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Anti-fungal activity of plant extracts for the management of *Fusarium oxysporum* f. sp. *elaiedis in vitro*

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Abstract

Fusarium oxysporum f. sp. *elaiedis* is the causal organism for vascular wilt, a major disease of oil palm. An experiment was conducted in Cameroon from 2014 to 2015, to assess the anti-fungal activity of aqueous and methanol extracts of *Eleusine indica*, *Diospyros crassiflora*, *Fagara heitzii* and *Milicia excelsa* for the management of the pathogen. The plants were tested at concentrations of 0 (negative control), 50 and 100% aqueous extracts and 100% methanol extract. The positive control was a commercial fungicide (Mancozan). Results revealed that at 3, 7 and 10 days after exposure (DAE), the fungicide and both extracts of most of the plants inhibited mycelia growth of the fungus significantly ($p<0.05$) compared to the negative control. The aqueous leaf extract of all the plants showed significantly better activity against the fungus than the methanol leaf extract. With the exception of *F. heitzii*, the fungicide and aqueous extract of all plants had more than 50% inhibitory effect on the pathogen at 7 and 10 DAE. The most effective aqueous extract of the plants against the pathogen were 100% *E. indica* and both concentrations of *M. excelsa* at 10 DAE. However, Mancozan was more effective at inhibiting fungal growth than *M. excelsa* by 20–27%, *E. indica* by 30–36%, *D. crassiflora* by 35–37% and *F. heitzii* by 63–98% at both concentrations tested at 10 DAE. In conclusion, *E. indica*, *M. excelsa* and *D. crassiflora* showed promising anti-fungal activity.

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Introduction

Fusarium oxysporum f. sp *elaeidis* is a soil borne pathogenic fungus, which causes vascular wilt disease of oil palm (*Elaeis guineensis* Jacq.). The disease is a major constraint to the production of oil palm in Cameroon and several other African countries. It affects the growth of the crop and reduces oil yield (Tengoua and Bakoumé, 2008; Ntsomboh-Ntsefong *et al*., 2012). It has been reported to cause up to 70% mortality of the crop (Renard and Revise, 1986).

The disease symptoms had been described extensively by early researchers. According to Flood (2006), the infected palms manifest through two types of symptoms: acute and chronic; in the first case, the palm dies three to four months after the appearance of the disease; in the second, the palm stays alive but it is weakened and rendered almost unproductive. The disease spreads when the plant roots get in contact with dead infected palm tissues (Renard and de Franqueville, 1989). When the fungus penetrates the roots of the oil palm, it develops in the xylem vessels where it causes water stress (Flood, 2006; Tengoua and Bakoumé, 2008). Expression of the disease depends on factors such as the age of the plant, its susceptibility, stage of the infection and environmental factors (Renard and de Franqueville, 1989).

The oil palm is a perennial crop that belongs to the family Arecaceae. It is considered as the most important oil crop in the world (Gascon *et al*., 1988; Cochard *et al*., 1993). Its cultivation is very important in Cameroon and it is mostly done by small-holders and agro-industries. According to Hoyle and Levang (2012), Cameroon produces approximately 230,000 tons annually; it is the world's 13th largest producer. In addition to palm oil which is extracted from the crop and used for cooking, palm kernel oil is also extracted and used in the cosmetic industry. The vascular wilt disease is a serious problem in the South West, Littoral, West, North West, Centre, and South Regions of Cameroon, where the oil palm is mostly cultivated (Ntsomboh-Ntsefong *et al*., 2012; Ngando *et al*., 2013).

Various methods have been employed to manage the vascular wilt disease of the oil palm. These include the use of tolerant cultivars (de Franqueville and Renard, 1990), cultural control and sanitary methods (Renard and Quillec, 1983; Renard and de Franqueville, 1991). The application of synthetic fungicides has been a very common method of managing fungal diseases in plants but these chemicals are expensive, toxic to humans and are not ecologically friendly. Despite all the efforts put in, the disease prevalence is still high. There is a need to identify appropriate management methods that are cost effective and environmentally friendly. The use of extracts derived from plants would offer farmers many benefits because they are affordable and environmentally friendly. Several plant extracts have been tested for anti-fungal activity against different formae speciales of *F. oxysporum* (Agbenin and Marley, 2006; Rongai *et al*., 2012, 2015; Dissanayake and Jayasinghe, 2013; Mishra *et al*., 2014). However, there is limited information on the anti-fungal activity of botanical extracts for management of *Fusarium oxysporum* f. sp. *elaiedis*. It is hoped that this study will provide information on suitable botanicals which can be used as components for a suitable integrated management package. The objective of this study was to determine the efficacy of aqueous and methanol leaf extracts of four plants (*Eleusine indica* L. Gaertn., *Diospyros crassiflora* Hiern, *Fagara heitzii* Aubr. et Pellegr. and *Milicia excelsa* Welw.) for the management of *Fusarium oxysporum* f. sp. *elaiedis in-vitro*.

Materials and methods

Study site

This study was conducted in November 2014 and January 2015. The fungus was isolated at the Institute of Agricultural Research for Development (IRAD) La Dibamba Phythopathology Laboratory located in IRAD Ekona in Cameroon. The effect of the extracts on fungal growth was determined in the Life Sciences and Chemistry Laboratories of the University of Buea in Cameroon. The Laboratories are located in Fako Division of the South West Region of Cameroon. The Division is located at the foot of the Mount Cameroon. The sites are found in the mono-modal

humid forest agro-ecological zone.

Isolation and identification of the pathogen

The fungus was isolated from infected oil palm trees in Ekona and identified with the aid of an optical microscope using the procedures outlined by Ntsomboh-Ntsefong *et al.* (2015).

Collection of plant materials

Fresh and disease-free leaf samples of four plants were used in this study. The plants consisted of one weed (*Eleusine indica*) and three forest species (*Diospyros crassiflora*, *Fagara heitzii* and *Milicia excelsa*). The weed was collected around the University of Buea Teaching and Research Farm while the other three plants were obtained from nearby forests. The plants were identified at the Botanical Garden in Limbe, Cameroon. All plant samples were taken to the Laboratories in sterile polythene bags. The plants were selected for this study because they have medicinal properties. For example, Obame *et al.* (2013) reported that *Fagara heitzii* Aubr. & Pellegr. is rich in phenolic compounds and displays good antimicrobial activity against several test microorganisms. Dzoyem *et al.* (2007) indicated that extracts from the stem bark of *Diospyros crassiflora* could be used traditionally in the treatment of fungal infections. *Eleusine indica* has been reported to possess anti-oxidant activity (Iqbal and Gnanary, 2012). Details about the plants are presented on Table 1.

Treatments and experimental design

The treatments were aqueous leaf extracts of the four plants, each at 50 and 100% concentrations; methanol leaf extracts of the four plants at 100%; a negative control (0%) set up using blank agar plates containing Potato Dextrose Agar with no extract and a positive control that consisted of the fungicide Mancozan (active ingredient mancozeb in the subclass dithiocarbamate pesticides). The experimental design was a randomized complete block with three replications.

Preparation of plant extracts

The leaves (250 g) of each plant species were weighed using an electronic balance. The leaves were thoroughly washed with tap water to remove debris and dust particles. They were surface sterilised using 1% sodium hypochlorite for about 20 minutes and then rinsed twice with distilled water. The sterilised leaves were chopped using a sterile scissors and blended with 250 ml of sterile distilled water to obtain an extract of 100% concentration (1:1 w/v). The solutions were filtered using filter paper into sterile beakers. The filtrate with a concentration of 100% was used for the test. After filtering, half of it was further diluted to a concentration of 50%. The methanol extract (100%) of each plant was also tested.

Anti-fungal activity of plant extracts

The anti-fungal screening of the different extracts was carried out using the poisoned food technique. Mixtures of PDA and the plant extracts were poured in Petri plates and allowed to set. The test fungus (7 mm) from a five days old culture was inoculated at the centre of the plates and placed in the reverse direction. The plates were incubated at room temperature immediately after inoculating the fungus and examined daily at 8:00 a.m. for development of fungal growth. Radial growth was measured at 3, 7 and 10 days after exposure (DAE). Colony diameter was obtained as described by Okigbo *et al.* (2009). The percentage of inhibition was determined according to the method proposed by Suleiman and Emua (2009). The formula used was: $((a-b)/a) \times 100$, where

a = radial growth of the pathogen in the control medium;

b = radial growth of the pathogen in the test medium.

The fungal biomass was determined by weighing the mycelium using an electronic balance.

Data analyses

All data collected were subjected to analysis of variance using the SPSS version 21 software, and the treatment means were compared using the Tukey HSD test at 5% level of probability. There were no significant differences in the year by treatment

interactions therefore the data for both years were combined.

Results and discussion

Effect of aqueous and methanol leaf extracts of four plants on the growth of Fusarium oxysporum

The results of this study revealed that the extracts exhibited different levels of antifungal activity against *Fusarium oxysporum* f. sp. elaeidis (Figs 1 to 3).

With the exception of *F. heitzii* at a concentration of 50%, the aqueous leaf extract of all the plants significantly ($p<0.05$) inhibited the growth of the fungus compared to the methanol leaf extract.

Table 1. Plants used in the study.

Family name	Scientific name	Common name	Description
Poaceae	*Eleusine indica* (L.) Gaertn.	Goosegrass	Grass Weed
Ebenaceae	*Diospyros crassiflora* Hiern	African ebony	Tree
Rutaceae	*Fagara heitzii* Aubr. & Pellegr.	Olon	Tree
Moraceae	*Milicia excelsa* Welw.	African teak	Tree

In general, the aqueous extract of all the plants had a higher ($p<0.05$) inhibitory effect at a concentration of 100% than 50%. The inhibitory effects of the aqueous extract of the four plants increased significantly ($p<0.05$) from 3 to 10 DAE but none of them completely inhibited fungal germination. The anti-fungal activity of *M. excelsa* increased from 31 to 73% at a concentration of 50% and from 34 to 80% at 100%. The inhibitory effect of *E. indica* increased from 14 to 64% at a concentration of 50%, and from 27 to 70% at 100%. The range for *D. crassiflora* was 33–63% at 50% and 44–65% at 100%. The aqueous extract of *F. heitzii* showed minimal or no anti-fungal activity at 50% (1–2% effect) and 100% (15–33% effect). The anti-fungal activity of the methanol extract of all plants increased only from 3 to 7 DAE. The fungicide completely inhibited the growth of the fungus throughout the sampling period.

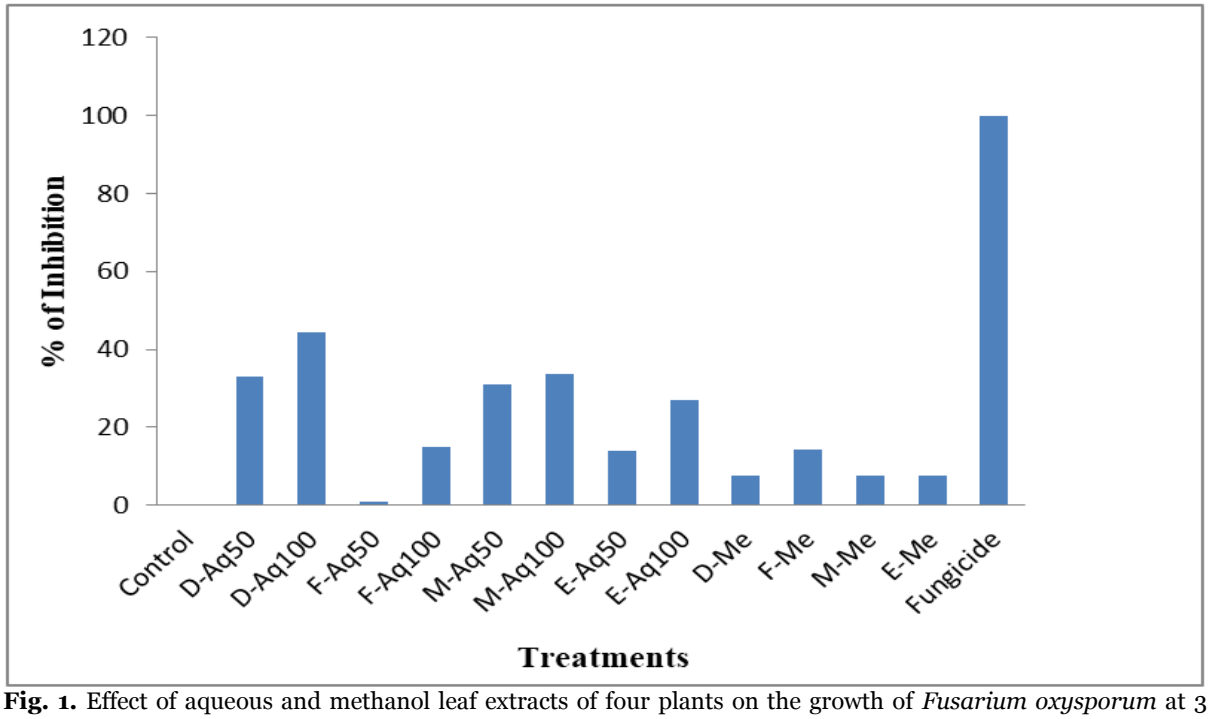

Fig. 1. Effect of aqueous and methanol leaf extracts of four plants on the growth of *Fusarium oxysporum* at 3 days after exposure (D=*D. crassiflora*; F=*F. heitzii*; M=*M. excelsa*; E=*E. indica*; Aq=Aqueous extract; Me=Methanol extract).

At 3 DAE, the plants exhibited different degrees of anti-fungal activity (Fig. 1). All the plants had less than 50% inhibitory effect on the fungus. With the exception of *F. heitzii* at a concentration of 50%, the aqueous extract of all the plants significantly ($p<0.05$) inhibited growth of the fungus compared to the negative control. Among the plants, *D. crassiflora* at a concentration of 100% had the highest inhibitory effect (44.4%).

At 7 DAE, there were significant ($p<0.05$) differences in anti-fungal activity among the treatments (Fig. 2). There was a significant ($p<0.05$) reduction in the growth of the fungus for treatments with the aqueous extract of all the plants when compared with the negative control. With the exception of *F. heitzii*, the inhibitory effect of all the plants on the pathogen was more than 50% but none was as effective as the fungicide. Among them, *M. excelsa* and *E. indica* extracted at both concentrations, and *D. crassiflora* at 100%, were most effective against the pathogen (60–70%). *Diospyros crassiflora* at a concentration of 50% was moderately effective (55%) against the fungus while 50% *F. heitzii* was the least effective with only very slight activity (2%).

The anti-fungal activity of 50% *F. heitzii* was similar to that obtained for the negative control. The inhibitory effect of the botanicals was in the following order: 50% *F. heitzii* < 100% *F. heitzii* < 50% *D. crassiflora* = 100% *D. crassiflora* = 50% *E. indica* < 100% *E. indica* < 50% *M. excelsa* < 100% *M. excelsa*.

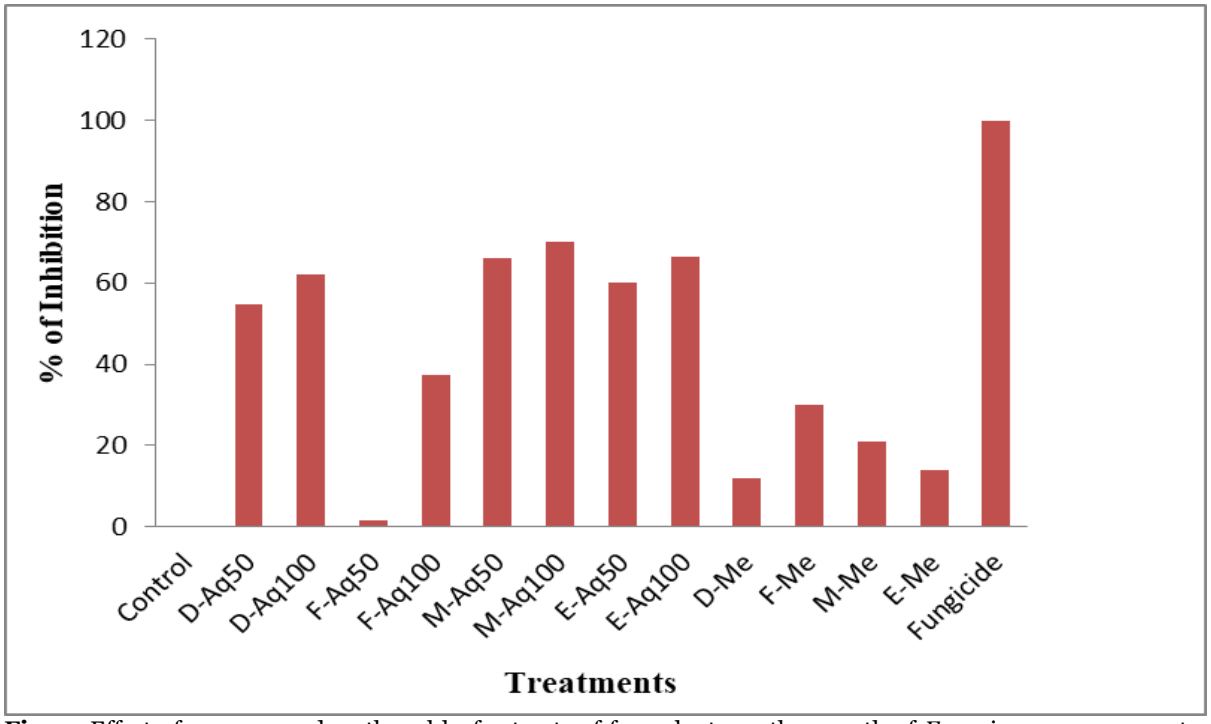

Fig. 2. Effect of aqueous and methanol leaf extracts of four plants on the growth of *Fusarium oxysporum* at 7 days after exposure (D=*D. crassiflora*; F=*F. heitzii*; M=*M. excelsa*; E=*E. indica*; Aq=Aqueous extract; Me=Methanol extract).

At 10 DAE, the aqueous extract of the different plants also exhibited different antifungal activity against the pathogen (Fig. 3). Growth of the fungus was highly inhibited (60–80%) at both concentrations of *D. crassiflora*, *M. excelsa* and *E. indica*. *Melicia excelsa* at 100% resulted in a higher inhibition (80%) of the fungal growth than all other concentrations of the different plants. *Fagara heitzii* had very low activity against the fungus especially when extracted at 50%. At a concentration of 100%, *F. heitzii* resulted in 37% inhibition of the fungus. Both concentrations of *D. crassiflora* gave similar inhibition of the fungal growth (63–65%) and this was comparable to 50% of *E. indica*.

This study indicated that the aqueous leaf extract of most of the plants tested inhibited the growth of the fungus significantly compared to the methanol leaf extract. This shows that the efficacy of the plants against the fungus differed with the solvent of extraction. The inhibitory effect of the aqueous extract of all the plants at a concentration of 100% was higher than at 50%. These findings agree with those of earlier scientists who indicated that at high concentrations, plant extracts caused maximum retardation in fungal growth (Mughal *et al*., 1996; Javed and Bashir, 2012).

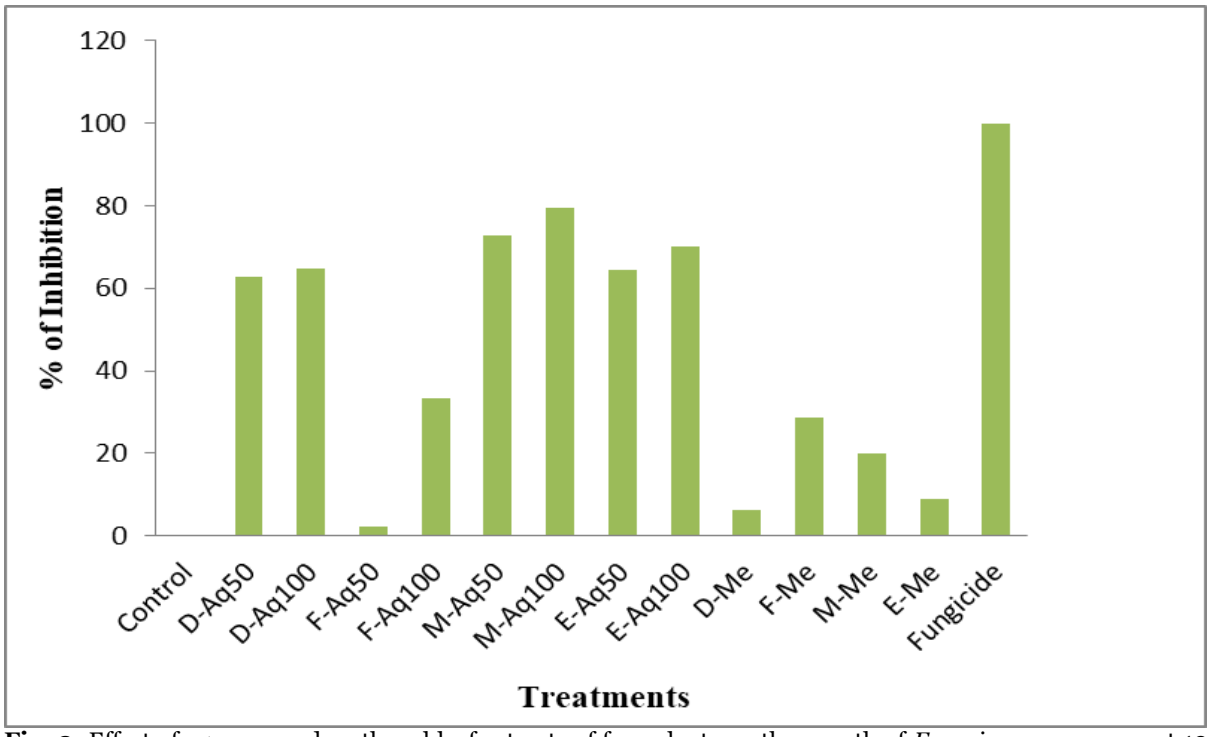

Fig. 3. Effect of aqueous and methanol leaf extracts of four plants on the growth of *Fusarium oxysporum* at 10 days after exposure (D=*D. crassiflora*; F=*F. heitzii*; M=*M. excelsa*; E=*E. indica*; Aq=Aqueous extract; Me=Methanol extract).

Effect of aqueous and methanol leaf extracts of four plants on the biomass of Fusarium oxysporum

The biomass of the fungus varied significantly among the treatments (Fig. 4). In general, the methanol extract of the different plants resulted in higher biomass than the aqueous extract at both concentrations. The fungicide and aqueous extract of all the plants reduced the fungal biomass significantly compared to the negative control treatment. The aqueous extract of all the plants at a concentration of 100% also gave a higher reduction of the fungal biomass than 50%. The lowest biomass was recorded for the fungicide treatment and both concentrations of the aqueous extract of *E. indica* and *M. excelsa*. Among the treatments with the aqueous extract, the biomass of the fungus was highest in treatments with 50% *F. heitzii*. This observation is not surprising because *F. heitzii* at a concentration of 50% had the least activity against the fungus, when compared to all other treatments. Overall, the negative control treatment had the highest fungal biomass which was twice that of 50% *F. heitzii*.

The fungicide Mancozan completely (100%) inhibited growth of the fungus up to 10 DAE and this was better than both concentrations of the aqueous extract of the different plants. However, serious concerns have been raised about synthetic fungicides and their use is being discouraged. For example, some are carcinogenic in nature; they may be persistent and leave residues in the environment. Although both concentrations of aqueous extracts of all the plants tested were not able to cause 100% inhibition of the fungus, *M. excelsa*, *E. indica* and *D. crassiflora* had

very significant effect. The fungitoxic effect of the aqueous extract of these three plants indicates their potential as sources of natural fungicides for the management of *Fusarium oxysporum* f. sp. *elaiedis*. These three plants could be further exploited for use, as a component of an integrated management strategy for the control of this fungus. It is recommended that the aqueous extract of these plants should be tested on-farm. In addition, phytochemical screening should be carried out to determine the secondary metabolites in them.

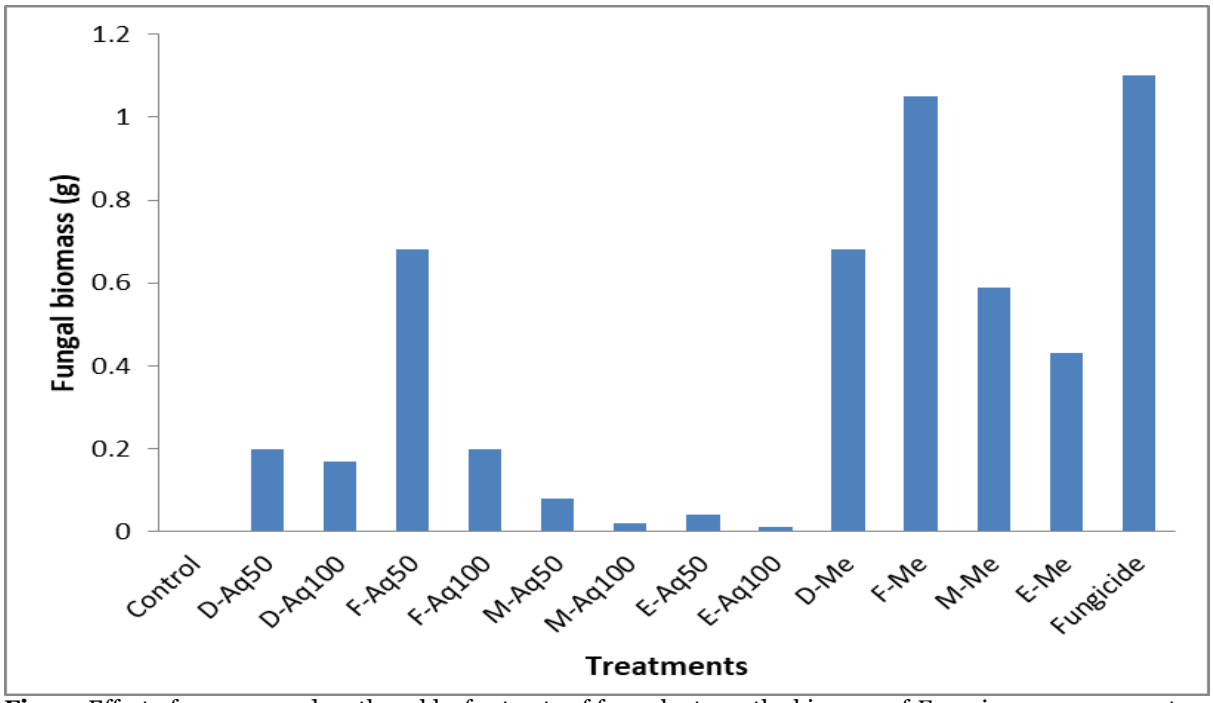

Fig. 4. Effect of aqueous and methanol leaf extracts of four plants on the biomass of *Fusarium oxysporum* at 10 days after exposure (D=*D. crassiflora*; F=*F. heitzii*; M=*M. excelsa*; E=*E. indica*; Aq=Aqueous extract; Me=Methanol extract).

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