

## CROPS AND SOILS RESEARCH PAPER

# Screening of plant extracts for antifungal activities against *Colletotrichum* species of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp)

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## SUMMARY

The aim of the present investigation was to evaluate the antifungal activities of plant extracts which can be used to control bean and cowpea anthracnose. Acetone, ethyl acetate and water extracts of *Ipomoea batatas*, *Carica papaya*, *Allium sativum*, *Syzygium cordatum*, *Chlorophytum comosum* and *Agapanthus caulescens* were screened *in vitro* for their antifungal activities against *Colletotrichum lindemuthianum* and *Colletotrichum dematium* of common bean and cowpea using the agar disc infusion and microtitre double-dilution techniques. The same extracts were then tested for antifungal activity *in vivo* as seed treatments against anthracnose disease. The water extracts of *Carica* and *Syzygium* were active against *C. lindemuthianum* and had minimum inhibitory concentrations (MICs) of 1.56 mg/ml. *Syzygium*, *Allium* and *Chlorophytum* water extracts were active against *C. dematium* and MICs were 3.13, 6.25 and 12.5 mg/ml, respectively. The MICs of *Allium*, *Syzygium* and *Agapanthus* acetone extracts were 0.78, 3.13 and 6.25 mg/ml, respectively, against *C. lindemuthianum* and 0.78, 6.25 and 3.13 mg/ml against *C. dematium*. *Agapanthus* water extracts and all the acetone extracts tested *in vivo* effectively reduced the incidence and severity of bean anthracnose disease in the greenhouse. *Agapanthus* acetone, *Allium* water, and both acetone and water extracts of *Carica* and *Syzygium* performed well *in vivo* in reducing cowpea anthracnose disease and compared well with reductions due to the application of the synthetic fungicide fludioxonil + mefenoxam (the commercial product Celest<sup>®</sup> XL) applied at 25 gai/l and also with levels in the non-inoculated control. The *Agapanthus*, *Carica*, *Syzygium* and *Allium* extracts were active on both *Colletotrichum* spp. *in vitro* and also reduced anthracnose disease of bean and cowpea and are potential seed treatments in anthracnose disease control. The easy seed treatment process and the accessibility of plants used in the present study could lead to high adoption of the use of the plant extracts as seed treatments by resource-poor, smallholder farmers.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp) are two important legume crops that are commonly grown in sub-Saharan Africa for food, cash, animal feed and as soil improvers. The seeds of the two crops are a cheap source of protein for the people of this region and ease of storage leads to their preference over meat products by smallholder farmers (Msuku *et al.* 2000), who

mostly lack cooling facilities. It has been reported that the two crops also have medicinal properties (Kritzinger *et al.* 2005; Wong *et al.* 2006; Jeng *et al.* 2009). Leaf extracts of cowpea (Kritzinger *et al.* 2005) and seed extracts of both crops have antifungal activities against some phytopathogens (Ng 2004), making them a potential source of agricultural fungicides.

Despite the importance of the two crops, farmers fail to realize maximum potential yields because of several limiting factors, among them the incidence and severity of diseases (Beaver *et al.* 2003;

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Adebanjo & Bankole 2004). Anthracnose is a common disease of legumes and is caused by *Colletotrichum* spp. Common bean anthracnose is caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., whereas cowpea anthracnose is initiated by both *Colletotrichum dematium* (Fr.) Grove var. *truncata* and *C. lindemuthianum*. Anthracnose is a seed-borne disease and complete crop failure can be expected under heavy infection (Allen 1983; Pastor-Corrales & Tu 1989). The pathogen has also been reported to be associated with humans and animals, making its control imperative (Cano *et al.* 2004; Mendiratta *et al.* 2005). For example, *C. dematium* has been implicated in corneal ulcers in humans (Mendiratta *et al.* 2005).

The current major means of controlling anthracnose is the use of synthetic fungicides (Tian *et al.* 2007) through seed dressing and foliar application. However, several problems pertaining to the use of synthetic fungicides have been reported, including accumulation of harmful chemical residues in seeds, water and soil and also the induction of resistance in the pathogens (Falandysz 2000; Shovan *et al.* 2008). Therefore, there is a need for safer alternative fungicides.

A survey by Mwine *et al.* (2011) in the Masaka district, southern Uganda, reported that 34 plant species belonging to 18 families were used in traditional plant production for plant protection by smallholder farmers. Among these were *Allium sativum* (garlic) water extracts, used against field and storage pests, and *Carica papaya* (pawpaw) water extracts, used against 'blight'. In a survey involving smallholder farmers in Zimbabwe, Sibanda *et al.* (2000) found that many of the farmers had tried or heard about spraying with botanical extracts from plants such as Mexican Marigold (*Tagetes* spp.), garlic, chillies (*Capsicum frutescens* L.) and tobacco (*Nicotiana tabacum* L. and other spp.) against pathogens and pests. The present authors were unable to find any reported surveys on the use of plant extracts as seed treatments by smallholder farmers.

There are many plants that are toxic to *Colletotrichum*; however, research in this field has focused upon the anthracnose diseases of other vegetables and fruits (Raghavendra *et al.* 2006; Chowdhury *et al.* 2007; Nduagu *et al.* 2008; Mdee *et al.* 2009) and little has been done on the anthracnose of beans and cowpea (Amadioha 2003; Shovan *et al.* 2008; Akinbode & Ikotun 2008). There is a need to search for plants that have fungicidal properties against

*Colletotrichum* on legumes. The plant species *A. sativum*, *Agapanthus*, *Syzygium*, *C. papaya*, *Chlorophytum* and *Ipomoea batatas* have been reported to possess antifungal properties against other phytopathogens (Pretorius *et al.* 2002; Taiga *et al.* 2008; Tegegne *et al.* 2008).

The present work therefore aimed to (1) evaluate plant extracts for their antifungal activities against *C. dematium* and *C. lindemuthianum* that could possibly lead to their use for the control of cowpea and bean anthracnose, (2) determine the minimum inhibitory concentrations (MICs) of different plant extracts extracted by different solvents and (3) evaluate the performance of some extracts as cowpea and bean seed treatments against *C. dematium* and *C. lindemuthianum*, respectively, *in vivo* in the greenhouse.

## MATERIALS AND METHODS

### Plant material

The plant materials used in the present study were *Syzygium cordatum* Hochst.ex Krauss. (fruits from a tree in the family Myrtaceae), *Chlorophytum comosum* (Thunb.) Jacq. cvar *variegatum* (whole plant, spider plant, herbaceous, family Asparagaceae), *Agapanthus caulescens* Spreng. (whole plant, herbaceous, family Amaryllidaceae), *I. batatas* (L.) Lam. (leaves, sweet potato, family Convolvulaceae), *A. sativum* L. (bulbs, garlic, family Amaryllidaceae) and *C. papaya* L. (leaves, pawpaw, family Caricaceae). Collections of *S. cordatum*, *C. comosum* and *A. caulescens* were made from the Manie van der Schijff Botanical Garden at the University of Pretoria, Pretoria, Republic of South Africa (RSA). The sweet potato leaves were collected from the University of Pretoria's Experimental Farm, whereas the garlic bulbs were bought at the Pretoria fresh market and the pawpaw leaves were collected from Durban in Kwazulu-Natal province, RSA. Samples of the collected plants were deposited in the University of Pretoria H.G.W.J. Schweickerdt Herbarium and voucher numbers were assigned (Table 1).

### Preparation of the crude plant extracts

All the plant materials were air dried in the shade and ground to powder using a pestle and mortar. Sequential extractions were performed on 1 kg of each plant powder by soaking them in 2 litres of 27.06 moles acetone, 20.30 moles ethyl acetate and

Table 1. The quantities (g) of crude extracts harvested from 1 kg plant material using acetone, ethyl acetate and water as solvents

Plant name	Voucher no.*	Solvent		
		Acetone	Ethyl acetate	Water
<i>Agapanthus caulescens</i>	PRU96528	7	13	53
<i>Allium sativum</i>	PRU96551	14	4	414
<i>Carica papaya</i>	PRU96552	33	15	103
<i>Chlorophytum comosum</i>	PRU96518	nd	nd	nd
<i>Ipomoea batatas</i>	PRU96529	nd	nd	nd
<i>Syzygium cordatum</i>	PRU96517	20	18	125

\* Voucher number assigned by the University of Pretoria H.G.W.J. Schweickerdt Herbarium.  
nd, not determined.

sterile distilled water (24 h in each). The plants were soaked in that order contingent to the polarity of the solvents. The organic solvents were removed using a Büchi Rotavapor (Model R-200, Switzerland) and water plant filtrates were concentrated to powder using a freeze drier (Edwards High Vacuum International, Sussex, England). Different quantities of crude extracts were harvested from different solvents as shown in Table 1. All the extracts were then stored in the fridge at 4 °C.

#### Collection of anthracnose infected plants and isolation of *Collectotrichum* spp.

Anthracnose-infected common bean and cowpea plants were collected from Middelburg (25°39'S and 029°45'E), Mpumalanga province, RSA and the Department of Agriculture experimental farm (25°46'S and 31°1'E), Nelspruit, Mpumalanga, RSA, respectively. Stems and leaves were surface disinfected in 0.15 mg/ml sodium hypochlorite for 3 min and rinsed three times in sterile distilled water, and then placed either on moist blotters in Petri dishes or on potato dextrose agar (PDA) under continuous ultraviolet light for 7 days at 24±2 °C. *Collectotrichum* spp. were morphologically identified using the Common Laboratory Seed Health Testing Methods for Detecting Fungi (Mathur & Kongsdal 2003) and were subcultured and stored in a fridge (4 °C) until needed.

#### Antifungal bioassays

##### Agar infusion assay

The antifungal activity of the extracts was determined by adopting the technique described by Kritzinger

*et al.* (2005) where appropriate amounts of each stock of crude extract was added to 100 ml of PDA before pouring into Petri dishes to yield final concentrations of 0.5, 1.0, 2.5 and 5.0 mg/ml. The amended PDA was poured into 65 mm Petri dishes with preset diametrical lines drawn on the bottom plate to identify the centre of the plate. Plugs (5 ml diameter) of *C. lindemuthianum* and *C. dematium* from 7-day-old fungal cultures were placed in the centre of the Petri dishes containing PDA amended with either water extracts, acetone extracts 0.05 ml/ml (v/v), ethyl acetate extracts 0.05 ml/ml (v/v), the fungicide fludioxonil + mefenoxam (as commercial product Celest<sup>®</sup> XL (25 gai/l) Syngenta SA, Midrand). The Petri dishes were then sealed with Parafilm<sup>®</sup> and incubated at 25 °C. Acetone, ethyl acetate and unamended PDA represented the negative controls. Celest<sup>®</sup> XL represented the positive control based on previous work (Aveling *et al.* 2012). The treatments were arranged in a completely randomized design and each treatment was replicated four times. The experiment was repeated twice. Evaluation of Petri dishes was executed by measuring the diameter of growth inhibition zones after 3, 6 and 9 days of incubation (DAI). The formula of Tegege *et al.* (2008) was used to calculate the proportional inhibition of mycelial growth:

$$\text{Inhibition} = \frac{dc - dt}{dc}$$

where dc is the average diameter of fungal colony of the negative control (PDA only) and dt is the average diameter of fungal colonies grown in the presence of plant extracts or standard fungicides.

The inhibition data were arc sine transformed and then analysed statistically using the Genstat computer package (VSN International 2008). The untransformed

and transformed data led to the same conclusion, hence the untransformed analysis data have been presented.

#### Microtitre double-dilution assay

Malt extract broth was inoculated with *Colletotrichum* fungi and incubated for 5 days at 25 °C. The broth was adjusted to 0.2 optical density (thus  $1 \times 10^5$  and  $1 \times 10^6$  spores/ml for *C. lindemuthianum* and *C. dematium*, respectively) using an electrospectrometer just before inoculation.

In order to yield a series of 50 mg/ml solutions of extracts, 50 mg of each of the plant extracts were dissolved in 1000 µl of 121.7 mg/ml dimethyl sulphoxide (DMSO). The 96-well microtitre plates were used and 100 µl of the broth was added to all the wells as follows: 100 µl of stock solution (50 mg/ml) of acetone plant extracts were added to the first three wells of row A, ethyl acetate plant extracts were added to the wells 4–6 and water plant extracts were added to wells 7–9. Nutrient broth was added to well 10 as a negative (sterility) control, while Celest® XL (100 µl of 2.5 ml/ml (v/v)) was added to well 11 as a positive control. To well 12, 100 µl of 121.7 mg/ml DMSO was added as a negative control. A series of dilutions of plant extracts, Celest® XL and 121.7 mg/ml DMSO were carried out to row H.

Six-day-old malt broth cultures (100 µl) of *Colletotrichum* fungi were added to each well. The plates were divided into two sets; the first set (six plates) was inoculated with *C. lindemuthianum* and the other with *C. dematium*. The plates were then covered with lids, sealed with Parafilm® and incubated at 25 °C for 48 h. Thereafter, 40 µl of 0.2 mg/ml iodinitrotetrazolium (INT) chloride was added to all the wells, with the exception of columns 3, 6 and 9 as a growth indicator to determine the MIC values for the 18 plant extracts. The microtitre plates were incubated for 24 h at 25 °C and evaluated. The first clear wells from A to H were regarded as the MICs of extracts. The experiment was repeated three times and the final MIC for the extracts was calculated as described by Fawole *et al.* (2009).

#### In vivo antifungal test

The varieties used in the present study were the common bean variety Jenny, and IT93K5132 (cream coloured) and Pan 311 (brown coloured) cowpea varieties. Bean and cowpea seeds were soaked in sterile distilled water for 30 min. A small hole of 3 mm

was made on each seed into the cotyledon using a sterile needle. The pricked seeds were then soaked in  $1 \times 10^6$  *Colletotrichum* spore concentration made from a 10-day-old culture to which 20 drops of Tween 20 was added to yield 0.075 mg/ml Tween 20 solution. Bean and cowpea seed were soaked in *C. lindemuthianum* and *C. dematium*, respectively, for 4 h in the dark. The suspension was drained after 2 h and the seeds were placed under high humidity conditions by placing them between moist sterile paper towels in a tightly sealed container overnight, to allow fungal infection to take place. The seeds were then soaked in 150 ml of selected plant extracts diluted to 5 and 15 mg/ml for 24 h at 25 °C and kept in the dark. The inoculated seeds soaked in Celest® XL (25 gai/l) and non-inoculated seeds soaked in distilled water served as positive controls. The negative controls comprised inoculated seeds soaked in distilled water and 121.7 mg/ml DMSO, respectively. The seeds were then sown in pots 150 (diameter) × 125 mm (depth) filled with a steam-sterilized mixture of sand and garden compost (Culterra (Pty) Ltd, Pretoria) in a ratio of 1:1 (m/m) at the rate of six seeds per pot. Each treatment was replicated four times and treatments were arranged in a randomized complete design. Water was applied daily in order to maintain soil moisture at field capacity. Each experiment was repeated twice. Data collected included anthracnose disease incidence and severity. The disease incidence was calculated by the formula below:

$$\text{Disease incidence} = \frac{\text{No. of infected plants per pot}}{\text{No. of plants per pot}}$$

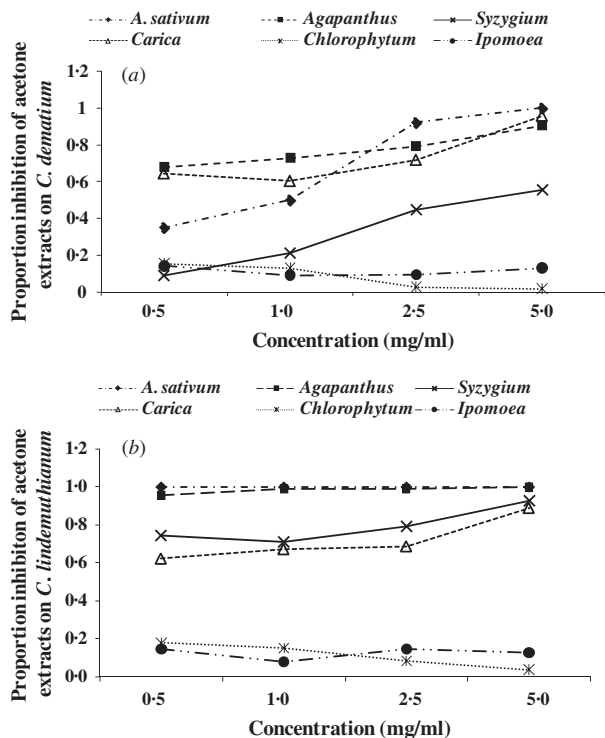
The anthracnose disease severity scale of 1–9 (CIAT 1987) was employed during the disease severity data collection. Arc sine transformation was performed on anthracnose disease severity data before statistical analysis. The Genstat Discovery statistical package (VSN International 2008) was used to analyse the transformed and untransformed disease data whose analysis was compared and gave similar results.

## RESULTS

#### Agar infusion technique

Figure 1a shows the inhibitory effect of acetone plant extracts on *C. lindemuthianum* at 6 DAI. *Allium* extract completely inhibited *C. lindemuthianum* at all concentrations and *Agapanthus* extract completely inhibited *C. lindemuthianum* at 5.0 mg/ml over the

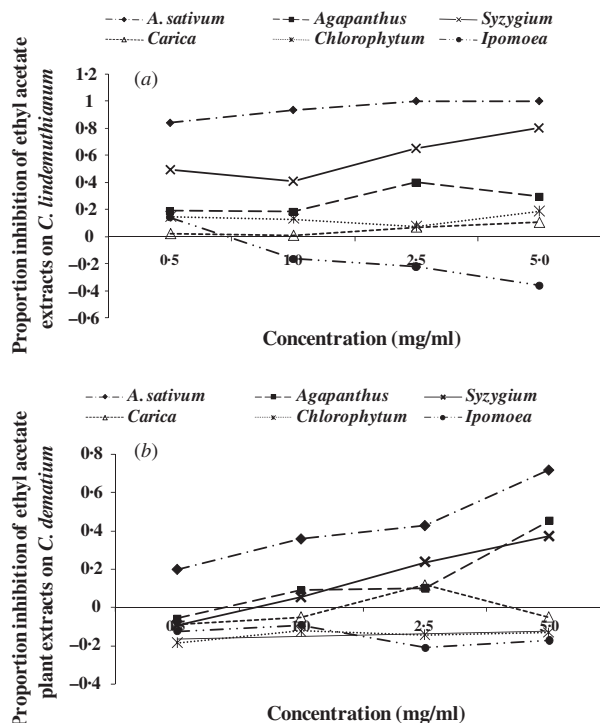




**Fig. 1.** The effect of acetone crude plant extracts on the colony growth of (a) *C. lindemuthianum* and (b) *C. dematium* at 6 days after inoculation.

control. The inhibition increased as the concentrations of all extracts increased, with the exception of *Chlorophyllum* and *Ipomoea* leaf extracts. In fact, *Chlorophyllum* extracts stimulated fungal growth as the concentrations increased. *Allium* acetone extracts completely inhibited the growth of *C. dematium* at 5.0 mg/ml only, whereas *Carica* and *Agapanthus* extracts inhibited the growth of *C. dematium* compared with the control by 96 and 90%, respectively, at the highest concentration (Fig. 1b). Likewise, in *C. lindemuthianum*, the increase in *Chlorophyllum* and *Ipomoea* extract concentrations promoted the growth of *C. dematium*.

*Allium* ethyl acetate extracts completely inhibited the growth of *C. lindemuthianum* at 2.5 and 5.0 mg/ml (Fig. 2a), whereas *Syzygium* ethyl acetate extract inhibited growth by 80% at 5.0 mg/ml compared with the control. All other ethyl acetate plant extracts (*Agapanthus*, *Carica*, *Chlorophyllum* and *Ipomoea*) failed to inhibit *C. lindemuthianum* by 50% or more at any concentration. *Allium* ethyl acetate extract inhibited growth of *C. dematium* by 87%, but the lower concentrations of extracts from this species inhibited growth by less than 50% compared with the control (Fig. 2b). Extracts of *Ipomoea* promoted the



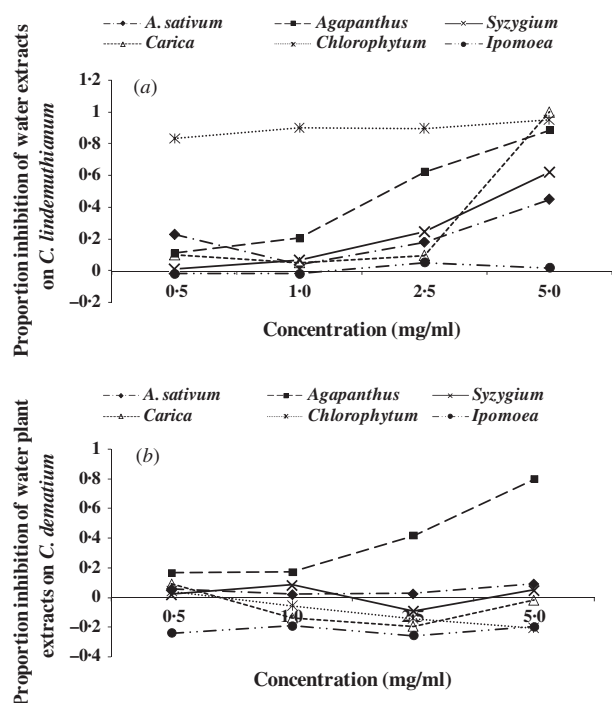
**Fig. 2.** The effect of ethyl acetate crude plant extracts on the colony growth of (a) *C. lindemuthianum* and (b) *C. dematium* at 6 days after inoculation.

growth of *C. lindemuthianum* and *C. dematium* as concentrations increased.

The *Carica* water extract completely inhibited the growth of *C. lindemuthianum* at 5.0 mg/ml followed by *Chlorophyllum* and *Agapanthus* extracts that inhibited the growth by 95 and 89%, respectively (Fig. 3a). *Agapanthus* water extracts inhibited growth of *C. dematium* by 80%, whereas the remainder of the extracts failed to reach 50% inhibition (Fig. 3b). *Ipomoea* extracts of all the concentrations, promoted growth of *C. dematium*. The *Chlorophyllum* water extracts promoted the growth of *C. dematium*.

*Microtitre double-dilution method*

Table 2 shows the MIC extracted with acetone, ethyl acetate and water extracts from different plant species. All the *Syzygium* extracts were active against *C. dematium* and *C. lindemuthianum*. *Allium* extracts were also active against *C. dematium* and *C. lindemuthianum*; however, the water extract failed to inhibit the growth of *C. lindemuthianum*. *Agapanthus* acetone and ethyl acetate extracts were active against both fungi with MICs of 1.56, 3.13 and



**Fig. 3.** The effect of water crude plant extracts on the colony growth of (a) *C. lindemuthianum* and (b) *C. dematium* at 6 days after inoculation.

6.25 mg/ml, respectively, but the range of concentrations of water extracts did not reveal a MIC. *Carica* and *Chlorophytum* water extracts were active against *C. lindemuthianum*, but their organic extracts were inactive against both *C. dematium* and *C. lindemuthianum*. All the *Ipomoea* extracts and DMSO were inactive against both *C. dematium* and *C. lindemuthianum*. Celest<sup>®</sup> XL had a MIC of 0.09 mg/ml, the highest inhibitory activity when compared with all the plant extracts.

#### In vivo antifungal test

The plants from bean seeds treated with water extracts of *Agapanthus* (5 and 15 mg/ml), acetone extracts from *Carica* (15 mg/ml) and *Syzygium* (5 mg/ml) extracts showed no anthracnose disease incidence (Fig. 4a). The above extracts and 5 mg/ml water extracts from *Allium* had low anthracnose disease incidence and compared well with the non-inoculated control and Celest<sup>®</sup> XL. All the acetone plant extracts, 5 mg/ml *Allium* and *Syzygium* water extract-treated seeds gave bean plants that had significantly ( $P \leq 0.05$ ) lower anthracnose disease severity than other water treatments and compared well with Celest<sup>®</sup> XL and the non-inoculated control (Fig. 4b).

Water extracts from *Allium*, *Carica* and *Syzygium* and acetone extracts from *Agapanthus*, *Carica* and *Syzygium* showed no or the lowest cowpea anthracnose disease incidence (Fig. 4c) and compared well with the non-inoculated control and Celest<sup>®</sup> XL. All the plant extracts and the positive controls had low cowpea anthracnose disease severity and were not significantly different ( $P \leq 0.05$ ) from each other, except for the plants from seeds treated with (15 mg/ml) *Allium* acetone extract and the negative controls (Fig. 4d).

#### DISCUSSION

Acetone and ethyl acetate extracts from *Allium* compared well with Celest<sup>®</sup> XL by completely inhibiting *C. lindemuthianum* and *C. dematium* in the agar infusion technique. The *Allium* water extract inhibited the growth of *C. dematium* in the microtitre double-dilution technique. The performance of *Allium* water extracts on *C. dematium* is similar to the findings of Shovan *et al.* (2008), where *Allium* water extracts inhibited the growth of *C. dematium*.

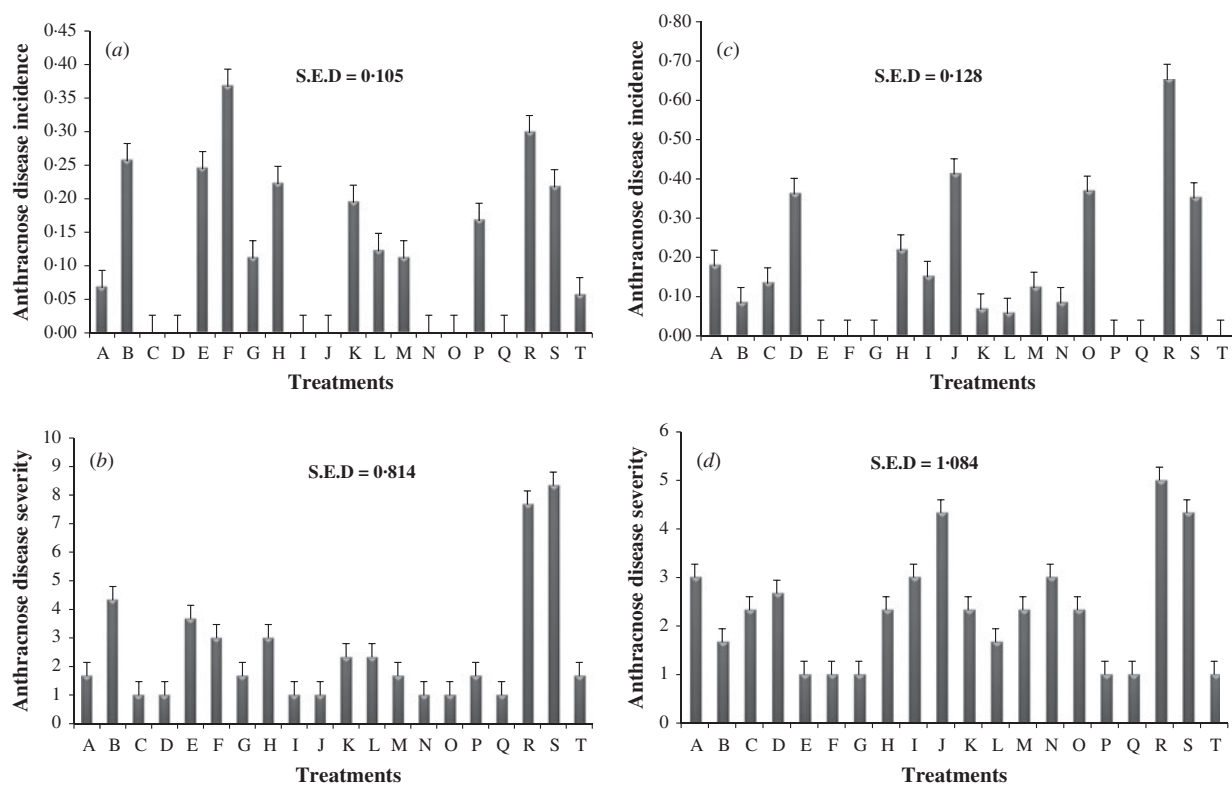
*Chlorophytum* water extracts had higher inhibition values on *C. lindemuthianum* than acetone or ethyl acetate extracts and it can be speculated that the *C. lindemuthianum* antifungal chemical in *C. comosum* is only soluble in water and not in acetone or ethyl acetate. *Chlorophytum* water extracts failed to inhibit the growth of *C. dematium*, which contrasted with the findings of Raghavendra *et al.* (2006) where aqueous extracts of *Chlorophytum borivilium* reduced the incidence of *C. dematium*. The difference in results could be due to differences in species of *Chlorophytum* used in the two studies.

*Carica* leaf water extracts at 5.0 mg/ml performed well and was comparable to Celest<sup>®</sup> XL, by completely inhibiting the growth of *C. lindemuthianum* in the agar infusion technique, but failed to inhibit the growth of *C. dematium*. The failure of *Carica* water extracts to inhibit the growth of *C. dematium* could be due to differences in chemical or genetic composition between the two *Colletotrichum* spp. used in the present study. The performance of *Carica* water extracts on *C. lindemuthianum* was superior to that of organic extracts in both *in vitro* techniques used in the present study and these results are in agreement with the findings of Anibijuwon & Udeze (2009), who reported that *Carica* leaf water extracts gave higher inhibition against bacteria than organic extracts.

Table 2. The MIC of selected plants extracts on *Colletotrichum dematium* and *Colletotrichum lindemuthianum*

Plant extracts	Solvent					
	Acetone		Ethyl acetate		Water	
	CL (mg/ml)	CD (mg/ml)	CL (mg/ml)	CD (mg/ml)	CL (mg/ml)	CD (mg/ml)
<i>Allium sativum</i>	0.78	0.78	0.78	3.13	0	6.25
<i>Agapanthus caulescens</i>	6.25	3.13	3.13	1.56	0	0
<i>Syzygium cordatum</i>	3.13	6.25	0.78	1.56	1.56	3.13
<i>Carica papaya</i>	0	0	0	0	1.56	0
<i>Chlorophytum comosum</i>	0	0	0	0	12.5	12.5
<i>Ipomoea batatas</i>	0	0	0	0	0	0
Celest <sup>®</sup> XL	0.09	0.09	0.09	0.09	0.09	0.09
DMSO	0	0	0	0	0	0

CL, *colletotrichum lindemuthianum*; CD, *colletotrichum dematium*; DMSO, dimethyl sulphoxide.



**Fig. 4.** (a) Proportion of bean seedlings affected with anthracnose: disease incidence. (b) Anthracnose disease severity of bean seedlings from seeds treated with plant extracts. (c) Proportion of cowpea seedlings affected with anthracnose: disease incidence. (d) Anthracnose disease severity of cowpea seedlings from seeds treated with plant extracts. Treatments A–H=water extracts and I–P=acetone extracts (A=5 mg/ml garlic extracts; B=15 mg/ml garlic extracts; C=5 mg/ml *Agapanthus* extracts; D=15 mg/ml *Agapanthus* extracts; E=5 mg/ml *Carica* extracts; F=15 mg/ml *Carica* extracts; G=5 mg/ml *Syzygium* extracts; H=15 mg/ml *Syzygium* extracts; I=5 mg/ml garlic extracts; J=15 mg/ml garlic extracts; K=5 mg/ml *Agapanthus* extracts; L=15 mg/ml *Agapanthus* extracts; M=5 mg/ml *Carica* extracts; N=15 mg/ml *Carica* extracts; O=5 mg/ml *Syzygium* extracts; P=15 mg/ml *Syzygium* extracts). Q=non-inoculated control; R=inoculated control; S=DMSO and T=Celest<sup>®</sup> XL.

However, *Carica* water extracts failed to maintain superiority *in vivo* by permitting a higher incidence and severity of bean anthracnose disease than other extracts, but was one of the best treatments in recording no incidence of cowpea anthracnose disease.

All *Syzygium* extracts were active against both *C. dematium* and *C. lindemuthianum* in the microtitre double-dilution experiment and the *in vivo* experiment where they had the lowest cowpea and bean anthracnose disease incidence and severity.

Generally, there was an increase in inhibition of fungal colony growth by the other plant extracts as concentrations increased and this could have been due to the increased availability of the antifungal chemicals in the media. Cao & van Bruggen (2001) reported the increase in inhibition of the growth of *Phytophthora infestans* in media when *A. sativum* extract concentrations were increased. However, the growth of *Colletotrichum* increased with concentration of *Ipomoea* leaf extracts and it can be speculated that these leaves have some constituents that are required for growth of *Colletotrichum*. Morris & Ward (1992) found that some plant chemical compounds such as the isoflavone daidzein stimulated the germination of zoospores of *Phytophthora sojae* (Kaufman and Gerdemann) and *Pythium irregulare* (Buisman) on *Glycine max* (L.) Merrill soybean (*G. max* (L.) Merrill).

Acetone plant extracts showed the highest inhibitory activity among the solvent extracts *in vitro* and also gave good results *in vivo*. Masoko *et al.* (2005) also found that acetone extracts were superior to other extraction solvents such as hexane, dichloromethane and methanol. The sequential extracting method could have a contribution towards the poor performance of ethyl acetate extracts compared with acetone extracts because most of the organic soluble chemicals in plants had already be extracted in the acetone and little remained in the residues. The water extracts gave poor results *in vitro*, but performed well *in vivo* because lower concentrations (0.5–5 mg/ml) were used *in vitro* and higher concentration (5–15 mg/ml) *in vivo*. The water extract results could be due to the solubility of compounds in water as speculated by Masoko *et al.* (2005), who stated that water fails to extract non-polar active compounds in plant materials. This explains a need for higher plant water extracts concentration to achieve fungal toxic level.

The present study has contributed an additional list of plants that can potentially be used by smallholder farmers to control anthracnose diseases of common bean and cowpea. All the plant extracts except *Carica* water extracts were effective in reducing anthracnose disease severity. The anthracnose disease incidences in some seedlings from extracts and Celest<sup>®</sup> XL treated seeds could be due to the deep-seated infections that escaped treatment. Extracts from the plant species used in the present study can be adopted easily as seed treatments by farmers because the plants from which the extracts were made can be accessed easily around their homesteads and the seed treatment process is simple to follow. The smallholder farmers can therefore use *A. sativum*, *A. caulescens* and *S. cordatum* extracts to treat their common bean and cowpea seeds by soaking to avoid the yield losses due to anthracnose disease. The use of the above-stated plant extracts by farmers can also result in the reduction of pesticide-related illnesses suffered by farmers after using the synthetic fungicides (Ngowi *et al.* 2007).

There is, however, a need to investigate further the performance of water and acetone *A. sativum*, *A. caulescens*, *C. papaya* and *S. cordatum* extracts *in vivo* as both seed treatments and foliar fungicides to control anthracnose disease of cowpea and common bean in the same way that *A. sativum* and *C. papaya* leaf extracts have been used as foliar fungicides to control pests and blight in Uganda (Mwine *et al.* 2011).

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