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EVALUATION OF SOME PLANT EXTRACTS ON MYCELIAL GROWTH AND SPORULATION DENSITY OF FUNGAL PATHOGENS OF GROUNDNUT (*Arachis hypogaea* L.) *IN-VITRO*

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ABSTRACT

The effect of autoclave and ultraviolet light-sterilized aqueous extracts of *Tithonia diversifolia*, *Chromolaena odorata* and *Tridax procumbens* on mycelial growth and sporulation density of fungal pathogens were also determined *in vitro*. Aqueous extracts of the test plants significantly ($p < 0.05$) reduced mycelial growth of the fungal pathogens. *Tithonia diversifoli*a extract inhibited mycelial growth of *C. arachidicola* by 96.17% while *C. odorata* extract reduced mycelial growth of *A. alternata* by 90.74%. *Tridax procumbens* extract suppressed mycelial growth of *C. personatum* by 92.4% at 7 days incubation. *Chromolaena odorata* extract reduced sporulation density of *C. arachidicola* by 81.16% while extract of *T. diversifoli*a induced 81.8% reduction on sporulation density of *A. alternata*. *T. diversifoli*a extract also curtailed sporulation density of *C. personatum* by 78.32%. Fungitoxicity attributable to ultraviolet light-sterilization of extracts was comparable to that of autoclave sterilization in all the pathogens. The study revealed that plant extracts can effectively control Cercospora leaf spot disease of groundnut and its causative organisms. However, *T. diversifolia C. odorata* and *T. procumbens,* should be used as a potential biocide in plant disease management, as they showed fungicidal and fungitoxic ability.

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the world's most important oilseed crops (Dwivedi *et al*., 2003), ranking the 13th most important food crop and 4th most important oilseed crop of the world (Surendranatha *et al*., 2011), being cultivated in more than 100 countries in six continents (Sharma and Mathur, 2006). Its cultivation is mostly confined to the tropical, subtropical, and warm temperate (zones) countries between 40° N and 40° S latitude (Ephrem, 2015). It is also an important cash crop in subsistence and commercial farming systems, as well as an important food source (Izge *et al*., 2007). Groundnut kernels contain 40-50% fat, 20-50% protein and 10-20% carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron and potassium (USAD, 2010). Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as

culinary oil (Ephrem, 2015). Oil pressings, seeds, and the haulms of groundnut are used as animal feed while the oil cakes are used as industrial raw material and fertilizer (Ayele, 2010). These multiple uses of groundnut plant makes it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (Ephrem, 2015). According to Trawalley (1998), its protein content is the cheapest source of dietary protein in places where meat is scarce and very expensive for large proportion of subsistent farming communities. The hay (vine) is a nutritious animal feed, particularly for the subsequent dry season when green forage is not available (Naab *et al*., 2005). In addition, groundnut seed and hay are often sold in local markets, providing income to resource-poor farmers (Naab *et al*., 2005; Nutsugah *et al*., 2007). Groundnut is affected by several diseases, such as early leaf spot (*Cercospora arachidicola* S. Hori), late leaf spot (*Phaeoisariopsis personatum* Berk. and Curt.), collar rot (*Aspergillus niger*), rust (*Puccinia arachidis* Speg), and bud necrosis (bud necrosis virus (BNV) (Ephrem, 2015). Early leaf spot (caused by *Cercospora arachidicola* S. Hori) and Late Leaf Spot (caused by *Cercosporidium personatum*) are most devastating and economically important foliar fungal diseases and major yield reducing factor of groundnut worldwide (Backman and Crawford, 1984; Khaleque, 1985, Smith *et al*., 1992 and Mirza, 1998). Leaf spots of groundnut are one of the most important diseases of this crop worldwide with annual yield losses of 15 to 50% (Lucas *et al*., 1992). Most farmers control these diseases using fungicides. However, the negative environmental impacts, mammalian toxicity and high costs are making their usage unattractive thereby searching for alternatives such as natural plant-based chemicals (Asawalam, 2006). Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1991). These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Das *et al*., 2010). Many research workers have tried to find out safe and economical control of plant diseases by using extracts of different plant parts (Hasan *et al*., 2005; Bdliya and Alkali, 2008). The use of plant extracts with antifungal activity offers an economical, safe, and easily available alternative method for the management of leaf spot disease of groundnut (Rahman and Hossain, 1996). Hence the objective of the study was to evaluate and determine the efficacy of plant extracts on mycelial growth and sporulation density of fungal pathogens *in-vitro*

MATERIALS AND METHODS

Experiments were carried in the laboratory of the Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, 2015/2016 season.

Preparation of culture media

Potato Dextrose Agar (PDA) (BAM Media M127) was prepared by dissolving 39 grams in 1 litre Erlenmeyer flask and then made up to 1 litre using sterile distilled water. The medium was autoclaved at 121^{o}C for 15 minutes at 15 lb. The sterilized medium was allowed to cool to 45°C, before supplemented with streptomycin sulphate (3 grams) and aseptically dispensed into sterilized 9 cm diameter glass Petri dishes.

Isolation and Identification of Fungal Pathogens

Fungal species were isolated from diseased leaves of groundnut showing characteristic symptoms of Cercospora leaf spot. The Cercospora leaf spot disease was characterized by necrotic lesions on leaves which were circular to angular spots or dots and vary in size from less than 1 mm to 10 mm in diameter. Margins of infected leaves (2 - 5 mm diameter) were cut to contain both diseased lesions and healthy uninfected tissues using flame-sterilized scissors and forceps. Cut out portions were surface-sterilized (1 % NaOCl for 5 min then rinsed in five changes of sterile distilled water) and blottered dry with tissue paper in the laminar flow. The dried diseased cut out were then inoculated on PDA. Inoculated Petri dishes were incubated at 28 ± 2 °C. Fungi grew from the plant parts were sub-cultured until pure cultures were obtained. The fungi were identified with the aid of colony and hyphal characteristics and measurement of conidial length was done using ocular micrometer (Holliday, 1980; Domsch *et al*., 1981; Barnett and Hunter, 1999).

Sources of plant materials

Leaves of three plant species namely *Tithonia diversifolia* (Hemsley) A. Gray (Mexican sunflower) (Plate 1a), *Chromolaena odorata* Linn, (Plate 1b), *Tridax procubens* Linn. (Coat button) (Plate 1c) were used in the experiment. These were obtained within the premises of the Federal University of Agriculture, Abeokuta, Nigeria.

Preparation of Extracts

Fresh leaves of *T. diversifolia*, *C. odorata* and *T. procumbens* were washed in tap water then surfaced-sterilized with (1% NaOCl for 5min and rinsed in five changes of sterile distilled water) and air dried at (28 ± 2 °C) for 1h. Fifty grams, seventy-five grams and hundred grams of each plant material were grounded using sterilized Brabantia 5-speed blender (Model BBEK 1051) in 100 ml distilled water, and then filtered through a Whatman® No. 9 filter paper separately into a 250 ml Erlenmeyer flask to produce 50 %, 75 % and 100% extract concentrations. One fraction of the crude extracts was autoclaved at 121 °C/15 psi for 15 min and another were exposed to ultraviolet radiation (wavelength 438 nm for 5 h) for sterilization.

Effect of plant extracts on mycelial growth and sporulation density of fungal pathogens of groundnut

Extract-media mixtures were prepared by mixing 1 ml extract with 9 ml molten PDA prior to solidification for each extract concentration. Media amended with mycelial disc of a 5- day-old cultures of each fungus were placed in the centre of the petri dishes. The control plates consisted of PDA mixed with 1 ml sterile distilled water. All treatments were in three replicate and incubated at 28 ± 2°C. Radial growth in treatments and control were measured at 24 h interval for seven days. This was expressed as the mean growth along two axes on two pre-draw perpendicular lines on the reverse side of each plate. Sporulation density was determined by adding 10 ml sterile distilled water to each petri dish and gently scraping with a sterile glass rod to dislodge the spores. The spores suspensions

obtained was filtered through sterile cheese cloth into a sterile 50 ml glass beaker and homogenized by manual shaking. The spores were then counted using a Neubauer Hemocytometer. The percentage inhibition of mycelial growth and sporulation density by each extracts were computed using formula.

$$I = 100\times (C - T)/C$$

Where;
I = percentage inhibition of mycelial growth
C = mycelial growth of fungus in control plate
T = mycelial growth of fungus in the treatment
(Sobia *et al*., 2011)

The percentage reduction (Sr) of sporulation by each extract was determined using the following formula of Nduagu *et al*. (2008)

$$Sr = \frac{(S1 - S2) \times 100}{S1}$$

Where;

Sr = percentage of reduction in sporulation;
S1 = Sporulation on the untreated medium (control); and
S2 = Sporulation on the treated medium.
Mean cumulative reduction in mycelial growth and sporulation density was obtained using the formula:

$$\overline{X} = \frac{1}{n}\sum_{i=1}^{m}\sum_{j=1}^{n_i} X_{ij}$$

Where;
$\overline{X}$ = Grand mean
n = Total number of observation
m = Number of days being compared with respect to its concentration
X_{ij} = jth observation in the ith group where i= 1, 2, …, m and j= 1, 2…, n_i

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS

Effects of ultraviolet sterilized extracts (aqueous) of *T. diversifolia, C. odorata* and *T. procumbens* on the mycelial growth of *C. arachidicola*

The effects of ultraviolet sterilized aqueous extract of *T. diversifolia, C. odorata* and *T. procumbens* on the mycelial growth of *C. arachidicola* are presented in Table 1.

Mycelial growth of *C. arachidicola* decreased with increase in concentration of the plant extracts used. On the third day, mycelial growth reduction by all extracts at 100% (w/v) were not significantly (p 0.05) difference from one another, with *C. odorata* induced highest mycelial growth reduction of 67.34%. The mycelial growth reduction of *C. arachidicola* was also comparable to that due to 75% (w/v) of *T. procumbens.* While extract of *T. diversifolia* at 50% (w/v) induced lesser mycelial growth reduction of 55.46%. On fifth day of incubation, *T. procumbens* exerted the highest mycelial growth reduction of 84.43%. Mycelial growth reductions at 75% (w/v) were not significantly (p 0.05) different across all extracts. On the seventh day of incubation, extract of *T. diversifolia* at 100% (w/v) reduced mycelial growth of *C. arachidicola* by 94.15% and this was significantly (p 0.05) higher than 89.67% induced by *T. procumbens* at 100% (w/v). *C. ordorata* induced the lowest mycelial growth of 75.35% on *C. arachidicola* . Values with different superscripts within the same column are significantly different (p 0.05) according to Duncan's Multiple Range Test.

Effect of autoclave sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *C. arachidicola*

Table 2 indicates the effect of autoclave sterilized aqueous extracts of *T. diversifolia, C. odorata* and *T. procumbens* on the mycelial growth of *C. arachidicola* after seven days of incubation. Mycelial growth reduction was influenced by increase in concentration of extracts. Values with different superscripts within the same column are significantly different (p 0.05) according to Duncan's Multiple Range Test. On the third day of incubation at 100% (w/v) of all extracts, mycelial growth reduction was at a peak with *C. odorata* inducing the highest mycelial growth inhibition of 67.34%, there was no significant (p 0.05) differences among plant extracts by same concentration. Mycelial growth of 64.94% induced by extract of *T. procumbens* at 75% (w/v) was comparable to those induced at the same concentration of other extracts.

On the fifth day of incubation, extract of *T. procumbens* at 100% (w/v) extended mycelial growth reduction to 84.43%. This was comparable (p 0.05) to the mycelial growth reduction observed with other extracts at the same concentration. Mycelial growth reductions induced at 75% (w/v) was not significantly (p 0.05) different from one another across all extracts. On the seventh day of incubation, extract of *C. odorata* at 50% (w/v) reduced mycelial growth of the pathogen by 75.35% and it was not significantly (p 0.05) different from other mycelial growth reduction at the same concentration across all extracts.

Table 1. Effect of ultraviolet light sterilized extracts (aqueous) of *T. diversifolia, C. odorata* and *T. procumbens* extracts on the mycelial growth of *Cercospora arachidicola*

		Mycelial growth inhibition (%)		
Treatment (Plant Extract)	Conc.% (w/v)	Day 3	Day 5	Day 7
Tithonia diversifolia	50	55.46^{c}	69.23^{d}	77.08^{c}
	75	63.17^{b}	75.03^{b}	88.75^{b}
	100	65.60^{ab}	81.09^{a}	94.15^{a}
Chromolaena odorata	50	56.80^{bc}	66.93^{d}	75.35^{b}
	75	64.91^{ab}	75.03^{bc}	87.75^{b}
	100	67.34^{a}	82.42^{a}	91.00^{ab}
Tridax procumbens	50	57.57^{c}	70.37^{cd}	79.23^{c}
	75	65.83^{ab}	76.35^{b}	89.06^{b}
	100	65.60^{ab}	84.43^{a}	89.67^{b}

Table 3. Effect of ultraviolet sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *Alternaria alternate*

		Mycelial growth inhibition (%)		
Treatment (Plant Extract)	Conc.% (w/v)	Day 3	Day 5	Day 7
Tithonia diversifolia	50	54.99^{e}	63.67^{c}	68.88^{f}
	75	62.96^{bc}	68.68^{b}	75.79^{de}
	100	68.54^{a}	73.00^{b}	83.16^{bc}
Chromolaena odorata	50	56.96^{de}	66.52^{cd}	70.08^{ef}
	75	63.97^{bc}	73.69^{b}	80.83^{cd}
	100	65.96^{a}	82.19^{a}	89.84^{a}
Tridax procumbens	50	56.40^{e}	63.80^{d}	79.13^{c}
	75	62.25^{cd}	68.72^{b}	81.78^{bc}
	100	70.38^{a}	76.25^{b}	86.45^{a}

Table 4. Effect of autoclave sterilized extracts of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *Alternaria alternate*

		Mycelial growth inhibition (%)		
Treatment (Plant Extract)	Conc.% (w/v)	Day 3	Day 5	Day 7
Tithonia diversifolia	50	54.99^{e}	63.68^{d}	68.88^{g}
	75	62.96^{bc}	68.68^{c}	75.79^{ef}
	100	68.54^{ab}	73.0^{b}	85.83^{bc}
Chromoleana odorata	50	56.96^{de}	66.52^{cd}	72.08^{fg}
	75	63.97^{bc}	73.69^{b}	80.83^{cde}
	100	65.96^{abc}	82.19^{a}	90.74a^{abc}
Tridax procumbens	50	50.40^{de}	63.80^{d}	79.13^{ef}
	75	62.25^{cd}	68.72^{c}	81.78^{bcd}
	100	70.38^{a}	76.58^{b}	86.12^{b}

The highest mycelial growth reduction of 96.17% was induced by extract of *T. diversifolia* at 100% (w/v) and was comparable to other mycelial growth reduction induced at same concentration across the extracts. Similarly, the mycelial growth reduction recorded at 75% (w/v) for all extracts were not significantly (p 0.05) different from one another.

Effect of ultraviolet light sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *A. alternate*

Table 3 shows Ultraviolet light sterilized aqueous extracts *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *A. alternata* after seven days of inoculation. The mycelial growth of *A. alternata* was significantly (p 0.05) reduced by the extracts at different concentrations. On the third day of incubation, extract of *T. procumbens* at 100% (w/v) reduced mycelial growth of the pathogen by 70.38% and this was significantly (p 0.05) higher than all mycelial growth induced at lower concentrations. It was however comparable to that due to other extracts at the same concentration.

The mycelial growth reductions induced by the different extracts was not significantly (p 0.05) different from one another. Similarly, at 50% (w/v), mycelial growth reductions were comparable. On the fifth day of incubation, extract of *C. odorata* induced the highest mycelial growth reduction of 82.19% at 100% (w/v). Mycelial growth reductions by extracts of *T. diversifolia* and *T. procumbens* at 100% (w/v) were not significantly (p 0.05) different from that due extract of *T. diversifolia* at 75% (w/v). Extract of *T. procumbens* reduced mycelial growth of the pathogen by 66.52% which was not significantly (p 0.05) different from that due to other extracts.

On the seventh day of incubation, extract of *C. odorata* at 100% (w/v), induced the highest mycelial growth reduction of 89.84% and it was comparable to 86.45% by extract of *T. procumbens* but significantly (p 0.05) higher than 83.16% by extract of *T. diversifolia* at the same concentration (Table 3). Values with different superscripts within the same column are significantly different (p 0.05) according to Duncan's Multiple Range Test.

Effect of autoclave sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *A. alternate*

Table 4 displays the effect of autoclave sterilized aqueous extracts of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *A. alternata* after seven days of inoculation. Mycelial growth of the pathogen was significantly (p 0.05) reduced by all extracts at the different concentrations. On the third day of incubation, mycelial growth reduction ranged from 50.40 to 70.38% across all treatment. The extract of *T. procumbens* induced the highest reduction, an effect that was comparable to that due to extracts of *T. diversifolia* and *C. odorata*. At 75% (w/v) concentration, mycelial growth reductions were not also significantly (p 0.05) different from each other though comparable to that due to extracts of *T. diversifolia* and *C. odorata* at 100% (w/v). On fifth day mycelial growth of the pathogen was reduced by 82.19% at 100% (w/v) concentration of *C. odorata*. The highest mycelial growth reduction was induced by 90.74% at 100% (w/v) of *C. odorata* extract. Similar trend was observable with *T. diversifolia* induced the highest mycelial growth reduction of 86.12% at 100% (w/v) on seventh day. Values with different superscripts within the same column are significantly different (p 0.05) according to Duncan's Multiple Range Test.

Table 5. Effect of ultraviolet light sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *Cercosporidium personatum*

		Mycelial growth inhibition (%)		
Treatment (Plant Extract)	Conc.% (w/v)	Day 3	Day 5	Day 7
Tithonia diversifolia	50	54.99^{e}	63.68^{d}	68.88^{f}
	75	62.96^{bc}	68.68^{c}	75.83^{de}
	100	73.35^{a}	73.0^{b}	87.43^{b}
Chromolaena odorata	50	56.96^{de}	66.52^{cd}	72.08^{ef}
	75	63.97^{b}	73.69^{b}	80.83^{cd}
	100	65.96^{a}	82.19^{a}	87.91^{b}
Tridax procumbens	50	56.40^{de}	63.80^{d}	79.13^{d}
	75	62.25^{cd}	68.72^{c}	85.11^{bc}
	100	70.38^{a}	76.58^{a}	90.64^{a}

Table 6. Effect of autoclave sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *Cercosporidium personatum*

		Mycelial growth inhibition (%)		
Treatment (Plant Extract)	Conc.% (w/v)	Day 3	Day 5	Day 7
Tithonia diversifolia	50	54.99^{e}	63.68^{d}	68.88^{f}
	75	62.96^{bc}	68.68^{c}	75.83^{de}
	100	73.35^{a}	73.0^{b}	87.43^{b}
Chromolaena odorata	50	56.96^{de}	66.52^{cd}	72.08^{ef}
	75	63.97^{b}	73.69^{b}	80.83^{cd}
	100	65.96^{a}	82.19^{a}	87.91^{b}
Tridax procumbens	50	56.40^{de}	63.80^{d}	79.13^{d}
	75	62.25^{cd}	68.72^{c}	85.11^{bc}
	100	70.38^{a}	76.58^{a}	90.64^{a}

Table 7. Mean cumulative effect of ultraviolet light and autoclave sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *Cercospora arachidicola*, *Alternaria alternata* and *Cercosporidium personatum*

	Mean cumulative reduction mycelial growth (%)		
Treatment (plant Extract)	*C. arachidicola*	*A. alternata*	*C. personatum*
T. diversifolia (uv)	74.30	68.85	69.15
(au)	74.74	69.15	69.87
C. odorata (uv)	74.17	72.64	72.19
(au)	74.73	72.93	72.2
T. procumbens (uv)	75.32	71.68	73.3
(au)	74.82	71.05	73.3
LSD (p 0.05)	1.78	3.59	5.73

Effect of ultraviolet light sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *C. personatum*

Table 5 shows the effect of ultraviolet light sterilized aqueous extracts of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *C. personatum* after seven days of inoculation. Mycelial growth reduction of *C. arachidicola* was influenced by increase in concentration of extracts. On the third day of incubation at 100% (w/v) concentration of all extracts, mycelial growth reduction induced by *C. odorata* was 67.34%. Mycelial growth of 64.94% induced by extract of *T. procumbens* was comparable to those induced at 75% (w/v) across all extracts. The least mycelial growth reduction of 56.19% was observed with extract of *T. diversifolia* at 50% (w/v) an effect that was comparable to those of other extracts at the same concentration. On the fifth day of incubation, extract of *T. procumbens* extended mycelial growth reduction to 84.43%. This effect was not superior to observe with other extracts at the same concentration. Mycelial growth reductions induced at 75% (w/v) was not significantly (p 0.05) different from one another across all extracts. They were also comparable to 70.37% due to 50% (w/v) of *T. procumbens* while the extract of *C. odorata* induced the lowest mycelial growth of 66.93% at 50w/v. On the seventh day of incubation, extract of *C. odorata* at 50% (w/v) reduced mycelial growth of the pathogen by 75.35% (w/v).

The highest mycelial growth reduction of 92.4% was induced by extract of *T. diversifolia* at 100% (w/v) and was comparable to other mycelial growth reduction induced at same concentration across the extracts. Similarly, the mycelial growth reduction recorded at 75% (w/v) for all extracts were not significantly (p 0.05) different from one another. Values with different superscripts within the same column are significantly different (p 0.05) according to Duncan's Multiple Range Test.

Effect of autoclave sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *C. personatum*

The effect of autoclave sterilized aqueous extracts of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *C. personatum* after seven days of inoculation are shown in Table 6. Generally, as concentration increased, the mycelial growth reduction of the pathogen increased for all extracts. Values with different superscripts within the same column are significantly different (p 0.05) according to Duncan's Multiple Range Test. On the third day, extract of *T. diversifolia* at 100% (w/v) induced (73.35%) the highest mycelial growth reduction. At 75% (w/v) mycelial growth reduction by all extracts within a range of (62.25 and 63.97%). On the seventh day of incubation, extract of *T. procumbens* at 100% (w/v) induced the highest mycelial growth reduction of

(90.64%). Extract of *C. odorata* and *T. procumbens* at 100% (w/v) reduced mycelial growth of the pathogen by (82.19 and 76.58%) respectively. Similarly, *T. diversifolia* exerted a lesser impact 70.0% significantly at the same concentration. On the seventh day *T. diversifolia* induced least mycelial growth reduction (68.88%) an effect that was comparable to that exerted by but not significantly different from (72.08%) induced by extract by *C. odorata*.

Mean cumulative effect of ultraviolet light and autoclave sterilized extracts (aqueous) of *T. diversifolia*, *C. odorata* and *T. procumbens* on the mycelial growth of *C. arachidicola*, *A. alternata* and *C. personatum*

Cumulative fungitoxic effect of ultraviolet and autoclave sterilized extracts of the plant extracts. The fungitoxic effect of all the plant extracts was comparable (p 0.05) except between ultraviolet sterilized *T. procumbens* and ultraviolet sterilized *T. diversifolia* extracts are indicated in Table 7

Mean cumulative reduction in mycelial growth is obtained from the formula:

$\bar{X} = \frac{1}{n} \sum_{i=1}^{m} \sum_{j=1}^{n_i} X_{ij}$, *Tithonia diversifolia*, *Chromolaena odorata*, *Tridax procumbens*, uv= Ultraviolet sterilized, and au= Autoclave sterilized

DISCUSSION

The results of the antifungal activity showed that the plant extracts had inhibitory effects on the growth and sporulation density of the fungi. These results revealed that antifungal activities of the extracts were enhanced by increasing the concentration from 50 to 100% (w/v), hence the inhibition activities of the extracts were concentration dependent. This is in agreement with the report of Ilondu (2012), Chiejina and Ukeh (2013) who stated that increase in the antifungal activities was observed with corresponding increase in concentration of plant extracts. Similarly, Benagi (1995) reported the efficacy of extracts of garlic, neem, and Tridax in inhibiting the mycelial growth of *Phaeoisariopsis personata* under *in vitro* conditions. Different plant extracts have been found to inhibit the conidial germination of *C. arachidicola* and *C. personatum* (Chary *et al*., 1984; Alam *et al*., 2002). According to Inderjit and Mukerji (2006), *Ageratum conyzoides* L. can produce and release many kinds of allelochemicals participating in their defense against pathogens. *In vitro* result in the study showed that all the plant extract exhibited fungistatic effect on the fungi pathogens. This correlates with the reports of Shetty and Prakash (1989) and Owolade *et al.* (1999) in which crude extracts from plant materials significantly inhibited mycelial growth of many pathogentic fungi. Alabi *et al.* (2005) also reported the fungitoxic and phytotoxic effect of extracts of *Venonia amaygdalina* L. *B. pinnatum* Kurz, *Ocimum gratissimum* L. and *Eucalyptus globules* Labill on the wilt pathogens in cowpea. The significant inhibitory effect of the plant extracts in the control of *C. arachidicola*, *A. alternata* and *C. personatum* showed that the fungitoxic components of these extracts (at concentration of 100% (w/v) effectively control the mycelial growth and sporulation density of the fungal pathogens. This is similar to observation by Daouk *et al*. (1995) who reported that the reduction in microbial population depends on high concentration which can completely inhibit the growth of microorganism. Nachman *et al*. (1994) also stated that the complete inhibition of mycelial growth of *A. ochraceus*, *A.niger* and *A. flavus* was achieved after exposing mycelial disc to oregano essential soil at high concentration.

Conclusion

This study revealed the used *T. diversifolia*, *C. odorata* and *T. procumbens* showed inhibitory effect on mycelial growth reduction and sporulation density of fungal pathogens as they showed fungicidal and fungitoxic ability. The use of plant extracts with antifungal activity offers an economical, safe, and easily available alternative method for the management of leaf spot disease of groundnut.

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