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Antimicrobial screening of twenty plant extracts on three pathogens of groundnut (*Arachis hypogaea* L.)

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Abstract: Twenty-one plants used in this study were collected from the local market in Benin City, Nigeria. Seventeen of them were dried before used, while the others were used fresh. Fusarium spp and *Macrophomina phaseolina* were isolated from groundnut seeds and maintained on PDA. Four replicates per extracts amended to PDA were inoculated at the center with the pathogen. Colony diameter was measured on the seventh day after inoculation. Of the twenty plant extracts used in this study, *Syzygium aromaticum* extract consistently resulted in total mycelial inhibition of the three pathogens and was significantly different from the other extracts except *Ocimum basilicum* on *F. solanii*. Percent inhibition (PI) of 90.91%, 80.73%, 61.82%, 60.36%, 58.91% and 59.64% were observed for *Xylopia aethiopica, Ocimum basilicum*, *Aframomum melegueta, Piper guinensis, Garcinia kola* and *Zingiber officinale* respectively on *F. oxysporum* and were effective. Percent inhibition of 100% (*O. balsilicum*) and 61.93% (*Garcinia kola*) exhibited on *F. solanii* were rated effective. *Zingiber officinale* (PI of 41.41%) extract exhibited moderate effectiveness on *M. phaseolina*.
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Keywords: Plant extracts, *Macrophomina phaseolina*, *Fusarium* species, Control management

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop of the tropical and subtropical world. It is used as a natural starting material for the production of soap and machine oil, treatments of various diseases such as loose cough, arthritis, constipation, recuperation after illness, (Mythily and Revathi, 2017). According to Ho (2000), the roots are used as poultry feed and fertilizer. It is the fifth world's most economically important oilseed crop with African continent accounting for about 31.6% of the world's groundnut production (Daudi et al., 2018). Despite the socio-economic and cultural importance of the crop, it is affected by plethora of pathogens which affects its productivity and quality. Leaf spot is of major constraint in its production in Tropical Africa. Groundnut rust and late leaf spot cause up to 70% yield losses in susceptible cultivars, which most smallholder farmers in developing countries often rely on (Khedikar et al., 2010).

Diseases arising in the field are usually controlled with synthetic fungicides. These fungicides apart from been expensive, often pollute the environment and threaten human health. Peasant farmers of the resource poor regions of the world can scarcely afford to buy chemicals for the control of pests and pathogens as such it is beneficial to them if studies are carried out which have the potential to identify possible plants species in their environment that may enhance their ability to control these pathogens at minimal cost.

In Africa, traditional medicine is well recognized and a great variety of plants are used by traditional healers in treatment of many ailments (Kamanzy et al., 2002). Plant extracts have also found their way into the treatment of plant diseases. Some researchers have had success controlling diseases of various plants with plant extracts (Ogwulumba et al., 2008; Sobolev et al., 2011; Martin et al., 2012; Soumya and Bindu, 2012; Bakeer et al., 2015; Ogbebor et al., 2015a, 2015b; Kim et al., 2016; Al-Azawi1 and Hassan, 2017). Extracts of leaves of papaya tree (*Carica papaya* L.) and vernonia (*Vernonia amygdalina* Delile) had been successfully tested against pathogenic fungi of groundnut (Ogwulumba et al., 2008). Extract of *Capsicum frutescens* (10 mg ml^{-1}) was also effective in controlling groundnut seed pathogens such as *Aspergillus niger*, *A. flavus*, *Penicillium* sp. and *Rhizopus* sp. (Soumya and Bindu, 2012).

Antimicrobial properties of plant extracts have shown great prospects for becoming an alternative to synthetic fungicides (Malkhan et al., 2012; Soumya and Bindu, 2012; Bakeer et al., 2015; Koita et al., 2017).

As such this study is part of an ongoing research into the identification of plants species that have fungitoxic effect on some of the major pathogen of groundnut.

2. Material and Methods

Collection of Plants/Preparation of Plant

The study area was Rubber Research Institute of Nigeria, main station at Iyanomo ($06^{0}09^{1}$ 26.9^{11} N, $05^{0}35^{1}$ 56.8^{11} E) about nineteen kilometers from Benin City, Edo State, Nigeria. Twenty plants used in this study were collected from the local market in Benin City, Nigeria. All plants except *Allium cepa* L, *Zingiber officinale* Roscoe and *Allium sativa* L. were dried in the oven at 35^{O}C. The samples were ground manually in the labouratory using hand grinding corona machine. The ground plants were packed in Mayonaise jars and stored in the refrigerator at about 4^{0}C till they were required for use. Ten gram of each sample was used per 100ml of Potato Dextrose Agar (PDA). The ground samples were introduced in the PDA solution in 250ml conical flasks. The flasks were plugged with cotton wool and wrapped with foil paper before sterilizing at 121^{O}C for 15min.

The extracts from samples of *A. cepa*, *Z. officinale* and *A. sativa* used fresh were prepared according to Ogbebor et al. (2007) by grinding in distilled water (100g of sample with 100ml of water) after which the grounded materials were squeezed in cheese cloth. The extracts were amended to PDA. The extract amended PDA were prepared by dissolving 3.9g of commercial PDA in some quantity of the filtered extract in a beaker and then made up to 100ml with more of the extract (Ogbebor et al., 2015a). This was dispensed into 250ml conical flasks, the mouth plugged with cotton wool, wrapped with foil paper and sterilized at 121 ^{O}C for 15 min.

Collection of Pathogen

The pathogens used in this study were isolated from groundnut seeds using the blotter method. The pathogen were established as a pure culture on PDA and maintained at 4^{0}C. Sub-culturing was done monthly to maintain fresh culture for during the course of the study. The amended PDA were dispensed into Petri plates, Four Petri dishes per extracts amended to PDA were inoculated at the center with a mycelial disc of 5mm in diameter taken from the periphery of actively growing 5- day-old culture of the pathogen. The control plate was devoid of plant extract. Petri dishes were incubated at 28±2°C. Colony diameter was measured on the seventh day after inoculation. Percent inhibition of growth in each of the treatment was calculated with respect to the control by the equation giving by Vincent (1927); Ogbebor and Adekunle (2005); Ogbebor et al. (2007).

$$\mathbf{I} = \frac{100(C-T)}{C}$$

Where I = Percent inhibition of mycelial growth with respect to control
C = growth in control
T = growth in treatment.

The percentage inhibitions were rated for their inhibitory effects using the scale described by Sagoyomi (2004); 0% inhibition: not effective, > 0-20%: slightly effective, > 20-50%; moderately effective, >50-<100%: effective, and 100% inhibition: highly effective.

The data generated during the course of the study were subjected to various descriptive and inferential statistics, ANOVA and means separation using Duncan multiple range test.

3. Results

The Twenty plant extract used- their common, Local and Botanical names are shown in Table 1.

Fusarium oxysporum Schltdl

The *in vitro* assay of twenty plants extracts against mycelial growth of *F. oxysporum* isolated from groundnut seeds are summarized in Fig. 1. Evaluation of the 20 plant species showed that some of the extracts were significantly effective reducing mycelia diameter of *F. oxysporum* (α>0.05). Significant among these extracts were *Syzygium aromaticum* (L.) Merr. & L.M.Perry (0.00cm), *Xylopia aethiopica* (Dunal) A.Rich (0.62cm), *Ocimum basilicum* L. & O. Canum Sim. (1.33cm), *Aframomum melegueta* K.Schum. (2.62cm), *Piper guinensis* Schum & Thonn. (2.73cm) and *Z. officinale* (2.78cm) when compared to the control (6.88cm). Extract of *S. aromaticum* gave a total inhibition of the mycelia growth of *F. oxysporum,* but the extract effect did not differ significantly with that of *X. aethiopica* (P<0.05) but differed from those of the other extracts (P>0.05). Mycelial growth recorded in extracts of *Chromolaena odorata* L. (7.80cm), *Azadirachta indica* A. Juss (8.48cm), *Spondias monibin* L.

(7.05cm) and *Tetrapleura tetraptera* (Schum & Thonn) Taub (7.10cm) were observed to be higher in value compared to growth in the control.

Table 1. Evaluated Twenty plant extracts - Their Common, Local and Scientific names

S/N	Common name	Local name	Scientific name
1.	Ginger (red)	Agio (Bini); Ata ile, Orin (Yor.); Jinja (Igbo); Cittar aho (Hausa)	*Zingiber officinale* Roscoe
2.	Pepper fruit	Ako (Bini); Igberi (Yor.); Nmimi (Igbo)	*Dennettia tripelata* G. Baker
3.	Alligator pepper, Grains of paradise	Ata-ire, Oburo (Yor.), Oziza (Igbo); Ehin-edo (Bini); Chitta, Gyan'damar Yaji (Hausa)	*Aframomum melegueta* K.Schum.
4.	Garlic	Ayo, Ayuu (Yor. & Igbo); Tafarnuwa (Hausa)	*Allium sativum* L
5.	Siam weed	Awolowo weed; Akintola-ta-ku (Yor.)	*Chromolaena odorata* L.
6.	Neem	Dogo Yaro (hausa); Eke-oyibo (Yor.)	*Azadirachta indica* A. Juss
7.	Bitter kola	Orogbo (Yor.); Adi (Igbo); Edun (Bini); Namiju; guoro (Hausa)	*Garcinia kola* Heckel
8.	Bitter leaf	Ewuro (Yor.); Oriwo (Bini); Olubu (Igbo); Shuwaka (Hausa)	*Vernonia amygdalina* Del.
9.	Chrysoba	Ikatee, Awonrinwan (Yor.)	*Chrysobalanus orbicularis Schum*
10.	Clove	Kanafuru (Yor)	*Syzygium aromaticum* (L.) Merr. & L.M.Perry
11.	Curry-leaf tree	Ebafo (Bini)	*Murraya koenigii* (L) Spreng
12.	Asthma weed, Cat's hair	Egele, Emi-ile (Yor.); Asin-uloko (Bini); Nonan kurchiya (Hausa)	*Euphobia hirta* L.
13.	Hog plum	Ihiegbe, Okhihan (Bini); Iyeye, Akika (Yor); Tsadar masar (Hausa)	*Spondias monibin* L.
14.	African nutmeg	Enenoyoba, Ukposa (Bini); Ariwo (Yor.); Gujiya danmiya (Hausa)	*Monodora myristica* (Gaertn) Dunal.
15.	Onions	Alubosa (Yor.); Alubarrha (Bini); Albasa (Hausa); Yabase (Igbo)	*Allium cepa* L
16.	African crocus	Oriema (Bini)	*Curculigo pilosa* Schum & Thonn.
17.	Climbing black or Benin pepper	Ata-Iyere (Yor.); Masoro (Hausa); Ebe-ahanhi akpoko (Bini); Ozeza (Igbo)	*Piper* guinensis Schum & Thonn.
18.	Scent leaf, Sweet basil	Aramogho (Bini); Efinrin-wewe, Efiri-ajija (Yor)	*Ocimum balsilicum* L. & O. Canum Sim.
19.	Tetrapleura	Aridan (Yor.); Ighimiakia (Bini); Oshosho (Igbo)	*Tetrapleura tetraptera* (Schum & Thonn) Taub
20.	Ethopian pepper	Eeru (Yor.); Unien (Bini); Uda, (Igbo); Kimba (Hausa)	*Xylopia aethiopica* (Dunal) A.Rich

Yor= Yoruba

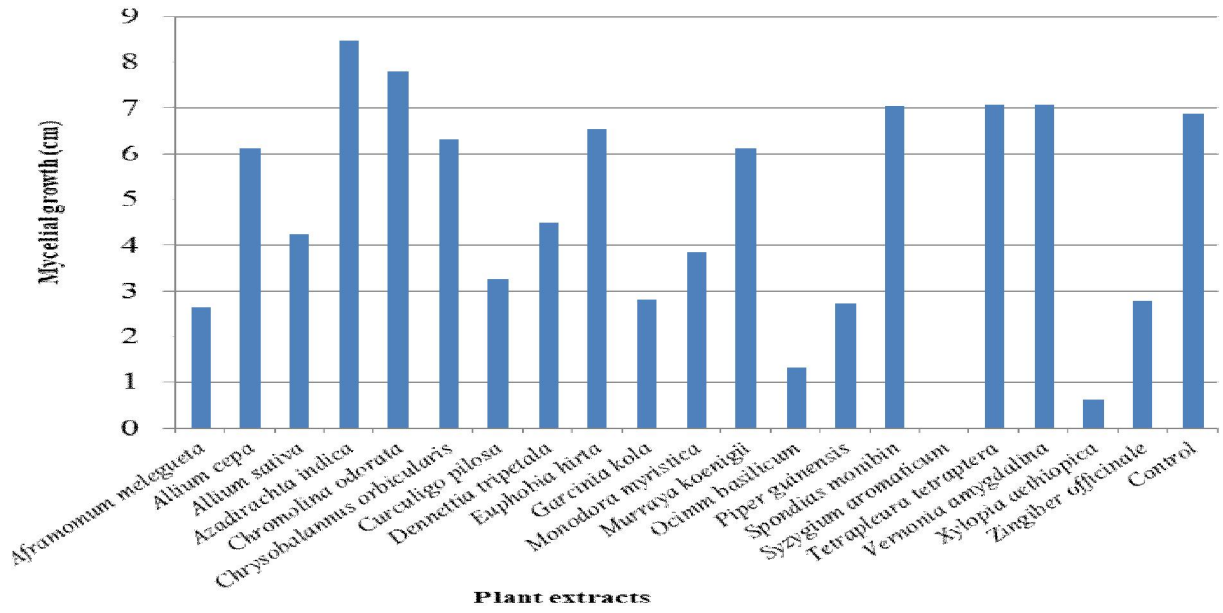

Figure 1. In vitro assay of twenty plants extracts against mycelial growth of *Fusarium oxysporum* isolated from groundnut. Bar is mean of four replicates. Lsd = 0.6177 at α = 0.05

***Fusarium solani* (Mart.) Sacc.**

Ten of the plant extracts tested controlled the mycelia growth of *Fusarium solani, S. aromatic* (0.00cm), *O. basilicum* (0.00cm), *Garcinia kola* Heckel (1.88cm), *X. aethiopica* (2.34cm), *P. guinensis* (2.83cm), *A. melegueta* (3.13cm), *Curculigo pilosa* Schum & Thonn. (3.30cm), *Z. officinale* (3.83cm), *Dennettia tripetala* G. Baker (4.20cm) and *Chrysobalannus orbicularis* (4.35cm) while the other 11 extracts did not control the pathogen (Fig. 2). Extracts of *S. aromatic* and *O. basilicum* gave a total inhibition of the mycelia growth of *Fusarium solanii* and their effects were not significant (P<0.05). The extract of *Ephorbia hirta* L. was least effective (8.10cm) compared to the control (4.93cm).

***Macrophomina phaseolina* (Tassi) Goid**

Significant control were observed by *Z. officinale* (5.28cm) and *A. sativum* (6.28cm) and highly significant *by S. aromatic* (0.00cm) and their effects differed significantly (P>0.05) (Fig. 3). Nine of the plant species tested did not control the mycelia growth of *Macrophomina phaseolina* (*Murraya koenigii*), *S. monibin, V. amygdalina, A. cepa, A. indica, C. odorata, C. orbicularis, E. hirta* and *Monodora myristica* (Gaertn) Dunal.

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The effects of the mycelia growth in the nine extracts did not differ with that in the control.

Percentage inhibition (PI) of the in vitro assay of the twenty plants extracts against mycelial growth of *F. oxysporum, F. solanii* and *M. phaseolina* isolated from groundnut seeds are summarized in Fig. 4. Total percent inhibitions were recorded on *S. aromaticum* extract on the three pathogens, and *O. basilicum* extract also recorded total PI on *F. solanii*; and their effect were rated highly effective (Fig. 4).

The percent inhibition of the effect of the extracts of *G. kola* and *X. aethiopica* on *F. oxysporum* and *F. solanii*; and the percent inhibition of the extracts of *Z. officinale*, *A. melegueta, C. pilosa, P. guinensis* and *O. basilicum* on *F. oxysporum* were rated effective and were not significantly different from each other (P<0.05); (Fig. 4).

The extract of *C. odorata, A. indica, V. amygdalina* and *Spondias monibin* on the mycelia growth of the three pathogens; extract of *Tetrapleura tetraptera* on *F. oxysporum* and *F. solanii*; extracts of *M. koenigii, E. hirta, A. cepa, M. myristica* on *F. solanii* and *M. phaseolina* and extracts of *A. sativa, Z. officinale* on *F. solanii; C. orbicularis* where not effective. The other extracts were either moderately or slightly effect on the pathogens (Fig. 4).

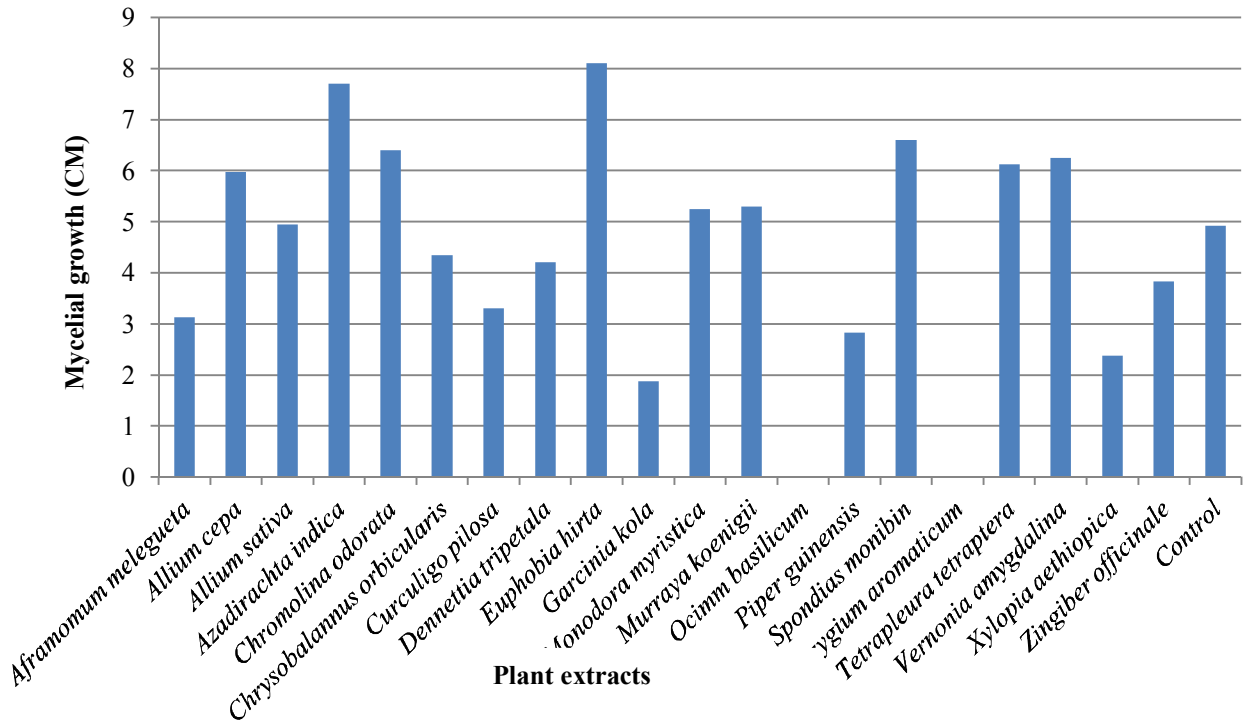

Figure 2. In vitro assay of twenty plants extracts against mycelial growth of *Fusarium solanii* isolated from groundnut. Bar is mean of four replicates. Lsd = 0.1878 at α = 0.05

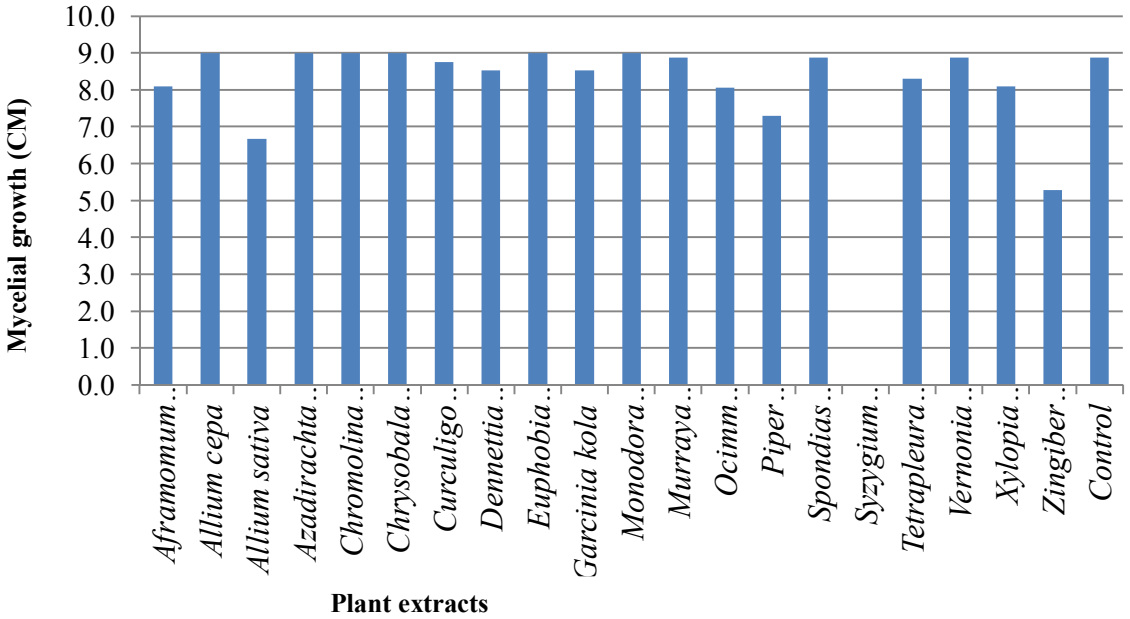

Figure 3. In vitro assay of twenty plants extracts against mycelial growth of *Macrophomina phaseolina* isolated from groundnut. Bar is mean of four replicates. Lsd= 0.1829 at α = 0.05

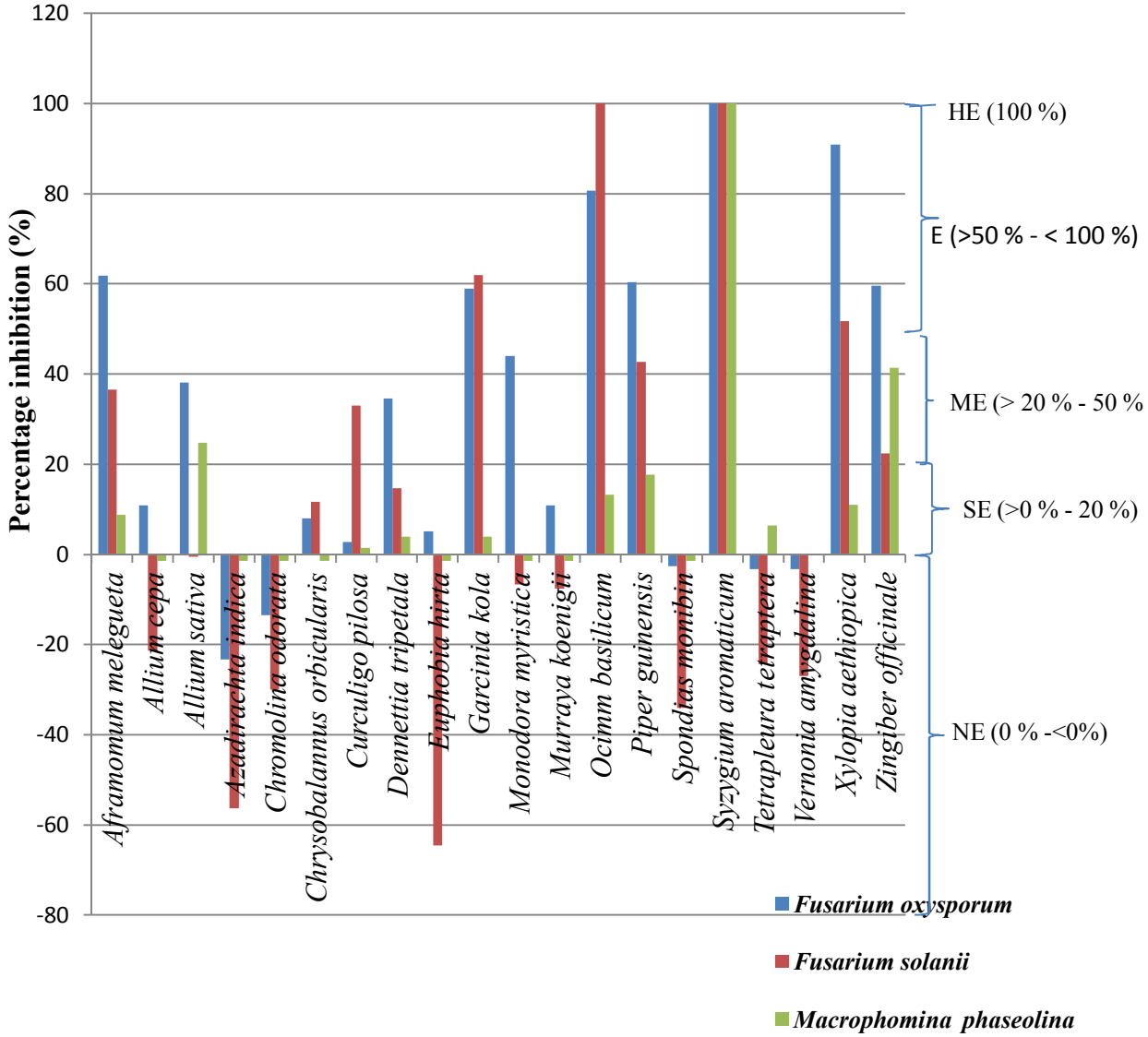

Figure 4. Percentage inhibition (PI) of *In vitro* assay of twenty plants extracts against mycelial growth of *Fusarium oxysporum*, *Fusarium solanii* and *Macrophomina phaseolina* isolated from groundnut. HE = Highly effective, E = Effective, ME = moderately effective, SE = Slightly effective, NE = Not effective. Lsd = 3.981, cv% = 15.7, α =0.05

Discussion and Conclusion

Many researchers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides (Israel *et al*., 2005; Ogwulumba *et al*., 2008; Omorusi *et al*., 2014; Bakeer *et al*., 2015; Ogbebor *et al*., 2015a, 2015c; Kim *et al*., 2016; Koita *et al*., 2017). Extracts of various plants used in this study demonstrated varied fungitoxic potency on the pathogens evaluated. Of the twenty plant extracts used in this study, *S. aromaticum* extract consistently gave total mycelial inhibition of the pathogens and its effectiveness was significantly different from the other extracts (P>0.5) except for extract of *O. basilicum* (P<0.5) on *F. solanii*. The result in this study on the antifungal potentials of *S. aromaticum* and *O. basilicum* are consistent with earlier studies by various authors (Tewari and Dath, 1984; Tewari,

1995; Udo *et al*., 2001; Ogbebor and Adekunle, 2005; Okigbo and Nmeka, 2005; Adedokun and Ataga, 2007; Shovan *et al.,* 2008; Ogbebor *et al*., 2015). Bowers and Locke (2000) showed that *S. aromaticum* extract reduced population density of *Fusarium oxysporum f. sp chrysanthemi* in soil by 97.5% in their study carried out in 1997 experiment. Ogbebor *et al*. (2015a) reported that *O. basilicum* extracts exhibited total inhibition of the mycelial growth of *Corynespora cassiicola* and that the concentration effect at 100% and 50% were not significantly different. Tewari (1995) observed that the leaf extract of *O. sanctum* was effective and inhibited conidia germination and mycelial growth of *Pyricularia grisea in vitro.* The toxic activities of *O. sanctum* oil were noticed even at a concentration of 1 ppm. The germ tube of *P. grisea* was completely deformed and grew shorter compared to their control with bulbular structure but not the appressoria formation at the end. Mycelial growth was completely inhibited at 50 ppm.

The other extracts demonstrated varied fungitoxic potencies. The extracts of *X. aethiopica* (PI of 90.01%), *O. basilicum* (PI of 80.73%), *A. melegueta* (PI of 61.82%) and *Z. officinale* (PI of 59.64%) on *F. oxysporum*; *O. basilicum* (PI of 100%), *G. kola* (PI of 61.93 %) on *F. solanii* were equally effective, while *Z. officinale* (PI of 40.56%) was moderately effective on *M. phaseolina.* Extracts of *X. aethiopica* and *Z. officinale* had earlier been implicated to posses' fungitoxic properties potent in the control of *F. oxysporum*. Okigbo and Nmeka (2005) used extracts of *X. aethiopica* and *Z. officinale* to control yam tuber rot caused by *F. oxysporum*, A. *niger*, and *A. flavus*.

Some of the extracts in this study were not effective on the pathogens evaluated. The result on *E. hirta* in this study is in line with earlier study by Ogbebor *et al.* (2007), and showed that extract of *E. hirta* gave mycelial growth diameter higher than that obtained from their respective control. However this does not necessary mean that they might not be fungitoxically potent to some other pathogens, as Gill (1992) implicated their fungitoxic uses in traditional medicine in the management of some fungal infections. The use of one or more of the less effective extracts in combination with each other or in combination with other method of disease control might give a better management of disease control of these pathogens. Israel *et al*. (2005) showed that treatment combination of mustard pod and oil-cake significantly reduced the infection of *M. phaseolina* and *Fusarium* propagule compared to their individual treatments. Bower and Locke (2000) experiments in the greenhouse tested this strategy and had favorable results, where the addition of a biological control agent in combination with the pepper/mustard extract resulted in increased symptomless plant stand over either the biological agent or the pepper/mustard extract used separately.

Many workers have compared the effectiveness of the use of biological control to chemical control in the management of plant pathogens (Tewari, 1995; Eksteen *et al.,* 2001). Eksteen *et al*. (2001) worked on crude extracts of *Eucomis autumnalis*, a plant native to South Africa, and it showed significant antifungal activity against seven plant pathogenic fungi comparable to broad-spectrum fungicides (Carbendazim/ Difenoconazole). Tewari (1995) demonstrated lesser cost of application of *Ocimum sanctum* (RS 375/ha) than synthetic fungicide of Ediphenphos (RS 1430/ha) or Carbendazim (RS 1580 /ha).

This study has demonstrated the possibility of using extracts from some the plants to control mycelial growth as *Fusarium oxysporum*, *Fusarium solanii* and *M. phaseolina*. The effectiveness of *S. aromaticum* and *O. basilicum* were clearly the highest of the twenty plants tested. Extract of *Xylopia aethiopica* was equally effective on *Fusarium oxysporum.* These plants exhibited promising potentials as alternatives to chemical fungicides for the control of the pathogens of groundnut.

The use of botanicals control may not be a panacea; however, they are useful tools for plant protection. For effective control to be achieved control measure should be applied on or before the onset of infection. However, disease prevention is often achieved with much less cost and trouble than treating the disease after it has set in. The large reservoir of natural fungicide in plants around us with continued research would provide less expensive, ecofriendly and could serve as a good alternative to synthetic fungicides.

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