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FUNGITOXIC ACTIVITY OF EXTRACTS OF SOME MEDICINAL PLANTS ON *Pythium aphanidermatum*, CAUSAL AGENT OF ROOT ROTS OF TOMATO (*Lycopersicon esculentum*)

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ABSTRACT
The in vitro fungitoxic activity of crude extracts of ginger (Zingiber officinale), bitter-kola (Garcinia cola), aloe (Aloe vera) and neem (Azadirachta indica) was tested on Pythium aphanidermatum isolated from root rot of tomato (Lycopersicon esculentum). The organic solvent (methanol) extracts of leaves of neem and aloe, seeds of bitter kola, and rhizomes of ginger at 40%, 60%, 80% and 100% concentrations were tested on Potato dextrose agar (PDA) for activity against the mycelia growth of Pythium aphanidermatum. The experiment was a completely randomized design with three replications per treatment. The results showed that mean percentage inhibition of mycelia growth was highest in plates containing ginger extract; followed by aloe. The fungitoxic components in bitter kola and neem were only effective in inhibition of mycelia growth of the pathogen at higher concentrations (80% and 100%) respectively.

Key words: Extracts, fungitoxic, inhibition, mycelia growth, root rot, tomato.

INTRODUCTION
Tomato plant, with its fruit is of great nutritional importance because it is an excellent source of vitamins like vitamin A, C, thiamine, niacin, riboflavin and of minerals like iron and calcium. Its fibres serve as roughage for the promotion of digestion. In view of its varied uses which include as salad vegetable, puree, paste, ketchup, juice and for cooking soups and stews, it can also be canned or pickled (Chiejina, 2005). The need to increase its production is therefore of paramount importance. Increased production has suffered some set back due to infection of the root and fruit by microorganisms in the field and in storage, respectively (Chinoko and Naqvi, 1989; Darahiyar, 1980 and Chiejina, 2005).

In Ngeria, tomato is mainly cultivated in the region north of 10^{o}N latitude. This covers the savannah belts where its cultivation is supported with irrigation. In the south, the excessive precipitation and the associated high relative humidity tend to limit tomato production during the raining season as it favours the multiplication of the pathogen responsible for the root rot. Among the pathogen responsible for root rot of tomato is *Pythium* sp (Agrios, 2005) and *Fusarium oxysporium* (Onyia *et al*, 2005); basal stem rot caused by *Sclerotium rolfsii* (Wokocha and Okereke, 2005). *Phythium*

aphanidermatum, implicated as the root rot disease of tomato, is a destructive soil-borne pathogen which attacks several other plants including moncotyledons and dicotyledons. With hot and humid weather being conducive for growth, the fungus is more devastating in tropical and sub-tropical regions of the world. In Nigeria, the pathogen is of major concern to vegetable growers in the northern savanna belts which potentially rank among the world's best vegetable production zones.

The study of medicinal plants used in folklore remedies in the treatment of microbial infections have attracted the attention of many scientists as possible alternatives to the existing drugs to which many infectious microorganisms have become resistant (Adegboye *et al.,* 2008). Likewise, the necessity to develop non-toxic, safe and effective biodegradable alternative to synthetic pesticides and fungicides have in recent years, led to global efforts at screening various plants for bioactivity against plant pathogenic organisms (Olaniyi, and Moermans, 2005; Wokocha and Okereke, 2005). It is however estimated that about 10% of the over 250,000 different plant species in the world today have been examined chemically for antimicrobial activity (Iwu, 1993). Therefore, a large reservoir of potential sources of botanical fungicides still exists especially in tropical forests awaiting exploitation. The continued use of natural plant products for plant disease protection is particularly important in countries like Nigeria where synthetic fungicides are not readily available, farmers are poorly equipped to handle them, and their use is uneconomical. In the continuous search for alternative control measures to the use of synthetic fungicides, the present study is aimed at evaluating the effectiveness of extracts of plants in controlling root rot of disease of tomato.

MATERIALS AND METHODS

Isolation of the pathogen

Tomato plants naturally infected by *P. aphanidermatum*, obtained from infected roots in eight locations in Kogi State, north central Nigeria, were washed free of soil and cut into 5mm segments where the pathogen was active. The segments were disinfected in 1% sodium hypochloride (NaOCl) solution for 3 minutes and rinsed in several changes of distilled water, dried in-between sheets of sterile filter paper and then plated on fresh potato dextrose agar (PDA) medium in 4.5cm Petri dishes; with three segments per plate. The dishes were incubated at 27^{o}C for seven days. Three sub-cultures were made to obtain pure culture of the pathogen.

Preparation of plant extracts

The following plant products were used for the preparation of the extracts: leaves of *Azadirachta indica* and *Aloe vera*, seeds of *Garcinia cola* and rhizome of *Zingiber officinale.* Fresh samples of each were used for the organic solvent (methanol) extraction according to (Epidi and Alamene, 2005; Ojo and Olufolaji, 2005 and Amadioha, 2003). Each of the plant samples was washed thoroughly in cold running tap water, sun-dried for seven days. Five hundred grammes (500g) of each was homogenized using warring blender, and placed in 1000ml flask containing 500ml methanol and thoroughly mixed together using glass rod and left for 24 hours to allow for extraction of active ingredients as cold extraction before being filtered into a fresh 500ml flask using four-fold cheese cloth as described by Wokocha and Okereke (2005). On the other hand, hot organic solvent extraction was carried out by weighing the same quantity of samples (500g), washed and soaked in 500ml of methanol in a 1000ml conical flask. They were then placed in pots of water and heated on the electric cooker at 100^{o}C. The filtrate was concentrated using the vacuum evaporator so as to regenerate the methanol. It

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was filtered using Buckner funnel and dried solidified extracts in percentage (%) was determined using the formula:

$$\frac{solid\ \ extracts}{samples} \quad x \quad \frac{100}{1}$$

From the crystal samples, 13.34g, 20g, 26.67g and 33.33g were weighed and dissolved in 50ml distilled water to give the final concentrations of 40%, 60%, 80% and 100%, using the modified method of Epidi and Alamene, (2005); Ojo and Olufolaji, (2005). Streptomycin was added at the rate of $125mg^{-1}$ to each of the plant extracts to check bacterial contamination and kept for the *in vitro* assay.

***In vitro* assay of plant extracts**

The bioassay of the different plant extracts to determine the effects on radial growth inhibition was carried out as described by Amadioha (2003). One (1ml) of solidified crude extracts was spread on the Petri dishes containing PDA as PDA/crude medium. The control was PDA on which 1ml of sterile distilled water was spread on the surface. Five mm diameter discs cut from the pathogen grown on PDA was placed at the centre of the Petri dish containing the PDA/crude extract, with three replicates and repeated once. A completely randomized design experiment at $27\pm2^{o}C$ was terminated at 7 days when the growth of mycelia in the control had reached the edge of the Petri dish. Percentage inhibition of mycelia or italicized growth was calculated using the formula of Pendey *et al.*, (1982) as follows:

$$\%\ \text{inhibition} \quad = \quad \frac{dc - dt}{dc} \quad x \frac{100}{1}$$

Where dc = average diameter of fungal colony in control plates, and dt = average diameter of fungal colony in treated plates.

Data analysis

All results obtained were analyzed using Simple Descriptive Statistics such as mean and standard error in accordance with Norman, (1995). Means were separated using analysis of variance (ANOVA). ANOVA statistical test used was tested at 5% level of significance. A two-tailed test was used for hypothesis testing. **SPSS 15.0** for windows was used for the statistical analysis. Honestly Significant Difference (HSD) was used for inferential statistical analysis while standard error was used for descriptive statistics.

RESULTS

The isolate

The isolated fungus from field infected roots, soil and soil-drenched with suspension from the various field locations studied in Kogi State, north central, Nigeria, initially as well as those planted in pots kept in the field was identified as *Pythium aphanidermatum*. The fungus was found to be associated with root rot of tomato plants in the study areas. The fungus has coenocytic (aseptate) hyphae with whitish vegetative mycelium that is richly branched, slender, and cylindrical profusely branching, hyaline and rapidly growing mycelium. The mycelium gives rise to terminal, or intercalary sporangia. The sporangia, which usually produced in vesicles during sexual reproduction, are globose to oval or at times irregular in shape and germinate directly by producing one to several germ tubes.

Inhibitory effects of aqueous extract of rhizome of ginger on the mycelial growth of *Pythium aphanidermatum*

The result in **table 1** shows that aqueous extracts of ginger at all concentrations completely inhibited mycelial growth of *Pythium aphanidermatum.* As there was no growth, the various concentrations applied were all significant compared with control (P 0.00 < 0.05). A low concentration of 40% of the hot extract of ginger is required to inhibit the mycelial extension of the fungus. Further to this, sporangial production and germination were equally inhibited at all levels of concentrations.

Table 1: Inhibitory effects of ginger rhizome extract on mycelial growth of *Pythium aphanidermatum*.

Concentration (%)	Mean percentage inhibition ± SE (%)
Control (0)	0.00 ± 0.0 [a]
40	100.0 ± 0.0 [b]
60	100.0 ± 0.0 [b]
80	100.0 ± 0.0 [b]
100	100.0 ± 0.0 [b]

Means followed by the same letter are not significantly different ($P \leq 0.05$).

Inhibitory effects of aqueous extract of aloe leaf on the mycelial growth of *Pythium aphanidermatum*

Aqueous extracts from aloe leaf was inhibitory to mycelia growth of *Pythium* during the first few days of inoculation. By the fourth day, however, their effects had reduced; especially at 40% concentration. There was mycelia growth at 40% concentration (**Table 2**) with a level of significant inhibition at 0.05% when compared with the untreated plates. The mycelium was scanty at the centre with whitish, feathery hyphae on the outer edges of the culture. Between 40% and other concentrations, there was a significant difference at (P 0.00 < 0.05). There was however no significant difference between 60%, 80% and 100% (P 1.00 > 0.05).

Table 2: Inhibitory effects of aloe leaf extract on mycelial growth of *Pythium aphanidermatum.*

Concentration (%)	Mean percentage inhibition ± SE (%)
Control (0)	0.00 ± 0.0 a
40	98.87 ± 0.4 [b]
60	100.0 ± 0.0 [c]
80	100.0 ± 0.0 [c]
100	100.0 ± 0.0 [c]

Means followed by the same letter are not significantly different ($P \leq 0.05$).

Inhibitory effects of aqueous extracts of bitter kola (*Garcinia cola*) fruits on the mycelial growth of *Pythium aphanidermatum*

The crude extract of bitter kola fruits showed significant reduction in mycelia growth of *Pythium* at different levels of concentration. High fungitoxicity *in vitro* was observed at 80% and 100% concentrations but no significant difference between them (P 0.22 > 0.05). The result shows that aqueous extracts at 80% and 100% concentrations from bitter kola completely inhibited mycelia growth in *Pythium*

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aphanidermatum for the first three days of inoculation (**Table 3**), but their inhibitory effects had worn off by the fourth day of inoculation. A highly significant difference was noticed in all the concentrations compared with control. Between 40% and 60%, there was no significant difference (P 0.37 > 0.05). A significant difference was noticed between 40% and 80%, between 40% and 100% at (P 0.01 < 0.05 and 0.00 < 0.05) respectively. Between 60% and 100% was a significant difference (P 0.04 < 0.05) compared with 60% and 80% of no significant difference (P 0.57 > 0.05).

Table 3: Inhibitory effects of bitter-kola fruit extracts on mycelial growth of *Pythium aphanidermatum*.

Concentration (%)	Mean percentage inhibition ± SE (%)
Control (0)	0.00 ± 0.0 [a]
40	50.92 ± 6.9 [b]
60	64.76 ± 7.8 [b c]
80	76.64 ± 5.4 [c d]
100	92.80 ± 2.8 [d]

Means followed by the same letter are not significantly different (P ≤ 0.05).

Inhibitory effects of aqueous extracts of neem (*Azadirachta indica*) leaf on the mycelial growth of *Pythium aphanidermatum*

The aqueous extracts of neem leaf at all level of concentrations significantly retarded mycelial growth of *Pythium aphanidermatum.* These results showed that the inhibitory effects of the extract on mycelial growth increased with increase in concentration (**Table 4**). Aqueous extracts from neem leaf was inhibitory to mycelial growth in *Pythium* during the first two days at 100% concentrations of the extract, but their inhibitory effect had generally worn off by the third day and by the fifth day, however, their inhibitory effect at 40% concentration had reduced when mycelial growth in them and in the untreated control almost completely filled the culture plate. There was however a significant difference between the control and other concentrations. But some concentrations showed no significant difference when compared; between 40%, 60% and 80%, at (P 0.98 > 0.05) and (P 0.90 > 0.05), and between 80% and 100% (P 0.57 > 0.05) respectively. However, 40% and 100% and between 60% and 100% were significant at (P 0.001 < 0.05) and (P 0.007 < 0.05) respectively.

Table 4: Inhibitory effects of neem leaf extract on mycelial growth of *Pythium aphanidermatum*.

Concentration (%)	Mean percentage inhibition ± SE (%)
Control (0)	0.00 ± 0.0 [a]
40	46.22 ± 7.9 [b]
60	50.82 ± 7.5 [b]
80	68.25 ± 6.1 [c b]
100	81.15 ± 5.4 [c]

Means followed by the same letter are not significantly different ($P \leq 0.05$).

DISCUSSION

The outcome of this study showed that the four plant extracts used significantly inhibited mycelial growth of *Pythium aphanidermatum in-vitro*. This suggests that the extracts possess bio fungicidal potential capable of controlling root rot disease of tomato in *in-vitro*. It was also observed that fungitoxicity of the extracts were higher at increased concentrations. The four aqueous plants extracts screened *in vitro* showed varying levels of toxicity on *Pythium aphanidermatum*, this was observed in percentage inhibitions of mycelial growth.

The fungitoxic effects of ginger and aloe extracts were significantly ($p \leq 0.05\%$) higher than those of bitter kola and neem extracts. These results agree with Wokocha and Okereke, (2005) on the inhibitory action of plant products employed in this investigation on mycelia growth. Treatments containing *G. cola* fruit and *A. indica* leaves were similar, showing mean percentage inhibition of 92% and 77% respectively. The crude extract of *G. cola* fruits showed significant reduction in mycelial growth of *Pythium aphanidermatum* at different levels of concentration similar to those of Wokocha and Okereke (2005). High fungitoxicity *in vitro* was observed at 80% and 100% concentrations but no significant difference between them ($P\ 0.22 > 0.05$). The result shows that aqueous extracts at 80% and 100% concentrations from bitter kola completely inhibited mycelial growth in *Pythium aphanidermatum* for the first three days of inoculation, but their inhibitory effects had worn off by the fourth day of inoculation. A highly significant difference was noticed in all the concentrations compared with control and between 40% and 60%, there was no significant difference ($P\ 0.37 > 0.05$).

In leaf extracts of neem, the mycelium was rather scanty at the centre with whitish, a luxuriant creamy-white on the edges of the culture. Growth habit was fluffy and in two concentric bands. Even though, neem leaf extracts seems to be the least effective among the extracts employed at various concentrations on mycelial growth of *Pythium aphanidermatum*, the percentage level of inhibition at various concentrations shows that there is level of significant at 80% and 100% concentration, compared with untreated plates. These results showed that the inhibitory effects

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of the extract on mycelial growth increased with increase in concentration similar to those of Ojo and Olufolaji (2005).

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This study has revealed the potential of botanicals in the control of root rot disease of tomato *in vitro*. However, it is pertinent to note that this finding is one among many other possibilities in disease control; nevertheless, more effort is required in integrating the study to other related findings. The use of plant products in integrated pests and fungi management could reduce over reliance on one source of agricultural chemicals to the farmer, as well as cut down the cost of production. The facts that these plants used in this study are easily available, with easy method of extraction, they can be exploited in the control of root rot disease of tomato.

CONCLUSION

This study provides information serving as a base line in establishing *Pythium aphanidermatum* as the causal pathogen of severe root rots of tomato and very rampant in the north central, Nigeria. The results at various concentrations showed that ginger (*Z. officinale*), aloe (*Aloe vera*), bitter kola (*Garcinia cola*) and neem (*Azadirachta indica*) contain antifungal properties which can be used to control *Pythium* root rot of tomato. Apart from being environmentally friendly, it is cheaper, affordable, biodegradable and therefore, preferable to commercial fungicides in agreement with previous findings.

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