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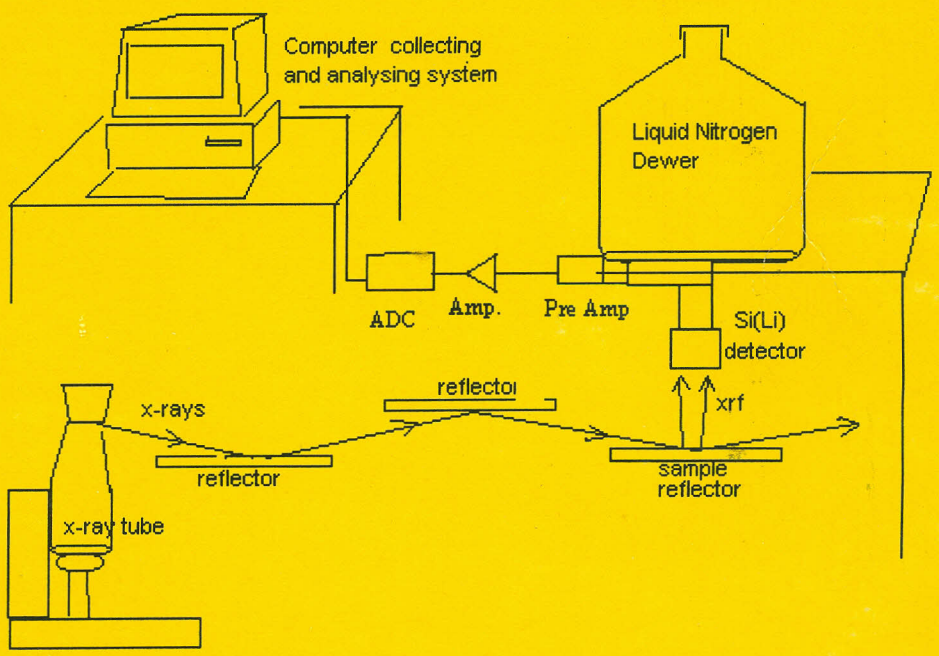

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Activity of some medicinal plant extracts against fungal plant pathogens

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Methanolic extracts of four plants traditionally used in herbal medicine were tested for antifungal properties against known plant fungal pathogens, *Mycorellusiela phaseoli, Aspergillus niger, Botryodiplodia* sp. and *Fusarium solani*. Results obtained by testing antifungal properties of the extracts on French bean, *Phaseolus vulgaris*, showed that *Azadirachta indica* (Meliaceae) (stem bark), *Ximenia caffra* (Olacaceae) (roots), *Entada abyssinica* (Leguminosae) (stem bark) and *Spilanthes mauritiana* (Compositae) (flowers), reduced fungal proliferation *in vitro*. In addition, *A. indica*, which was most active against fungal growth, reduced root-rot disease in French bean seedlings.

Key words: medicinal plants; fungi; pathogens

INTRODUCTION

Plant fungal pathogens are known to be among the agents that substantially reduce yields in food production (Jeyaranjan *et al.*, 1986). These pathogens also destroy stored foods. As a result, considerable quantities of fungicides are applied to crops and stored grain to prevent or reduce losses (Thapliyal, 1979). However, the use of fungicides on plants and stored food for disease control may have some negative impact on the environment and the consumer. For example, several fungicides are not biodegradable and may disrupt the ecosystem or end up in the food chain, thereby poisoning the consumer. The search for biodegradable fungicides is therefore urgent (Ascher, 1980). The use of biological control on plant fungal pathogens will in addition greatly benefit many developing countries whose financial resources are too low to be used for importing synthetic fungicides (Jayarajan *et al.*, 1986).

It is known that plants have played important roles in traditional medicine and in pest control (Lwande & McDowell, 1988). For example, *Ehretia amoena* Klotzsch is ground and mixed with oil and then rubbed on skin known to have a fungal infection (Chhabra *et al.*, 1984). For pest management, unique sesquiterpene dialdehydes have been isolated from *Warburgia ugandensis* Sprague and found to act as insect antifeedants (Taniguchi *et al.*, 1988). It is therefore reasonable to look for extracts of known medicinal plants that can cheaply control plant fungal pathogens and are also friendly to the environment.

The medicinal plants selected for this study are traditionally used against fungal and bacterial diseases in humans and livestock. *Azadirachta indica* A. Juss. has an insect anti-feeding compound azadirachtin (Schmutterer *et al.*, 1980) and is traditionally widely used along the east coast of Africa against several diseases. Roots of *A. indica* are specifically used for treatment of syphilis and gonorrhoea. The seed oil is used against worm infections and chronic forms of skin diseases, stomach ulcers and rheumatism. The seed paste is used as a shampoo to remove lice from the head (Schmutterer & Ascher, 1986).

Ximenia caffra Sond. (Olacaceae) is a remedy for infected eyes (Watt & Breyer-Brandwijk, 1962). *Entada abyssinica* A. Rich. (Leguminosae) is used for the treatment of *Mycobacterium tuberculosis* infections (Kokwaro, 1976) and *Spilanthes mauritiana* DC. (Compositae) is used for the treatment of candida infections (Watt & Breyer-Brandwijk, 1962). The aim of the present study was to evaluate the effect of some of these plant extracts on known plant fungal pathogens by assessing the effect of the plant extract on spore germination. Further tests were carried out using *A. indica* extract on actual diseased French bean, *Phaseolus vulgaris* L.

MATERIALS AND METHODS

Plant Materials: *A. indica* and *X. caffra* were collected from Lamu District, Coast Province. *E. abyssinica* was collected from Kakamega Forest in Kakamega District and *S. mauritiana* from Bungoma District (these geographical locations are in the Republic of Kenya). They were all authenticated by Mr. Simon Mathenge of the University of Nairobi Herbarium, Nairobi, where pressed duplicate specimen are held for reference. Plant materials were dried under shade and crushed to a powder using a crushing machine model 8 lab. mill (Christy & Norris Ltd., Chelmsford, England).

Extraction: Dried plant materials (50g) were separately soxhlet extracted with methanol for 10 hours (Chahbra *et al.*, 1982). The solvent was removed under reduced pressure below 50° C to give the crude extracts. These were further dried in a dessicator over anhydrous copper sulphate to give the dry solid for the bioassay.

Fungal Strains: The fungal strains were obtained from Kabete Campus, University of Nairobi, Nairobi, where they were isolated from the soil and maintained. The strains used were: *Mycorellusiela phaseoli, Aspergillus niger, Botryodiplodia* sp. and *Fusarium solani* fsp. *phaseoli.*

***In vitro* bioassay:** The fungi were grown on potato dextrose agar (PDA) enriched with bean extract. The bean extract was formulated by boiling beans in distilled water and this extract was used for dissolving the PDA. Sporulation of the cultures was checked by cutting mycellial disks with a 5 mm cork borer. Each mycellial disk was placed downwards in a drop of water on a slide, agitated, and the spore suspension observed under the microscope to asses the level of sporulation. Where sporulation was good, spores were harvested using 0.85% sterile saline, by gently scraping the sporemat with a sterile needle. The spore suspension was filtered through layers of muslin cloth to remove hyphal fragments (Deverell, 1981). Spore density was adjusted by use of a haemocytometer to about $1.5 \times 10^2 - 4.5 \times 10^2$ spores/ml.

Each extract (1.28g) was dissolved in N,N-dimethyl formamide (DMF) (E. Merk, Darmstadt, Germany) and then diluted with 3ml Mueller-Hinton broth (MHB) (E. Merk, Darmstadt, Germany). Dilutions of the above extract solutions were prepared and placed into test culture tubes containing MHB with 25% DMF to cover the range 3.2–0.4 g/ml (Stock solutions). 2ml of each stock solution was added to 17ml of molten potato dextrose agar (Oxoid) at 50° C, enriched with bean extract and mixed thoroughly. Immediately, 1ml of fungal spores were added. This made a final extract concentration range of 0.32–0.04 g/ml. Dithane M–45 (Twiga Chemicals, Kenya) was diluted according to manufacturers' recommendations, inoculated, and used as positive control. Negative controls had no extracts and no fungicides.

The plates were made in triplicate and incubated at room temperature (18–24° C). The time of incubation was controlled by the appearance of growth on negative control plates. The level of growth inhibition for each extract or fungicide was determined as follows:

$$\% \text{ inhibition of mycelia growth} = \frac{NT-NC}{NT} \times 100$$

where NC = Average number of fungal colonies on media with plant extract or chemical fungicide, and NT = Average number of fungal colonies on the negative control.

***In vivo* bioassay:** Black polythene bags, 15 cm in diameter were ¾ filled with soil collected from Kenyatta University Botanical Garden in Nairobi, Kenya. Two sets of ten replicate bags were prepared for the treatment, and a similar number prepared for the controls. All the soils were infected with the fungus *Fusarium solani.* The soils were kept in the greenhouse and maintained at 70% water holding capacity for one week to allow the fungus to establish. Test soils were sprayed with 12% W/V of *A. indica* extracts, dissolved in water, until most of each test soil was adequately covered (Streets, 1975). Positive control soils were similarly sprayed with 2.5g Dithane M–45 (Twiga Chemicals, Kenya)

dissolved in 1 litre of water. Negative control soils were sprayed with water only. Immediately after spraying, all the soils in each bag were planted with three seeds of French beans. They were all kept in the greenhouse, maintained at 70% water holding capacity, and examined for cases of root-rot disease after eight weeks.

RESULTS

As shown in figure 1, *A. indica* extracts had low inhibition (0–30.4%) on the growth of *M. phaseoli, A. niger* and *Botryodiplodia* sp. at concentrations of 0.04–0.24 g/ml. However, inhibition against *F. solani* was higher, up to 66% at a concentration of 0.24 g/ml. The maximum inhibition for the three fungal species, *M. phaseoli, A. niger* and *Botryodiplodia*, was 60% at 0.32 g/ml. *F. solani* was, however, completely (100%) inhibited by *A. indica* extracts at 0.28 g/ml.

Figure 2 shows the % inhibition for *X. caffra* extracts on the four fungal species tested. *X. caffra* extracts showed very low inhibition (less than 40%) in the concentration range 0.04–0.24 g/ml, rising to a maximum of 35.9% in *A. niger*. However, the % inhibition increased sharply to about 90% in the four fungal species tested as the concentration increased.

Figure 3 represents results obtained when *E. abyssinica* extracts were used. The inhibition shown by the extracts increased slowly to 46.4% at a concentration of 0.28 g/ml. It then sharply reached maximum percentages of 48.2, 60.5, 92.7 and 99 against *M. phaseoli, Botryodiplodia* sp., *F. solani* and *A. niger* respectively at 0.32 g/ml.

Results for *S. mauritiana* extracts are shown in figure 4. The level of percentage inhibition by *S. mauritiana* extracts was low, with a mean of 44.5%, and then increased gradually to a mean of 80.9% at a concentration of 0.32 g/ml.

Figure 5 shows the incidence of root-rot in French beans treated with *A. indica* extracts as a protective agent and Dithane M–45 .The results indicate that 20% of the plant population had 5% of their root tissue affected when they were protected by *A. indica* extracts. Only 7% of the population had 5% of the root tissue affected when Dithane M–45 was the protective agent. Incidentally, at one point both *A. indica* extracts and Dithane M–45 had 20% of the plant population affected by root-rot disease. In both cases the effect was up to 25% of the root tissue. The root-rot intensity was reversed when the affected tissue reached 75%. In such cases only 3% of *A. indica* protected plants were involved compared to 7% of Dithane M–45 protected plants. All the French beans in the soils sprayed with water had over 75% of the tissue affected (positive control).

DISCUSSION

The results obtained show various levels of inhibition for all the plant extracts and pathogens tested. For example *A. indica* extracts were most effective on *F. solani*, which they inhibited by 100% at a concentration of 0.28 g/ml. *E. abyssinica* extracts inhibited the growth of *A. niger* by 99% at a concentration of 0.32 g/ml. This is expected since the plant extracts contain alkaloids and coumarins (Okemo, 1996). *E. abyssinica* methanolic extracts, however, contain no alkaloids but few coumarins. Extracts of *X. caffra* and *S. mauritiana* were less inhibitory. Other research workers have reported similar antifungal variations (Shadomy *et al.*, 1985; McGinnis & Rinaldis, 1986; Pfaller *et al.*, 1989) Shadomy *et al.* (1985) and Pfaller *et al.* (1989) have further explained that differences in the effect of antifungal susceptibility will also depend on the composition of the media used, the pH, size of the fungal inoculum, and incubation temperature.

Results obtained also show that methanolic extracts of *A. indica* inhibited the growth of *F. solani* by 100% at a concentration of 0.32 g/ml. At the same concentration the it inhibited *A. niger* by 60%. *S. mauritiana*, on the other hand, inhibited *F. solani* by 82% at 0.32 g/ml and *A. niger* by 87.5% at the same concentration. Fabry *et al.* (1996) found similar activity when *A. indica* and *S. mauritiana* were tested for antifungal activity on a wide range of fungi. Such observed differences for *A. indica* extracts could suggest that the permeability for effective compounds in *A. indica* is more for *F. solani* than for *A. niger*. It could also mean that the effective compounds in the extracts affect the

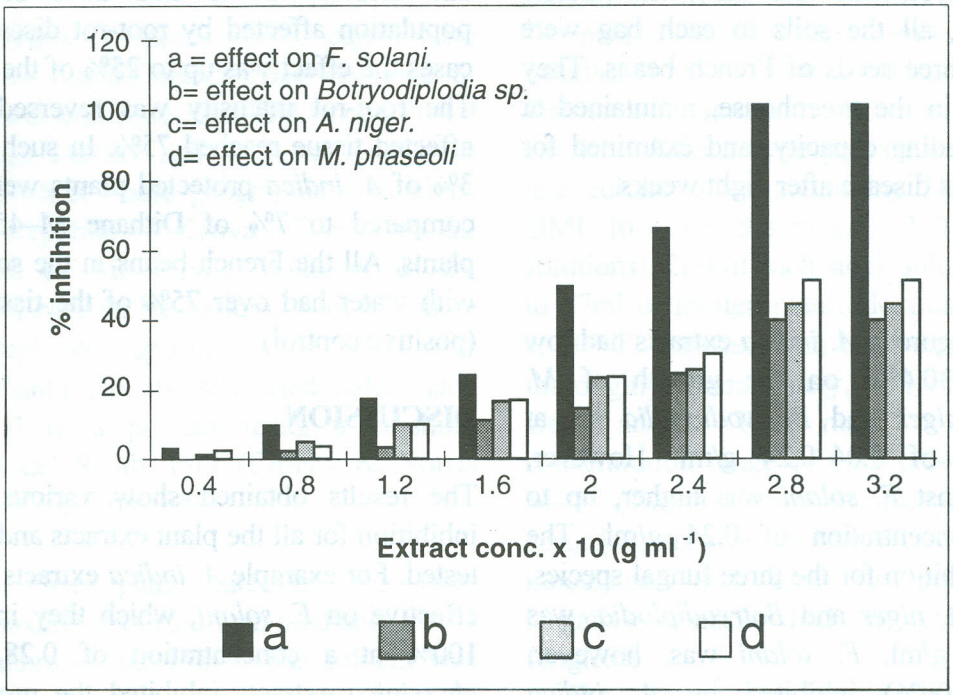

Figure 1. Effects of *A. indica* extracts on the growth of various plant fungal pathogens.

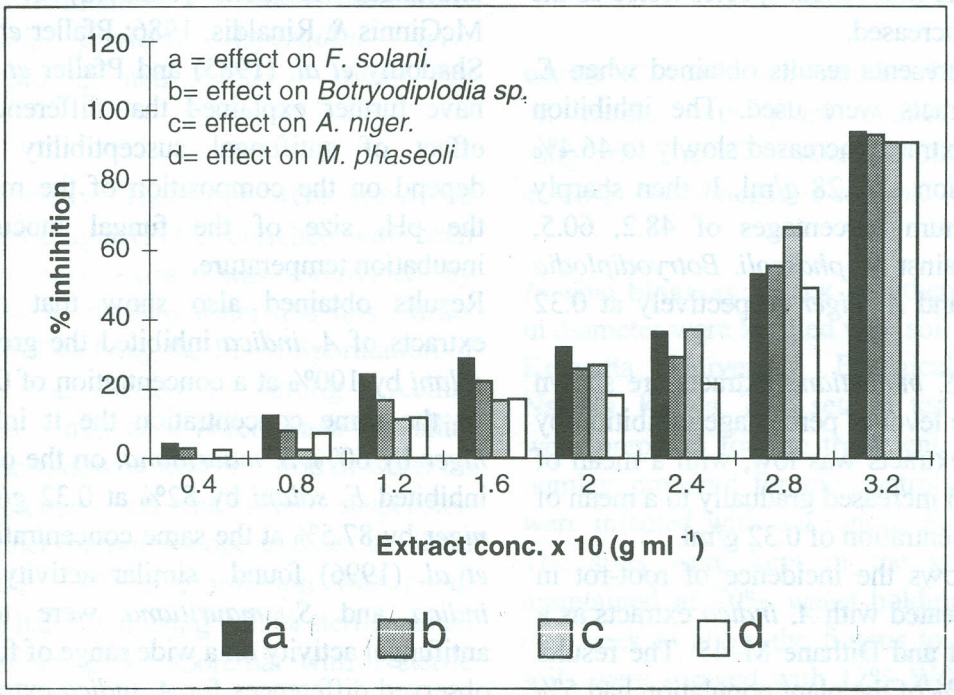

Figure 2. Effects of *X. caffra* extracts on the growth of various plant fungal pathogens.

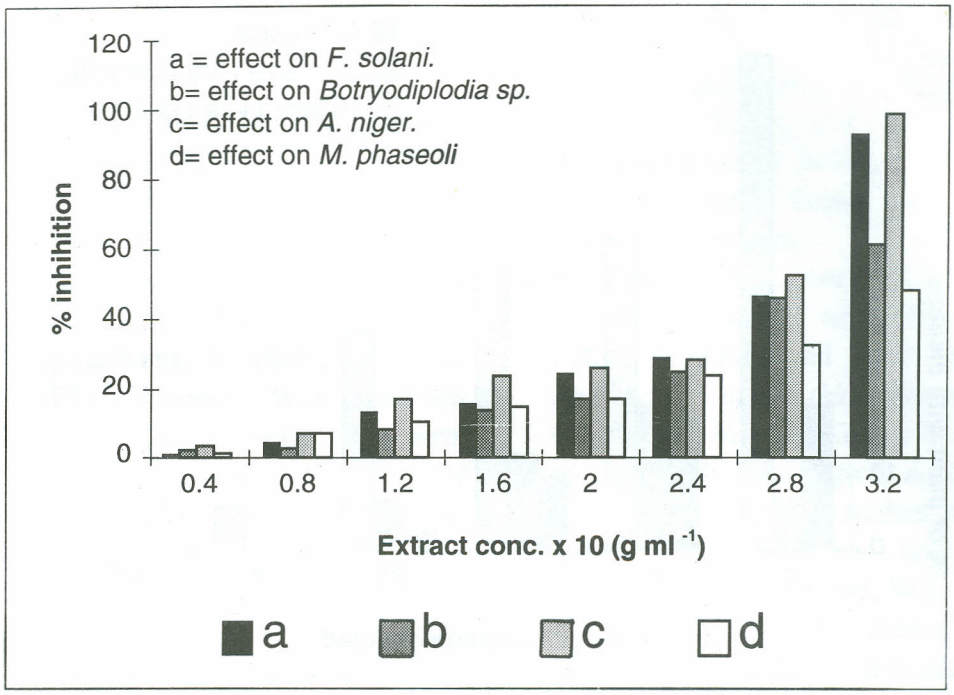

Figure 3. Effects of *E. abyssinica* extracts on the growth of various plant fungal pathogens.

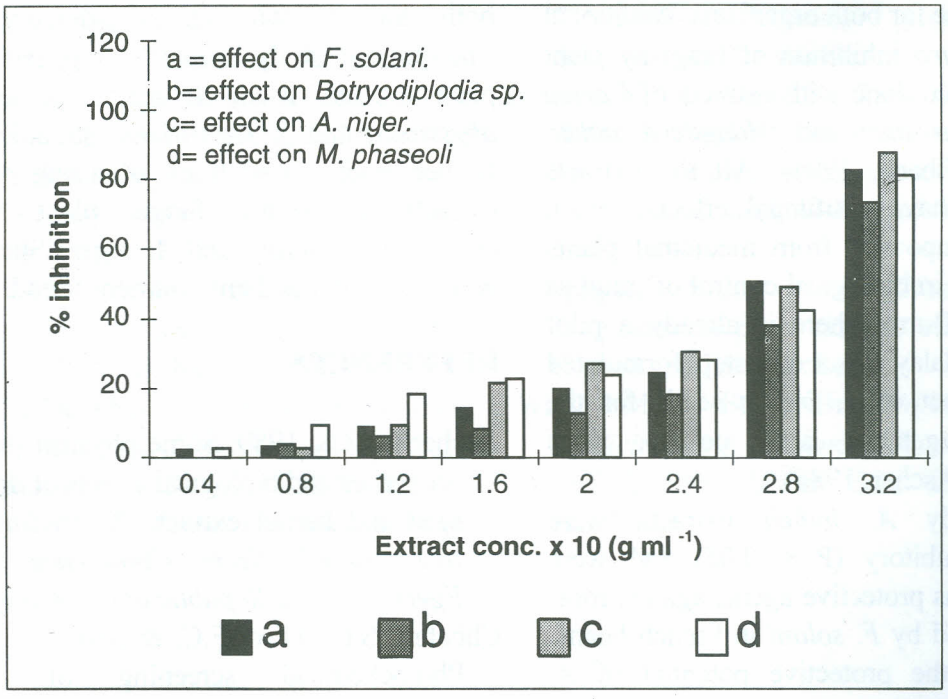

Figure 4. Effects of *S. mauritiana* extracts on the growth of various plant fungal pathogens.

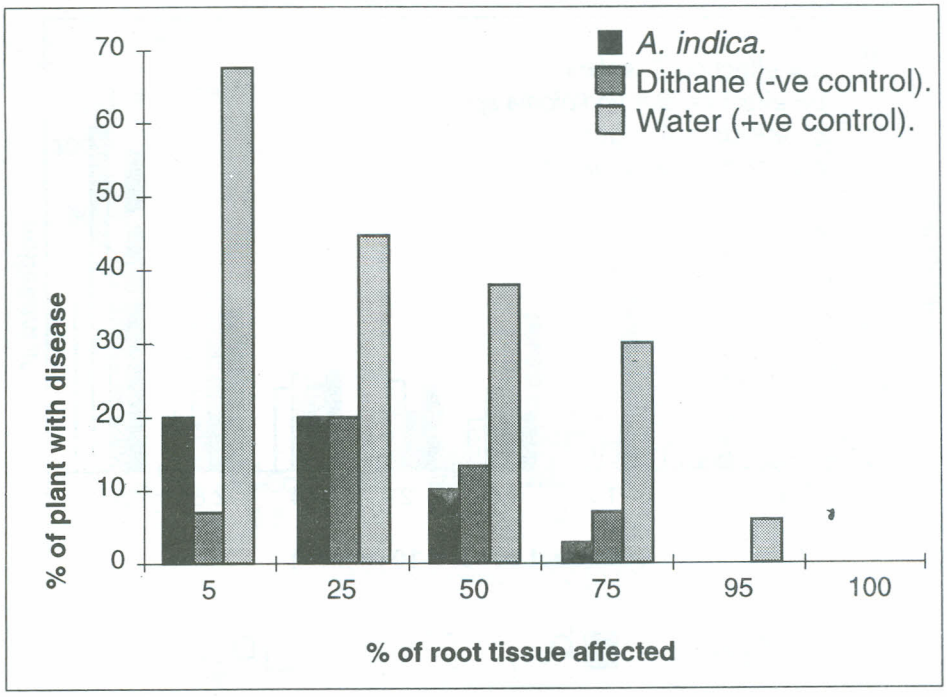

Figure 5. Effects of *A. indica* extracts and Dithane M–45 on French beans infected with *Fusarium solani*.

organisms similarly. For *S. mauritiana*, the permeability and/or effect of active compounds could be the same for both organisms. Additional studies for *in vitro* inhibition of fungi by plant extracts have been done with extracts of *Carica papay+a, Costus afer* and *Mangifera indica* (Madanagu & Ebana, 1994). All the extracts were found to have antifungal effects, which implies that compounds from medicinal plants can also be used in biological control of fungi as plant pests. In Burma there is already a pilot factory in Mandalay for a one-step formulated methanolic extract of *A. indica* seeds for the control of vegetable and peanut pests (Schmutterer & Ascher, 1986).

In this study *A. indica* extracts were significantly inhibitory ($P < 0.05$) and were therefore tested as protective agents against root-rot disease caused by *F. solani* in French beans. Comparatively, the protective potential of *A. indica* extracts against root-rot disease were found to be nearly as potent as Dithane M–45, which is commonly used as an antifungal agent in Kenya. Singh & Singh (1980) and Jayarajan *et al.* (1986) examined the effect of adding neem cake to soil that had high a incidence of Crossandra flower wilt caused by *F. solani*. In both cases the wilt was considerably reduced. The present study similarly suggests that the plant extracts used, *A. indica*, *X. caffra*, *E. abyssinica* and *S. mauritiana*, should be tested further with possibilities of using the crude extracts to control fungal plant pathogens especially *F. solani* and *A. niger*. Such control will be cheaper and environment friendly.

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