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Short Communication

Efficacy of four plant extracts in the control of root rot disease of cowpea (*Vigna unguiculata* [L.] Walp)

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A study on the use of ginger (*Zingiber officinale*), aloe (*Aloe ve*ra), bitter kola (*Garcinia cola*) and neem (*Azadirachta indica*) extracts in the control of root rot of cowpea caused by *Pythium aphanidermatum* was carried out *in vitro* and in the field (*in vivo*). They were evaluated for their antifungal activity over *P. aphanidermatum*, a rot fungus of many economic crops. Vegetative growth values over the fungus at 40, 60, 80 and 100% concentrations were generally low compared with the control, complete inhibition of fungal mycelia growth was exhibited at all concentrations in ginger extract. Aloe at 60% completely inhibited mycelial growth; this was followed by bitter kola with only retardation of mycelial growth while neem was the least effective. Highest mycelial dry weight was noticed on bitter kola extract but more sporangia formation in neem after a prolong incubation. However, the extracts were sparingly effective for a short period on the field experiment. A statistical evaluation of variance showed a significant difference between mycelial radial growths values recorded on the various plant extracts concentrations used compared with the control.

Key words: Plant extract, fungal mycelial growth, cowpea root rot, *in vitro, in vivo*.

INTRODUCTION

Cowpea are susceptible to a wide range of pests and pathogens that attack the crop at all stages; rust caused by *Uromyces appendiculatum* (Onuh et al., 2005), soft stem and root rots caused by *Pythium aphanidermatum* (Emechebe and Shoyinka, 1985; Dutta, 2005; Gale, 2002), blotch caused by *Colletotrichum capsici* (Croft, 2007) and blight caused by *Ascochyta phaseolorum* (Adeyeye and Olufolaji, 2004). Pythium root rot, sometimes called damping off, or wilt, may be caused by several species of *Pythium* (Agrios, 2005; Zaumeyer and Thomas, 1957). The authors reported that those most commonly found on beans, especially cowpea, are *Pythium ultimum*, *P. debaryanum*, *P. mytiotylum*, *P. helicoids*, *P. aphanidermatum*, *P. oligandrum*, *P. rostratum*, *P. pulchrum*, *P. vexans*, *P. anandrum* and *P. acanthicum*. *Pythium* is a soil pathogenic fungus belonging to the family pythiaceae, it usually attack seedlings at the base and root under condition of over crowding and over-watering (Dutta, 2005). In Nigeria, fungi constitute the major limiting factor to the production of cowpea. Losses caused by fungi attack vary from 20 to almost 30% (William, 1975).

Since the end of the Second World War, there has been a great boom in the use of fungicides throughout the world. After the great justified alarm in the early 60s about the dangerous consequences to man and environment in the area of phytotoxicity (Williams, 1975), there is an urgent need for alternative method of plant disease control. This scenario necessitates the search for and the development of ecologically sustainable fungi control method which are effective against the target species but create minimal adversity for non-target species.

Historical successes have been recorded in the use of azadirachtin (from neem) and similar alkaloids, flavorniods, terpenoids from aloe, ginger and bitter kola as biopesticides and fungicides. According to Santreal international (1998), aloe contains some active ingredients that are fungicidal. Cold-water extracts of *A. indica* (neem), *Garcinia cola* (bitter kola) and *Zingiber officinale* (ginger) at various concentrations posses fungicidal activity against the mycelial growth and sclerotial germination of a soil fungus, *Sclerotium rolfsii* as reported by Wokocha and Okereke (2005).

Due to identifiable problems (e.g. chemical residues,

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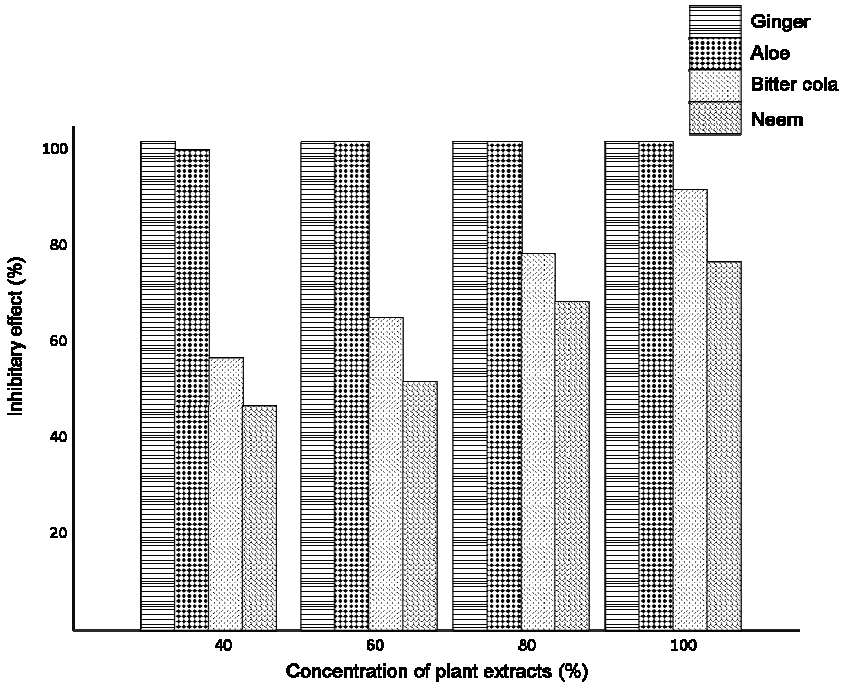

Figure 1. The *in vitro* effect of plant extracts on cowpea root rot disease.

biodegradation, phytotoxicity, pollution, etc) associated with chemical control strategies, alternative control methods are being attempted. The aim of this research is to provide useful information on cheaper, affordable, natural and environmentally friendly pesticide in the control of root rot disease of *V. unguiculata*.

MATERIALS AND METHODS

Based on previous biological activities, rhizome of ginger leaves of neem, aloe and seeds of bitter kola were collected for this study. The fungus (*P. aphanidermatum*) was isolated from infected soil and roots of cowpea from the study areas. Pathogenicity test were carried out according to Koch's postulate. Fresh matured leaves and seeds from each of the plants were plucked, thoroughly rinsed in running tap water before they were air-dried in the laboratory and pounded in a mortar to facilitate extraction. Hot water extraction was obtained by infusing 40, 60, 80 and 100 g each of aloe, bitter kola, ginger and neem powder separately in 100 ml of sterile distilled water, using a 250 ml Erlenmeyer's flask in a water bath set at 100°C for 30 min. This was allowed to cool and the crude extract obtained from the infusion by filtration through 4 folds of sterile cheese cloth, to give concentrations of 40, 60, 80 and100% respectively as described by Wokocha and Okereke (2005). Each of the extract concentration was kept aseptically in 150 ml conical flasks. The contents in the flasks were exposed to U/V light for further sterilization. The linear growth was carried out on extracts of bitter-kola, neem, aloe and ginger on potato dextrose agar. Mycelia A disc of 5 mm diameter (using a sterile cork-borer) of the fungus was placed on the thin film formed on the PDA just at point of experiments had distilled water in place of plant extract respectively. The treatments and control were incubated for 5 days at room temperature (27 ± 2°C). The diameter of the radial growth of the fungi were measured at the end of incubation period and then used to determine the inhibition of each extract using the formula:

Mycelial growth inhibition (%) = [(dc-dt) / dc] × 100

Where dc = average diameter of fungal colony in the control and dt = average diameter of fungal colony in treatment group.

For the *in vivo* experiment, seeds of cowpea were sown at the rate of 3 seeds in each plastic pot containing sterilized field soil. Potted plants were randomly arranged in 3 groups and watered twice daily with tap water. Plants in the first group were drenched inoculated with the sporangia suspension (3×10^4 sporangia/ml distilled water) 2 days after *Z. officinale* (ginger) extracts were applied as soil drench, going by the method described by Amadioha (2003). Plants in the second group were inoculated with sporangia suspension 2 days before application of plant extracts, a modified method of Fernando and Linderman (1993). The 3 replicated pots per treatment and the control were set up in a completely randomized block design. The disease incidence was determined using the formula:

$$\text{Disease incidence (\%)} = \frac{No.\ of\ \text{inf}ected\ \ plants}{Total\ \ No.\ of\ \ plants}\ x\ \frac{100}{1}$$

RESULTS AND DISCUSSION

P. aphanidermatum was pathogenic as shown from the pathogenicity test. The 4 aqueous plant extracts screened *in vitro* showed varying levels of toxicity to the fungus, expressed as mean inhibition of mycelial growth. Result in Figure 1 showed that the mean inhibittion of mycelial growth was highest (100%) in plates containing *Z. officinale* extract at 40% concentration. Aloe

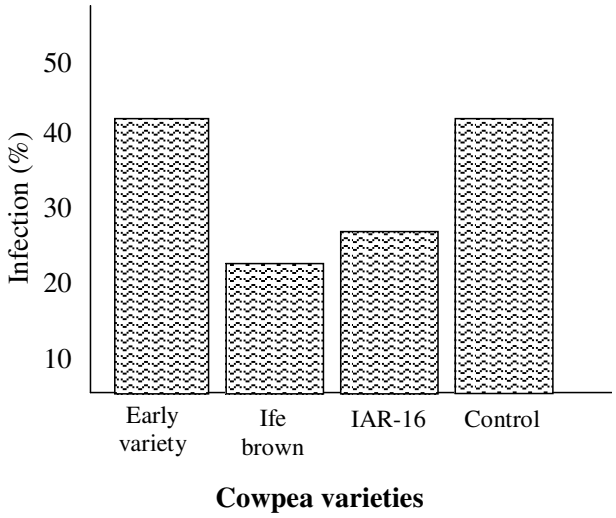

Figure 2. The *in vivo* effect of *Zingiber officinale* (ginger) extracts on cowpea root rot disease.

ranked second in fungicidal property as linear mycelial growth was completely inhibited at 60% concentration. Treatments containing *G. cola* seeds and *A. indica* leaves show mean inhibition of 92 and 77%, respectively, at 100% concentration. There was a significant difference in statistical test at ($P < 0.01$) between mycelia radial growths values recorded on the various plant extracts concentrations used compared with the control, this observation is in agreement with that of Wokocha and Okereke (2005). This suggest that there is difference in the water soluble elements in the respective leaves that are fungicidal, which do not only inhibit or retard mycelial growth but also reduces (to some extent) the incidence and severity of root rot disease of cowpea in the field (*in vivo*) as indicated in Figure 2. It was also observed that fungitoxicity of the extracts was higher at increased concentrations and hot water extractions more effective than cold-water extractions, which is in agreement with Onuh et al. (2005).

This study has revealed the potential of botanicals in the control root rot disease of cowpea in the field. The reduction of infection to 20% in Ife-brown indicates the effectiveness over the other varieties, followed by IAR-16, while the extract had little effects on the early variety. There was no difference in infection in early variety and the control experiment. It is worthy to note that despite the highest concentration (100%) of the 4 extracts applied, the above results were obtained in ginger crude extracts. Ginger crude extract drenches provided the best control, since it cannot completely replace synthetic fungicides. Nevertheless, more efforts are required in integrating the study to other related findings. Ojo and Olufolaji (2005) reported that this may go a long way in providing better alternative to the over dependency on synthetic fungicides. The use of plant products in integrated pests and fungi management could reduce over reliance on one source of agricultural chemical to the farmers, as well as cut down cost production. The plants used in the study are readily available and with easy method of extraction it can be exploited in the control of root rot disease of cowpea.

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