., Aspergilus spp., Penicillium spp. and Rhizopus spp.* respectively on treated wheat seeds, whereas the highest percentage of *B. sorokiniana* (11.67%), *Fusariun spp.*(24.33%), *Aspergillus spp.* (17.07%), *Penicillium spp*. (7.5%) and *Rhizopus spp.*(4.5%).

Fusarium oxysporium

Fig. (1). Leaves extract

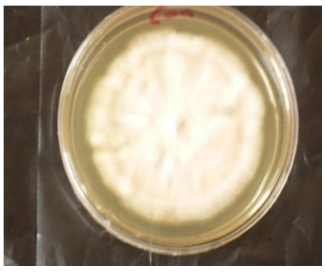
Fusarium oxysporium

Fig. (2). Stem extract



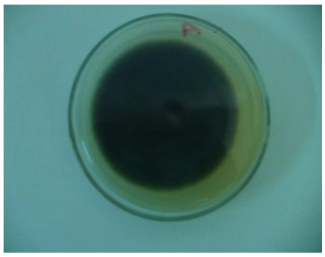
Fusarium oxysporium

Fig. (3). Alcohol 70%



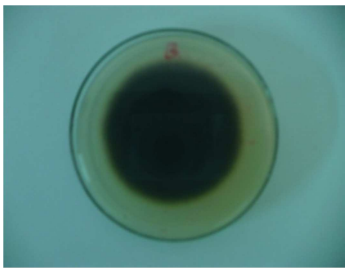
Fusarium oxysporium

Fig. (4). Control



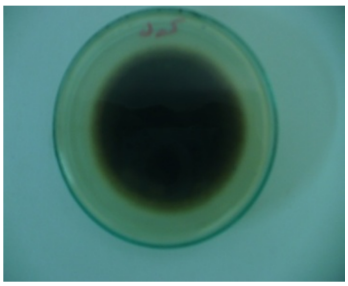
Alternaria spp.

Fig. (5). Stem Extract



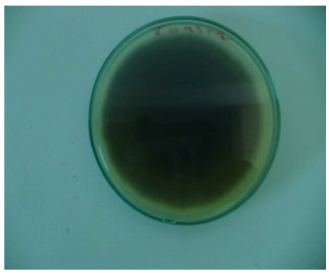
Alternaria spp.

Fig. (6). Leaves



Alternaria spp

Fig. (7). Alcohol 70%



Alternaria spp

Fig. (8). Control

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