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The evaluation of six fungicides for reducing *Alternaria cassiae* on cowpea seed

Noëlani van den Berg[a,b,*], T.A.S. Aveling[a], S.L. Venter[b]

[a] *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa*

[b] *ARC-Roodeplaat, Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria 0001, South Africa*

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Abstract

Alternaria cassiae (Juriar and Khan) was found to be a destructive seed-borne, foliar pathogen of cowpea (*Vigna unguiculata* (L.) Walp) in Southern Africa. Six fungicides at three concentrations were evaluated for their efficacy in reducing the pathogen in culture and on seed. The fungicides included benomyl, bitertanol, captab (captan), mancozeb, propiconazole and triforine. An untreated control was included for comparison. Seeds were artificially inoculated with *A. cassiae*, treated with the different fungicides and percentage germination and infection was determined in vivo. Percentage emergence and disease incidence were also determined in greenhouse experiments. None of the treatments eradicated *A. cassiae* from cowpea seeds. Captab at 30 g/101 (1.5 × the recommended rate) proved to be the best treatment for reducing the pathogen.

Keywords: *Alternaria cassiae*; Cowpea; Seed treatment

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an important food and fodder crop and is increasingly being cultivated by small-scale farmers in South Africa (Van den Heever et al., 1996). Cowpea seeds are particularly rich in protein and fulfil in many of the dietary needs of rural people (Quass, 1995). However, there are several reports of seed-borne fungi associated with cowpea diseases (Onesirosan, 1975; Sinha and Khare, 1977; Emechebe, 1980). Therefore, the need arises for research on diseases of cowpea, especially those transmitted via seed and those resulting in significant yield losses. Field surveys of cowpea growing areas in Southern Africa revealed the presence of a destructive foliar disease. *Alternaria cassiae* (Juriar and Khan) was reported as the causal pathogen of this new disease on cowpea (La Grange and Aveling, 1998). The pathogen has previously been recorded on *Cassia* spp., *Rhynchosia* sp. and *Crotolaria* sp. (Juriar and Khan, 1960; Walker and Boyette, 1985; David, 1991).

Alternaria spp., in general, are seed-borne (Neergaard, 1977). *Alternaria porri* (Ellis) Ciferri is seed-borne in onion (Neergaard, 1945; Simmons, 1967). Singh et al. (1977) found that *A. tenuis* Nees is present in all layers of the pericarp and also invades the endosperm and embryo of sunflower seeds. Maude and Humpherson-Jones (1980) reported that *Alternaria brassicicola* (Schwein.) Wiltshire was present on brassica seed and *A. sesamicola* Kawamura on sesame seed. To date little research has been done in South Africa to evaluate seed treatments for the control of seed-borne diseases of cowpea. Smith et al. (1999) evaluated eight chemical fungicides and one biocontrol method for the treatment of *Colletotrichum dematium* (Pers) Grove on cowpea. The objectives of this study were to determine whether *A. cassiae* is seed-borne in cowpea, to test various chemical fungicide treatments for control of *A. cassiae* in vitro and in vivo, and to evaluate the most promising treatments in the greenhouse.

*Corresponding author. Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa. Tel.: +27-12-420-3852; fax: +27-12-420-3960.

E-mail address: noelani.vdberg@fabi.up.ac.za (N. van den Berg).

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2. Materials and methods

2.1. Standard agar seed health test

The standard agar seed health test according to the rules of the International Seed Testing Association (ISTA) (1999) was used to determine whether *A. cassiae* is seed-borne in cowpea. Two cowpea cultivars (Rhino obtained from Agricultural Research Council, Grain Crop Institute and IT82D889 obtained from International Institute for Tropical Agriculture) were evaluated. Four replicates of 100 seeds of each cultivar were surface sterilized in 1% (w/v) sodium hypochlorite for 2 min. Seeds were plated out (10 per Petri dish) onto cornmeal agar (CMA) amended with 0.025% chloramphenicol and incubated at 25°C under a 12 h UV-light/12 h dark regime for 7 days. The seeds were examined for fungal growth using a Nikon SMZ 645 stereomicroscope. The species identification of *A. cassiae* was confirmed by staining conidia with lactophenol blue and viewed with a Zeiss Standard 20 light microscope. Conidia were compared with the isolate PPRI 6393 obtained from the National Collection of Fungi, Plant Protection Research Institute, Pretoria. The cultivar with the highest infection of *A. cassiae*, IT82D889, was used for subsequent experiments.

2.2. Fungicide treatments

Fungicides were used at the registered rates in the in vitro and in vivo experiments. They are listed by common name and active ingredient (the trade name, manufacturer and recommended dosage are given in parentheses). The fungicides were: benomyl WP, 500 g a.i./kg (Benlate, du Pont, Halfway House, RSA—5 g/10 l); captab 80% WG, 500 g a.i./l. (Captan, Kombat, Greytown, RSA—20 g/10 l); propiconazole, 500 g a.i./l (Novel, Novartis, Isando, RSA—2.5 ml/10 l); mancozeb, 800 g a.i./kg (Dithane M45, Starke Ayres, RSA—20 g/10 l); bitertanol, 300 g a.i./l (Baycor, Bayer, Isando, RSA—4 ml/10 l) and triforine, 190 g a.i./l (Denarin, Cyanamid, RSA—15 ml/10 l). The above rates were also used as standard rates per litre of medium in the in vitro experiment.

2.3. In vitro experiment

An isolate of *A. cassiae* (PPRI 6393) was maintained on V8-agar at 25°C in a 12 h UV-light/12 h dark cycle. The six test fungicides, at 0.5×, 1× and 1.5× the registered rate, were each incorporated into CMA after autoclaving. Discs (7 mm diameter) from actively growing cultures of the isolate were transferred aseptically to the centre of amended plates. Control plates containing no fungicides were included in the experiment. Four replicate plates of each treatment were incubated at 25°C in 12 h UV-light/12 h dark regime. Colony diameter was measured after 5 and 8 days. Data were analysed using the analysis of variance test, and significant differences were determined using the Student's T-test at $P = 0.05$.

2.4. In vivo experiment

Eleven samples of 100 g of cowpea seed (IT82D889) were artificially inoculated with *A. cassiae* by slurry coating with a 10^5 conidia/ml water spore suspension and left to air dry in a laminar flow cabinet. Based on the results obtained from the in vitro experiment the 10 best fungicide treatments at the tested concentrations were used in this experiment. All three test concentrations of mancozeb and propiconazole, 1.0× and 1.5× triforine, 1.5× bitertanol and 1.5× captab were used. Each of the chemicals was suspended in 5 ml sterile distilled water and applied as a slurry to the seed samples. The control was also inoculated with *A. cassiae* as previously described and treated with 5 ml sterile distilled water.

2.4.1. Seed germination assays

The effect of the different treatments on percentage germination was determined. Four replicates of 100 treated seeds from each chemical treatment were placed on moist Whatman No.1 blotter paper in Petri dishes, sealed with parafilm, and incubated at 25°C in a 12 h UV-light/12 h dark regime. Percentage germination was rated on days 5 and 8. Data was analysed as previously described.

2.4.2. Agar seed health test

The standard agar seed health test was conducted (ISTA, 1999). Four replicates of 100 seeds from each of the previously described treatments were used. Seeds were plated onto CMA supplemented with 0.025% chloramphenicol (10 seeds/plate). Plates were incubated at 25°C in a 12 h UV-light/12 h dark regime. Seeds were observed under a dissecting microscope for the presence of fungal growth.

2.4.3. Greenhouse trial

Based on the results of the in vivo seed germination and agar seed health tests, three replicates of 20 seeds of the eight best treatments used in the in vivo experiments were planted in seedling trays containing a peat-based growing medium. Plants were maintained in a glasshouse (18/25°C (±1°C) night/day temperature) and watered daily. The treatments were; bitertanol 1.5×, captab 1.5×, mancozeb 1.0× and 1.5×, propiconazole 1.0× and 1.5× and triforine 1.0× and 1.5×. The control sample was treated with sterile distilled water. Seven weeks after planting, percentage emergence, shoot and root length and percentage abnormality

(abnormalities such as chlorosis, deformed roots, stems or leaves and stunted growth) and disease incidence of seedlings was determined. Experimental layout in the greenhouse was a randomised block design, which was repeated once. Data was analysed as previously described.

3. Results

3.1. Standard agar seed health test

A. cassiae was found to be seed-borne on Rhino and IT82D889 cowpea seeds, with respective infection percentages of 6% and 14%. As IT82D889 cultivar seeds had the highest infection of *A. cassiae,* this cultivar was used for further experiments.

3.2. In vitro experiment

All fungicides except benomyl at 0.5 ×, 1 × and 1.5 × significantly inhibited mycelial growth of *A. cassiae* in culture (Table 1). All the plates amended with benomyl showed a significantly higher rate of fungal growth than the control plates (Table 1). Mancozeb and propiconazole at all concentrations tested, totally inhibited mycelial growth. Triforine at 1 × and 1.5 × the registered rate was also effective in inhibiting the growth of the fungus, a very slight growth of 0.5 and 0.25 mm, respectively, was measured (Table 1).

Table 1
Colony diameter of *Alternaria cassiae* grown on cornmeal agar amended with various fungicides

Treatment	Diameter of colonies (mm)
Control	28.25[a]g
Benomyl 0.5 ×	34.0i
Benomyl 1 ×	31.25h
Benomyl 1.5 ×	31.5h
Bitertanol 0.5 ×	7.63ef
Bitertanol 1 ×	4.5c
Bitertanol 1.5 ×	2.5b
Captab 0.5 ×	8.5f
Captab 1 ×	6.25de
Captab 1.5 ×	2.5b
Mancozeb 0.5 ×	0a
Mancozeb 1 ×	0a
Mancozeb 1.5 ×	0a
Propiconazole 0.5 ×	0a
Propiconazole 1 ×	0a
Propiconazole 1.5 ×	0a
Triforine 0.5 ×	5.13cd
Triforine 1 ×	0.5a
Triforine 1.5 ×	0.25a
LSD	1.585

[a]Each value is the mean of four plates measured after 8 days' growth. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to the Student T-test.

Table 2
Percentage germination and infection of cowpea seed by *Alternaria cassiae* treated with various fungicides

Treatment	Germination (%)	*A. cassiae* infection (%)
Control	66[a]b	42e
Bitertanol 1.5 ×	47a	5a
Captab 1.5 ×	62b	7ab
Mancozeb 0.5 ×	64b	19cd
Mancozeb 1.0 ×	57ab	14abc
Mancozeb 1.5 ×	72b	7ab
Propiconazole 0.5 ×	62b	24d
Propiconazole 1.0 ×	67b	15bcd
Propiconazole 1.5 ×	69b	8ab
Triforine 1.0 ×	61ab	13abc
Triforine 1.5 ×	61ab	5a
LSD	14.15	9.979

[a]Each value is the mean of 4 replications of 100 seeds. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to the Student T-test.

3.3. In vivo experiments

3.3.1. Seed germination assays

Only bitertanol at 1.5 × the recommended dosage significantly reduced percentage germination of cowpea seed (Table 2).

3.3.2. Agar seed health test

All the fungicides tested significantly decreased the percentage of *A. cassiae* infection of cowpea seeds artificially inoculated with the pathogen (Table 2). Bitertanol 1.5 ×, captab 1.5 ×, mancozeb 1.5 ×, propiconazole 1.5 × and triforine 1.5 × were most effective in reducing the incidence of *A. cassiae* (Table 2).

3.3.3. Greenhouse trial

All treatments except bitertanol 1.5 ×, captab 1.5 × and mancozeb 1.0 ×, significantly decreased the percentage emergence (Table 3). None of the treatments except bitertanol 1.5 × showed a difference in shoot and root length (Table 3). Propiconazole 1.5 × showed a significant decrease in root length (Table 3). Bitertanol 1.5 ×, mancozeb 1.0 × and triforine 1.5 × showed a significantly higher percentage abnormality. Percentage disease incidence was significantly reduced by all treatments, except triforine 1.0 × and 1.5 × (Table 3).

4. Discussion

A. cassiae was found to be seed-borne in cowpea seeds. Thirty-six seed borne *Alternaria* spp. have been listed (Neergaard, 1977) and according to Rotem (1994), this might suggest that practically all *Alternaria* spp. pathogenic to foliage, also infect seeds.

Table 3
Effect of six fungicide treatments of cowpea seed on percentage emergence, shoot and root length, percentage abnormality and diseased seedlings

Treatment	Emergence (%)	Shoot length (mm)	Root length (mm)	Abnormality (%)	Disease (%)
Control	75.00[a]c	305.3bc	106.3c	10.33a	33.67b
Bitertanol 1.5 ×	64.67bc	253.3a	88.3ab	30.00c	16.67a
Captab 1.5 ×	69.00bc	310.0c	106.0c	15.00ab	18.00a
Mancozeb 1.0 ×	69.00bc	275.7abc	98.67bc	24.33bc	18.00a
Mancozeb 1.5 ×	54.33a	274.3abc	98.33abc	15.00ab	12.50a
Propiconazole 1.0 ×	50.00a	280.7abc	93.33abc	11.5ab	15.67a
Propiconazole 1.5 ×	58.67ab	264.3ab	82.33a	21.00abc	15.00a
Triforine 1.0 ×	56.33ab	278.3abc	92.33abc	21.00abc	21.67ab
Triforine 1.5 ×	50.00a	279.7abc	93.33abc	24.00bc	21.33ab
F-value	4.358	1.471	2.209	2.733	2.231

[a] Each value is the mean of 3 replications of 20 seeds. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to the Student *T*-test.

According to Neergaard (1977), effective seed treatments must eliminate pathogens without being toxic to seeds. All the fungicides tested in the in vitro study, except benomyl were effective in inhibiting mycelial growth of *A. cassiae* in culture. These results correlate with those of Smith et al. (1999) who reported that benomyl was not very effective in inhibiting growth of *Colletotrichum dematium* also isolated from cowpea when tested in vitro. However, Aveling et al. (1993) reported that benomyl at 1.0 × and 1.5 × the recommended rate significantly inhibited the mycelial growth of *A. porri*. According to Sivapalan (1993), benomyl significantly reduced the germination of conidia of *A. brassicicola* in vitro, when compared to the control. Benomyl is a systemic benzimidazole-based fungicide effective against a large number of fungal pathogens (Maude, 1978); however, this group of fungicides has no effect on some of the dark-spored imperfect fungi, such as *Helminthosporium* spp., *Phoma* spp. and *Alternaria* spp. (Maude, 1978; Agrios, 1988). Mancozeb and propiconazole were most effective in preventing fungal growth of *A. cassiae* in vitro. Sivapalan (1993) reported a significant reduction in the germination of conidia of *A. brassicicola* treated in vitro with mancozeb.

Bitertanol, captab, mancozeb, propiconazole and triforine at the highest concentration were all effective in reducing the percentage infection of seeds artificially inoculated with *A. cassiae*. Captab and mancozeb are protective, non-systemic fungicides and thus prevent fungal growth on the external surface of seeds thereby inhibiting further infection of the seed (Smith et al., 1999). Bitertanol and propiconazole are systemic fungicides and are often applied as seed or soil treatments. According to Agrios (1988) both bitertanol and propiconazole show long protective and curative activity against the imperfect fungi, such as *Alternaria* spp. Triforine is a systemic fungicide and is effective against many ascomycetous and imperfect fungi. In South Africa mancozeb, captab (captan) and triforine are some of the fungicides generally recommended for the control of alternaria diseases on vegetables, fruits and flowers (Nel et al., 1999).

None of the fungicides eradicated *A. cassiae* from cowpea seed in the in vitro experiment indicating that they are possibly not fungitoxic to the pathogens or did not penetrate the seed tissue to kill internal mycelium.

In the greenhouse, seeds treated with mancozeb showed the lowest disease incidence. However a significant increase in abnormality and a decrease in emergence were also recorded. Sivapalan (1993) reported that broccoli seed artificially inoculated with *A. brassicicola* showed a higher percentage emergence and a lower percentage fungal infection when treated with mancozeb.

Captab at the highest concentration was the best treatment overall. According to Wu et al. (1979) captan at 500 ppm was able to completely inhibit the germination and sporulation of *A. brassicicola*. Captab also showed promising results when tested for the control of *C. dematium* on cowpea (Smith et al., 1999). Captab has been commercially used as a seed and soil treatment since the 1950s (Jacks, 1951) and has remained in the market since then. In South Africa, captab (captan) is registered on many vegetables and fruits, but it is, as yet, not registered on cowpea. This fungicide looks promising as a seed treatment for cowpea, but will need to be tested under field conditions.

Currently, there are no fungicides registered as seed treatments for cowpea in South Africa. However, a major role of seed treatments is the disinfection of seeds, thus preventing the local and international spread of infected seed samples. The results presented in this study provide valuable data, which may assist in the registration and development of a seed treatment for cowpea.

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