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AQUEOUS AND ETHANOLIC EXTRACTS OF *Vernonia amygdalina* L. IN THE CONTROL OF FUNGI ASSOCIATED WITH *Arachis hypogeae* L.

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ABSTRACT

A study was carried out on the use of Vernonia amygdalina del. extract to control fungi associated with groundnut (Arachis hypogeae L) seeds. Aspergillus niger van Tiegh, A. flavus link ex fries, Cercospora arachidicola Hori, Phoma exigua desm., Macrophomina phaseolina (Tassi) Goid, Fusarium oxysporium schl., Cercosporella sp and Phyllosticta sp were isolated from groundnut seeds obtained from Choba market in Rivers state. The water and ethanolic extracts of Vernonia amygdalina significantly (P=0.05) inhibited the spore germination and vegetative growth of Phoma exigua and Macrophomina phaseolina. Water extract was most active for Phoma exigua with an ED_{50} of less than 12.0%, followed by Macrophomina phaseolina 12.8%. The ethanolic extract showed fungitoxicity with ED_{50} of 13.2% (M. phaseolina) and ED_{50} of 15.9% (P. exigua). The results showed that Vernonia amygdalina leaf extract would be effective in controlling the leaf spot and charcoal rot of groundnut caused by Phoma exigua and Macrophomina phaseolina respectively.

Key words: Bitter Leaf Extract, Fungal Disease, Groundnut.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a member of the family *Fabaceae*. Groundnut has been used traditionally since the origin of humanity. It is one of the most important sources of edible vegetable oil and in Nigeria the oil next only to palm oil as edible oil (Oyenuga, 1968).

Nigeria is among the major exporting countries of groundnut in shell and unshelled forms (FAOSTATE, 2000). This has been contributing to a large percentage of the country's foreign exchange. Large quantities of groundnuts are consumed locally in the area of production. The nuts can be eaten raw, whole roasted and salted, boiled or chopped in confectioneries or ground into peanut butter.

Groundnut seeds are susceptible to major pathogenic attack in the field and storage. Diseases of groundnut include pre and post emergence damping off of seedlings caused by *Pythium myriotylum* (Porter, 1970). Some of the most destructive pathogens of groundnut are spread or transmitted by seed. Broadbent *et al*., 1969 reported the following fungi *Aspergillus niger*, *Aspergillus rubber*, *Aspergillus penicillioides*, *Aspergillus repens*, *Aspergillus tereus*, *Aspergillus flavus*, *Aspergillus nidula*, *Macrophomina phaseolina*, *Fusarium acuminatum*,

Fusarium moniliforme, *Penicillium citrinum*, *Phomopsis sp* and *Corticum solani* to be associated with stored groundnut seeds in Nigeria. The charcoal rot of groundnuts caused by *Macrophomina phaseolina* is distributed round the world and cause disease in a wide range of crops (Porter *et al*., 1984). *Macrophomina phaseolina* is seed borne in several crops.

A. flavus, *A. fumigates*, *A. niger*, *A. tereus* and *R. stolonifer* were reported by Abdel Hafez and Hissy, (1981) to be among the ten common seed- borne fungi of groundnut. They reported that these fungi induce complete mortality after four weeks and 25% moisture content after eight weeks and 15 and 20% moisture content. According to them, the increase in the moisture content of the seed accelerated the rate of fungus invasion and mortality of the embryo. On the other hand, investigated changes in the composition of the seed-borne fungal floral at various levels of relative humidity. They found *Aspergillus* species as the main component of fungal floral in stored seeds. At 40-80% relative humidity, they found *A. flavus* days at 100%. They reported a decline in germ inability of seeds with the rise of the relative humidity. Germ inability was completely lost after 30 days at 100% relative humidity and after 180 days at 80% relative humidity.

The studies on the use of plant extracts have opened a new avenue for the control of plant diseases (Madunagu and Ebena, 1994; Ihejiaka, 2001, Pattnaik *et al*; 2012). A natural plant product with fungicidal properties could be less expensive and environmentally friendly than synthetic fungicides.

This study was therefore undertaken to identify the fungi associated with groundnut seeds and to determine the use of plant extracts (*Vernonia amygdalina*) for the control of leaf spot and charcoal rot diseases of Groundnut.

MATERIALS AND METHODS

Groundnut seeds (*Arachis hypogaea*) were obtained from Choba market in Rivers State, Nigeria. The fungi associated with groundnut seed were isolated using the standard blotter method as recommended by International Seed Testing Association (ISTA, 1996).

The groundnut seeds were surface sterilized with 70% ethanol for 5minutes and rinsed with three changes of sterile distilled water to remove surface contaminants. The surface sterilized groundnut seeds were plated on three layers of moistened Whatman filter paper(No 1, diameter 9cm) in sterilized Petri dishes and incubated at ($28^{O}C \pm 2^{O}C$) for 7days.

After incubation period, the identification of the isolated fungi was carried out based on their habit characteristics, such as type, colour, septation of spores etc, using the description of Barnett and Hunter (1992).

Pure cultures of the isolated fungi was prepared using a sterile inoculating needle to transfer the fungal spores from culture plates and inoculated on Potato Dextrose Agar (PDA). The name of each fungus after incubation period, the identification of the isolated fungi was carried out based on their habit characteristics, such as type, colour, septation of spores using the description of Barnett and Hunter (1992).

Pure cultures of the isolated fungi was prepared using a sterile inoculating needle to transfer the fungal spores from culture plates and inoculated on Potato Dextrose Agar (PDA). The name of each fungus was labeled on the plates. The plates were incubated at room temperature ($28^{0}C \pm 2^{0}C$) for 7 days

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Preparation of Extract

Aqueous and ethanol extract of *Vernonia amygdalina* were prepared using the methods of Sexena and Mathela (1996). In the aqueous extract method, fresh leaves of *Vernonia amygdalina* were collected, ground into paste using a blender. Different concentrations of the plant extract were prepared by weighing 20g, 30g, 40g, and 50g of the paste and added to 100mls of distilled water in a beaker. The mixture was covered with aluminium foil and allowed to stand for 24 hours. The content of the flask was filtered using Whatman filter paper (No. 1, 9.0cm). The extract in the flask was sterilized in an autoclave at 121^0C for 15 minutes. The filtrate was allowed to attain room temperature, before use.

In the ethanol extract method, 20g, 30g, 40g, and 50g of the *Vernonia amygdalina* paste were added to 100mls of 70% ethanol in separate flasks and allowed to stand at room temperature for 24 hours.

After 24 hours, the extract was filtered using Whatman no. 1 filter paper. The filtrate was sterilized in an autoclave at 121^0C for 15minutes. The filtrate was allowed to attain room temperature (28^OC± 2^0C) before use (Owolade and Osikanlu, 1999).

Antifungal Activity of the Extracts

The effect of the plant extract on the growth of *Phoma exigua* and *Macrophomina phaseolina* was determined by measuring the mycelial dry weight. Hundred millilitres of potato dextrose broth (PDB) was poured into each flask containing 5ml of different concentrations (0, 0.2, 0.3, 0.4 and 0.5g) of the respective extracts. With a sterile cork borer (3mm) mycelia disc of 7-day old cultures of *Phoma exigua* and *Macrophomina phaseolina* were inoculated in the flask and incubated at 28 ± 2^0C for 7 days.

RESULTS

The fungi isolated and identified to be associated with the groundnut seeds include: *Aspergillus niger* Van Tiegh, *Aspergillus flavus* Link ex Fries, *Cercospora arachidicola, Macrophomina phaseolina* Tassi Goid, *Phoma exigua* Desm., *Fusarium oxysporium* Schl., *Cercosporella sp,* and *Phyllostica sp* .

The results of the effect of *Vernonia amygdalina* water and ethanolic extracts on the mycelia growth of *Phoma exigua* and *Macrophomina phaseolina* are presented in Fig 1. The water extract and ethanolic extracts at (P=0.05) reduced the mycelia growth of *Phoma exigua* and *Macrophomina phaseolina* at all concentrations when compared with the control.

The water and ethanolic extracts of *Vernonia amygdalina* reduced the germination of *Phoma exigua* and *Macrophomina phaseolina* spores (Figs2 and 3). The water and etanolic extracts of *V.amygdalina* significantly inhibited (P=0.05) *Phoma exigua* by 80% and 93% and *Macrophomina phaseolina* by 66% and 75% respectively. Water extract was most active for *Phoma exigua* with an ED_{50} of less than 12.0% followed by *Macrophomina phaseolina* with an ED_{50} of less than 12.8%. The ethanolic extract showed fungitoxicity with ED_{50} of 13.2% for *Macrophomina phaseolina* and ED_{50} of 15.9% for *Phoma exigua*.

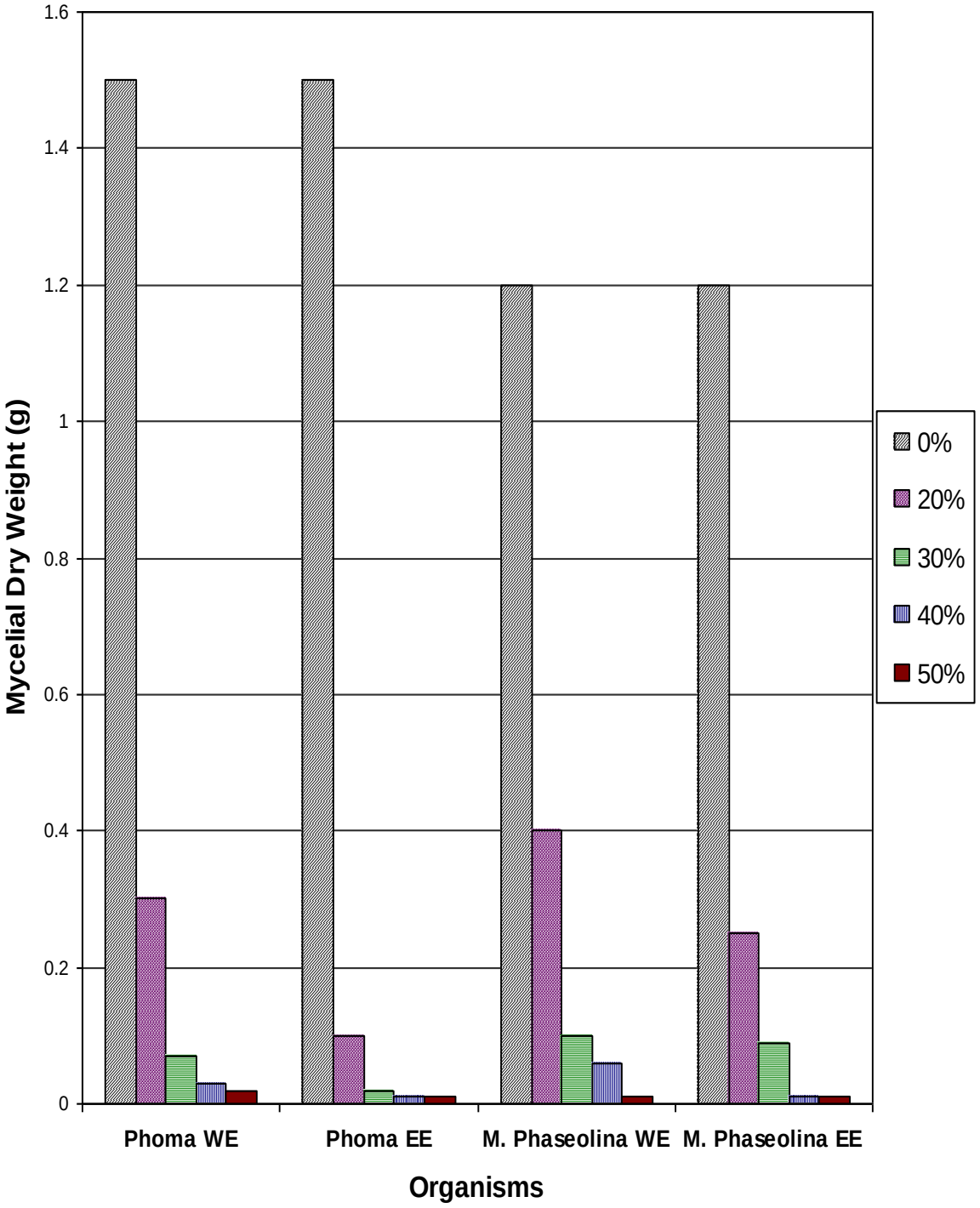

Fig. 1: Effects of *Vernonia amygdalina* water and ethanolic extracts on the mycelial growth of *Phoma exigua* and *Macrophomina phaseolina.*

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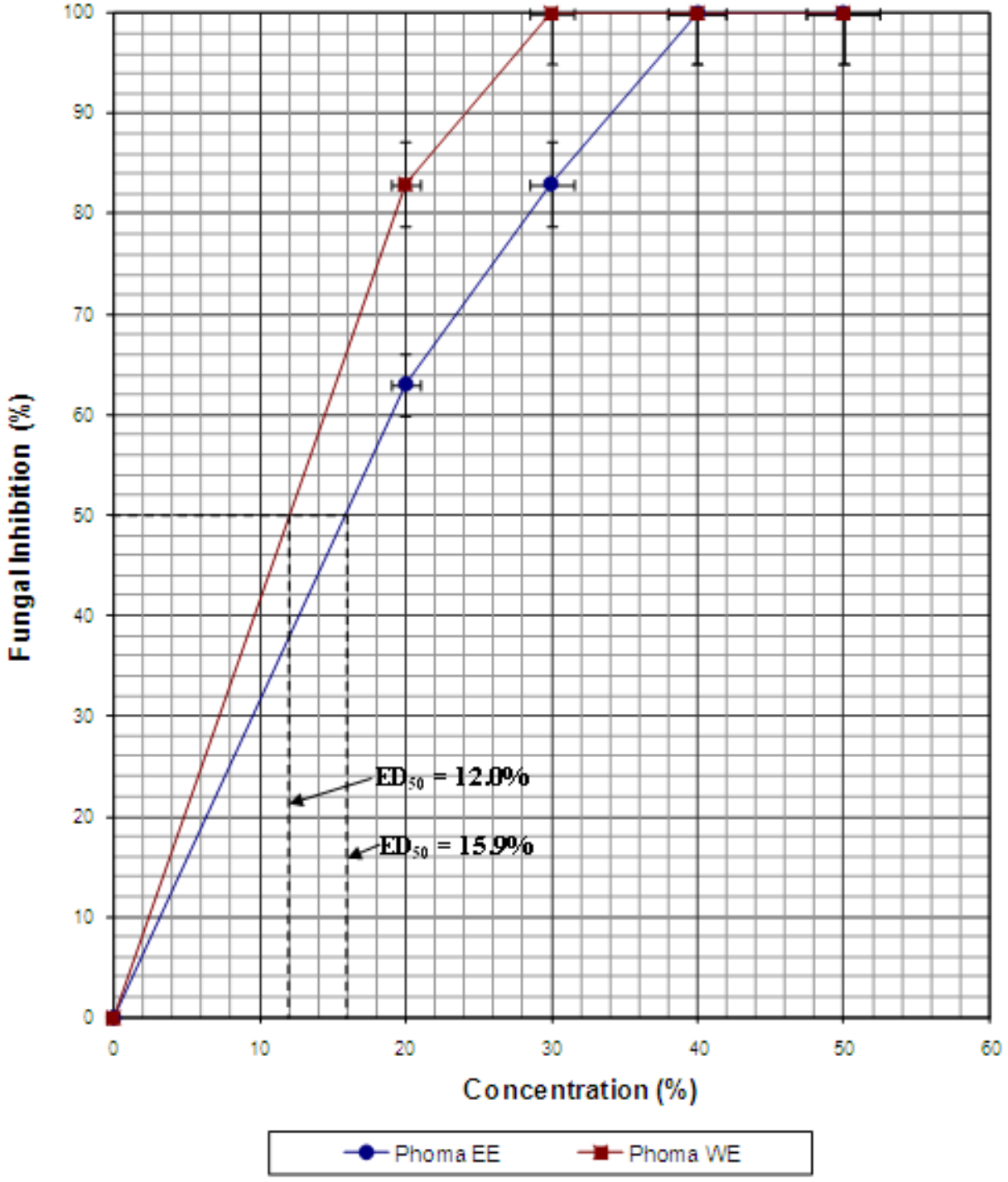

Fig. 2: Effects of *Vernonia amygdalina* water and ethanolic extracts on spore germination of *Phoma exigua*

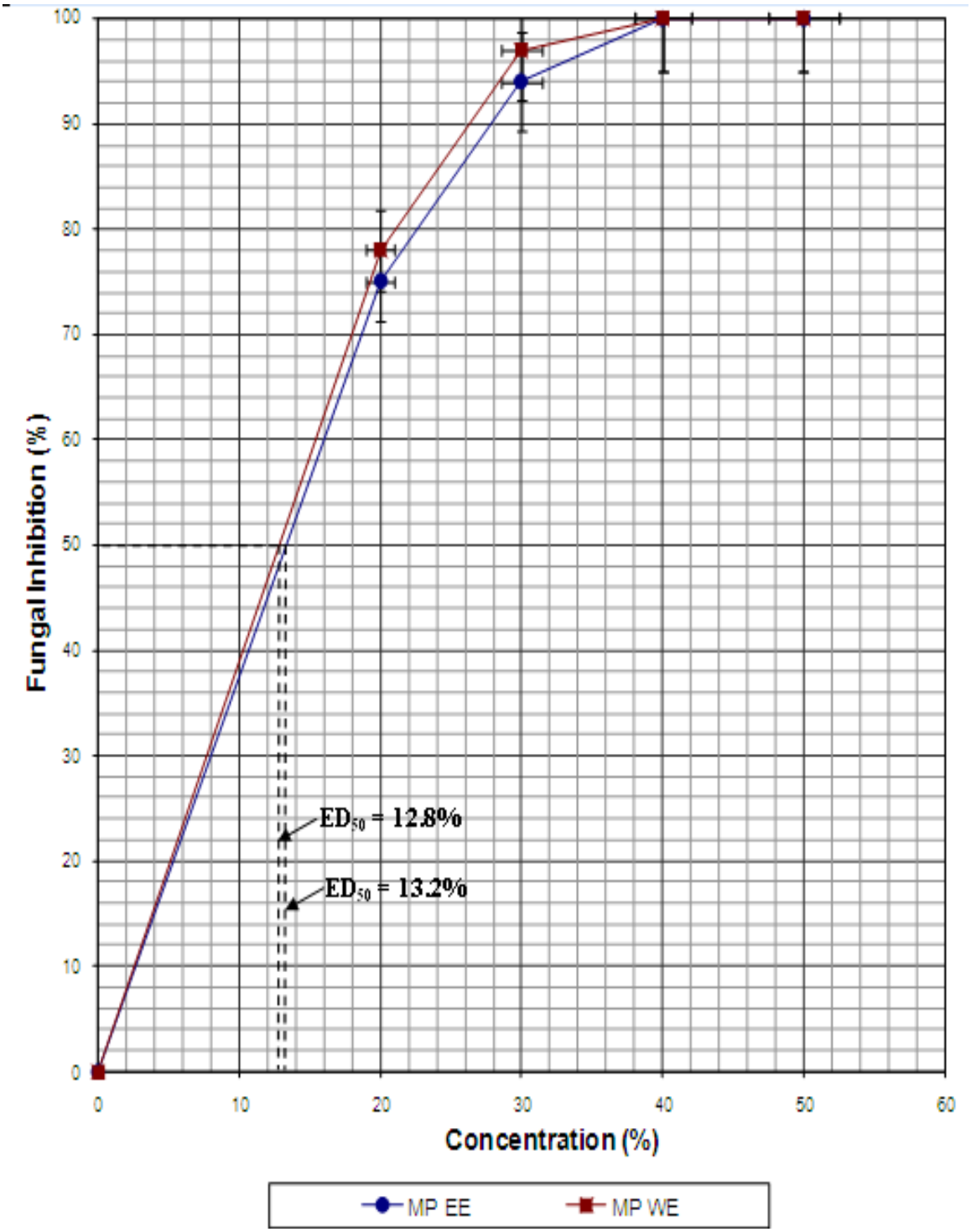

Fig. 3: Effects of *Vernonia amygdalina* water and ethanolic extracts on spore germination of *Macrophomina phaseolina*

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DISCUSSION

Groundnuts are susceptible to fungal attack in the field and storage. In this study, the following fungi: : *Aspergillus niger* Van Tiegh, *Aspergillus flavus* Link ex Fries, *Cercospora arachidicola, Macrophomina phaseolina* Tassi Goid, *Phoma exigua* Desm., *Fusarium oxysporium* Schl., *Cercosporella sp*, and *Phyllostica sp* were found to be associated with groundnut seeds collected from retailers in Choba Market, Rivers State. These fungi are known to cause diseases in ground nut such as discolouration of pods, charcoal rot and leafspot (Broadbent et al 1969).

The *in vitro* studies on the effect of *Vernonia amygdalina* extract on mycelial growth of *Phoma exigua* and *Macrophomina phaseolina* showed reduction in mycelial weight as the plant extracts inhibited spore germination and fungal growth. The reduction of spore germination and mycelial growth could be due to the production of aromatic volatile oils which contains thymol and eugenol. The presence of tannins, alkaloids, flavonoids and saponins suggest antimicrobial activity, also the bitter glycoside vernonin from the leaves and root of *V. amygdalina* which is known to be responsible for antimicrobial activities of the plant. Similar significant antifungal effects on mycelial growth with the use of some plant extract has been reported by Onifade (2000), Udo *et al* (2001), Owolade and osikanu (1999), Obagwu *et al* (1997), Olufolaji (2002) and Adekunle (2001).

In this study, there was significant inhibition of *Phoma exigua* and *Macrophomina phaseolina* mycelia growth with increase in the concentration of the *Vernonia amygdalina* water and ethanolic extracts. Owolade and Osikanu (1999) made a similar report on the effectiveness of plant extracts increasing with increased concentration. Similarly, Udo *et al* (2001) reported the inhibition of growth of fungi at different concentrations (20%, 30%, 40%, 50%) of plant extracts, but there was growth at 0% (control). The report concluded that phytochemical elements were present in the extracts of plants and their potency depended on extract solvent.

In this study both water and ethanol extracts inhibited the mycelial growth of the fungi in varying degrees. Onifade (2000) had observed the superiority of water extract to organic solvents which he explained may react with the active ingredients of the plant forming inactive compounds

There were obvious differences and deviations on the effect of the plant extracts on different fungi as shown by the significant differences (P = 0.05) in the mycelial growth of *Phoma exigua* and *Macrophomina phaseolina* using 20%, 30%, 40%, 50% concentration of the extracts of *Vernonia amygdalina*. These differences in antifungal activities according to Marsh *et al* (1997) are mostly due to inherent differences in the normal physiology of the fungi.

The following fungi: *A. niger, A. flavus, M. phaseolina, P. exigua* etc were isolated and identified to be associated with post- harvest groundnut. *Vernonia amygdalina* leaf water and ethanolic extracts significantly inhibited mycelial growth and spore germination of *Phoma exigua* and *Macrophomina phaseolina*. This findings show that *Vernonia amygdalina* extract can be used in plant disease control.

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