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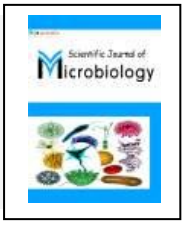

Original article

Host range and control of *Cryptosporiopsis spp*, a cashew blight pathogen

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ABSTRACT

Ten fungicides and three adjuvants were evaluated on spore germination and mycelial growth of *Cryptosporiopsis spp* isolated from cashew (*Anacardium occidentale*) *in vitro*. Effects of selected pesticides and adjuvants were determined using food poisoning technique and conidial germination assays as indicators of sensitivity. The pesticides were tested according to labeled rates. Among the pesticides tested, Chlorothalonil (720g/L) inhibited *Cryptosporiopsis spp* mycelial growth and conidial germination. Tebuconazole (0.75 lbs), Tebuconazole (4.5%) + sulphur (70%), and Triadimefon 25% + Metalaxyl 20% reduced mycelial growth but did not reduce conidial germination, while sulphur had no adverse effect on either the mycelial growth or conidial germination of *Cryptosporiopsis spp*. Mancozeb (80%), Mancozeb 480g/kg + Metalaxyl 100g/kg, Mancozeb 680g + Metalaxyl 40g/kg and Picoxystrobin (250 g/l) inhibited conidial germination but did not reduce mycelial growth. Among the adjuvants, potassium chloride inhibited both spore germination and mycelial growth. Long-term exposure of the fungus to the pesticides by growing it on malt extract agar amended with pesticide resulted in inhibited mycelial growth. A study was carried out to determine the host range of *Cryptosporiopsis* spp. The fungus which causes cashew leaf and nut blight infects young tender shoots, pseudo-fruits and nuts. Host range studies of *Cryptosporiopsis* spp were conducted on potted plants in a glasshouse using non-target

plant species belonging to nine plant families. The tested species, in addition to sorghum, comprised crop species reported to be intercropped in cashew farms as well as economically important cultivated crops. Five of the tested non-target plant species were immune; none developed any symptoms of infection when inoculated with Cryptosporiopsis spp. The test plants were mango (Mangifera indica L), cassava (Manihot esculenta Crantz), and sorghum (Sorghum bicolor L.) Moench), pigeon pea (Cajanus cajan (L.) Millsp), sweet potato (Ipomoea batatas (L.) Lam), lemon (Citrus limonum Risso), mung bean (Vigna radiata (L.) Wilczek) and Eucalpytus spp. Artificial inoculation of the test plants with spore and mycelial suspension of Cryptosporiopsis spp showed that leaf symptoms were produced in sorghum, mung bean, sweet potato, eucalyptus and cassava. Blight symptoms caused massive damage on the foliage of affected plants that caused wilting and eventual death of inoculated plants. The severity of symptoms varied in the different plants tested in the study.

1. Introduction

Cashew, *Anacardium occidentale L.*, belongs to the *family* Anacardiaceae. It is widely cultivated in the coastal zone, a great increase in the area of cashew production is occurring in semi-arid conditions, due to favourable conditions for higher quality of both apples and nuts (Emilson *et al.,* 2006). The important part of the crop is nuts and apples that are regarded as a healthy source of protein and vitamins and are being consumed in increasing quantities in both developed and developing countries. Three main cashew products are traded on the international market - raw nuts, cashew kernels and cashew nut shell liquid. A fourth product - the cashew apple is generally processed and consumed locally. The cashew industry ranks third in the world production of edible nuts. India and Brazil are the major cashew exporters, with 60 percent and 31 percent respectively of world market share (FAOSTAT, 2012). The major importers are the United States (55 percent), the Netherlands (ten percent), Germany (seven percent, Japan (five percent) and the United Kingdom (five percent). Some reported diseases of cashew are powdery mildew caused by *Oidium anacardii* Noak (Waller *et al*. 1992), cashew gummosis, caused by *Lasiodiplodia theobromae* (Emilson *et al.,* 2006), Inflorescence dieback caused by *Lasiodiplodia theobromae* (Adeniyi *et al.,* 2011) and cashew blight caused by *Cryptosporiopsis spp* (Sijaona *et al.,* 2006). In East Africa, yield loss of 80-100% has been attributed to fungal diseases and this affects the income of farmers in terms of foreign exchange. *Cryptosporiopsis* spp fungus was first described by Sijaona *et al.,* (2006) and has been the focus of much research in recent years because it is the causal agent of cashew blight. This disease, which is now spreading in cashew growing areas in East Africa, may cause losses in cashew yield of upto 48.4% (ACRR, 2006) and therefore render the growing of cashew uneconomic. It was first recorded in Tanzania in 2003 (Sijaona et al., 2006) and since then outbreaks have been detected in cashew growing areas. It is considered as one of the most important cashew pathogen in the humid areas of the world. In the Southern Tanzania were temperatures are warmer, leaf and nut blight has been reported (Sijaona et al., 2006) and the pathogen causes significant yield losses. High temperature and high relative humidity favour the outbreak of the disease. Plants with blight produces fewer nuts and of low quality. Under favourable conditions, flower panicles and stems may be affected, causing nut shrivelling. Due to this destructive pathogen, the yield loss was estimated at 48.4% in Tanzania (ACRR, 2006).

In vitro studies have demonstrated that some fungicides restrict or prevent the growth of fungal pathogens (camilla *et. al.,* 2006). Chemical control measures have been tested and found effective in the control of diseases (Ogundana and Denis, 1981; Plumbley, 1985). Cashew leaf and nut pathogen was first described from Tanzania by Sijaona *et al.,* 2006 and elaborated by Global Plant Clinic (GPC). *Cryptosporiopsis* spp agent causing cashew blight has been isolated from leaf and nut parts in Tanzania and the Caribbean. In the literature there are no reports

about the influence of fungicides on mycelial growth of *Cryptosporiopsis spp* from Cashew plant. In view of the importance of the crop and the effect of fungal diseases on the yield, there is a need to identify management options for this new disease associated with this important crop. The aim of the study was to determine in vitro effects of selected fungicides on spore germination and mycelia growth on the mycelial growth of *Cryptosporiopsis spp* isolated from cashew. The presence of other hosts plays an important role in disease epidemic. The primary inoculum of *Cryptosporiopsis* spp comes from several sources such as weed hosts, soil, crop debris which enhances the disease level. Cashew blight's host range is important because it is the means by which it classified. There are no consistent morphological distinctions between *Cryptosporiopsis* spp infecting cashew and other species. The aim of the work described here was to determine the host range of *Cryptosporiopsis* spp in cashew growing areas, especially in relation to major arable crops, and examine the host specificity of the pathogen and to ascertain its epidemiology.

2. Materials and methods

2.1. Source of isolate

Cryptosporiopsis spp isolate used in this study was originally isolated from diseased cashew plants collected in Naliendele, Mtwara. Mycelial plugs and conidia for the experiments were obtained from 10 day-old *Cryptosporiopsis spp* cultures grown on Potato dextrose agar. The pesticides used in this study were fungicides Tebuconazole, Mancozeb, Metalaxyl, Chlorothalonil, Potassium chloride, Picoxystrobin, Triadimefan and Sulphur. The bases for selection were (1) recommended for use in crops infected with blight, (2) use in areas adjacent to cashew fields, or (3) to compare the effect of different chemical classes/active ingredients to *Cryptosporiopsis spp.*

2.2. Effect of pesticides and adjuvants on conidial germination

To determine the effect of the pesticides on conidial germination, conidia of *Cryptosporiopsis spp* harvested from 10-day-old cultures on Malt extract agar were mixed with sterile water amended with the various pesticides. Pesticide concentrations used in these assays were used in accordance with the recommended rates (Table 1). Inhibition of spore germination technique was adopted. Single drop of conidial suspension of *Cryptosporiopsis spp* was added to the well of cavity slides, to which a single drop of double the concentration of different fungicides was added to get the required concentrations. Later cover slip was placed on the cavity slide. The periphery of the cavity was smeared with vaseline to prevent contamination and evaporation of water. Each concentration was replicated thrice in a separate cavity slide. A control treatment was maintained with distilled water. These cavity slides were kept in the Petridishes lined with moist blotting paper and were incubated at room temperature ($27\pm1^{o}C$). After 24 h, observations were taken in ten microscopic fields for each slide and the total numbers of spores germinated in each microscopic field were recorded and per cent germination was calculated. Further, the per cent inhibition of spores was calculated by using formula given by Vincent (1947). Per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947);

$I = 100(C-T)/C$

Where, I = Inhibition percentage,

C = Growth in control (check),

T = Growth in treatment

2.3. Effect of pesticides and adjuvant on mycelial growth

To determine the effect of the pesticides on mycelial growth, 90 mm petri dishes containing 15 ml of MEA per plate were prepared, with and without pesticides (control). The pesticides tested and their rates are listed in Table 1.

Fungicides and adjuvants viz., Tebuconazole, Mancozeb, Metalaxyl, Chlorothalonil, Potassium chloride, Picoxystrobin, Triadimefan, sodium hydroxide, sodium hypochlorite (NaOCl) and Sulphur were evaluated for their efficacy on mycelial growth of *Cryptosporiopsis spp* by food poisoning technique (Nene, 1979). Nine mm diameter of culture disc of *Cryptosporiopsis spp* was kept at the center of each Petriplate containing the fungicides of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at $27\pm1^{o}C$ for ten days and colony diameter was recorded. The experiment was performed twice in a completely randomized design (CRD) with five replications per treatment.

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

Where dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group. Data from similar trials were pooled when the variances were homogenous. All data were analyzed using the Proc general linear model procedure of SAS. Conidial germination data were transformed using the arcsine square-root transformation before analysis was performed. The means were compared using Duncan's multiple range tests.

Table 1
Pesticides and adjuvants tested and their rates.

Sl. No.	Common name	Systemic/ Non-systemic	Trade name	Rate/50ml
1.	Tebuconazole (0.75 lbs)	S	Buzz Ultra	0.15g
2.	Sulphur 80% w/w	NS	Thiovit Jet	400 g/100 L
3.	Tebuconazole (4.5%) + sulfur (70%)	NS +S	Unicorn DF	0.15g
4.	Mancozeb 480g/kg + Metalaxyl 100g/kg	NS +S	Bagonal Super WP	0.75g
5.	Mancozeb 640g/kg + Metalaxyl 80g/kg	NS +S	Ebony M72 WP	1.25g
6.	Triadimefan 25% + Metalaxyl 20%	NS +S	Bellet 450 WP	0.15g
7.	Mancozeb 680g + Metalaxil 40g/kg	NS +S	Unilax 720WP	0.15g
8.	Mancozeb 80%	NS	Bagonal 80 WP	0.15g
9.	Chlorothalonil 720g/L	NS	Mo-Bankoner 500SC	0.1125ml
10.	Potassium chloride	NS	Potassium chloride	1g
11.	Picoxystrobin (250 g/l)	S	Acanto 250 SC	0.0375ml
12.	sodium hypochlorite (NaOCl 3.85% m/v)	NS	JIK	
13.	Sodium hydroxide (NaOH) pellets	NS	Sodium hydroxide	

S – Systemic NS – Non-systemic

2.4. Host Range test

The plants tested in this study were Mango (*Mangifera indica* L), Cassava (*Manihot esculenta* Crantz), Sorghum (*Sorghum bicolor* (L.) Moench), Pigeon Pea (*Cajanus cajan* (L.) Millsp), Sweet potato (*Ipomoea batatas* (L.) Lam), Lemon (*Citrus limonum* Risso), mung bean (*Vigna radiata* (L.) Wilczek) and Eucalyptus spp. Seeds and tubers of these plants were collected from Naliendele Research Institute, Mtwara, Tanzania. Seedlings of plants used for the tests were raised in sterile plastic bags in the green house, Naliendele. Sources of inoculum were cultures of *Cryptosporiopsis* spp. The organisms were grown in potato dextrose agar (PDA) incorporated with 1% volume of streptomycin to prevent bacterial growth. Pure cultures of test fungi were multiplied on several plates to produce the required inoculum loads. Cultures of *Cryptosporiopsis* spp were blended into a suspension with sterile distilled water. Suspensions were filtered through sterile muslin cloth.

The possibility of existence of alternative hosts was studied. The plants found in fields in and around the Agricultural Research Station, Naliendele areas were included in this study. The weeds and grasses were identified according to Hosmani (1995), Krishnashastry *et al*. (1984) and Narashimachar (1976). In determining the host range of the leaf and nut blight (*Cryptosporiopsis* spp), sterile soil samples were packed in polythene bags. Soil sterilization was achieved by fumigation with a soil drench chemical. Seeds of the test plants were sown in polythene bags, two seeds per bag. Five polythene bags (10 stands) were planted for each test plant. Seedlings were inoculated eight weeks after germination by drenching the soil with spores suspensions of *Cryptosporiopsis* spp. Two polythene bags per test plant were inoculated while two polythene bags were used as controls. The inoculated plants were sprayed with water to maintain high relative humidity to achieve favourable conditions for disease expression. All the polythene bags, both for inoculated plants and controls, were covered with polyethylene bags to ensure a humid environment. Regular monitoring was done and observations recorded 4 days after inoculation for the development of symptoms. The pathogen from infected hosts were reisolated on PDA and observed under microscope for morphological characters.

3. Results

3.1. Effects of fungicides and adjuvants on the inhibition of spore germination

Fungicides and adjuvants were evaluated in the laboratory for their efficacy against *Cryptosporiopsis spp* through poison food technique (Fig 1 and 2). The results presented in Table 2 revealed that, there was a significant difference between the fungicides, adjuvants and interactions. It was revealed from the results (Table 2) that all treatments at different concentrations significantly inhibited spore germination of *Cryptosporiopsis spp*. However, Mancozeb 80% caused highest spore inhibition (98%) followed by Mancozeb 480g/kg + Metalaxyl 100g/kg (96%), Mancozeb 680g + Metalaxil 40g/kg (96%) and Picoxystrobin (91%).

Table 2
Effect of different fungicides and adjuvants on *Cryptosporiopsis spp* spore germination.

Fungicides and adjuvants	Spore inhibition (%)
Picoxystrobin (250 g/l)	(91)72.49±2.1^{a}
Mancozeb 80%	(98)81.72±1.3^{a}
Mancozeb 480g/kg + Metalaxyl 100g/kg	(96)78.95±0.8^{a}
Triadimefon 25% + Metalaxyl 20%	(6)14.25±1.0^{e}
Tebuconazole (0.75 lbs)	(27)31.20±2.6^{d}
Control	(0)0.00±0.0^{c}
Mancozeb 640g/kg + Metalaxyl 80g/kg	(73)59.10±3.7^{b}
KCL	(57)48.92±4.6^{bc}
Chlorothalonil 720g/L	(90)72.31±4.6^{a}
NaOCl	(59)49.95±1.9^{bc}
NaOH	(50)44.72±2.2^{c}
Sulphur 80% w/w	(22)25.71±10.6^{de}
Tebuconazole (4.5%) + sulfur (70%)	(58)49.77±5.6^{bc}
Mancozeb 680g + Metalaxil 40g/kg	(96)78.30±0.7^{a}
LSD	11.57
CV%	13.69

Means (Values are arcsine transformed values) followed by the same alphabets in a column is not significantly different according to Least Significant Difference ($P < 0.05$) test.

Data presented in Table 2 reveal that Mancozeb 80%, Mancozeb 480g/kg + Metalaxyl 100g/kg, Picoxystrobin and Mancozeb 680g + Metalaxyl 40g/kg were the most effective fungicides against *Cryptosporiopsis spp* spore germination while sulphur and Tebuconazole had the least effects. Sodium hydroxide and potassium chloride revealed significant mycelial and conidia inhibition (Table 2 and 3). All chemical fungicides and adjuvants exhibited significant inhibition of spore germination against *Cryptosporiopsis spp* ($F_{5, 20}=12.52$; $P<0.01$).

3.2. Effect of pesticides on mycelial growth of cryptosporiopsis spp

The chemical fungicides and adjuvants exhibited significant inhibition of mycelial growth of *Cryptosporiopsis spp* ($F_{5, 20}=12.52$; $P<0.01$). The pesticides Chlorothalonil (720g/L), Tebuconazole (4.5%) + sulphur (70%), Triadimefon 25% + Metalaxyl 20% and Tebuconazole (0.75 lbs) completely inhibited the growth of *Cryptosporiopsis spp* grown on pesticide-amended MEA (Table 3). Mancozeb had little effect on mycelial growth unlike the large effect on spore inhibition. Among all the pesticides and adjuvant tested, only NaOCl had no adverse effect on mycelial growth. Reduction of *Cryptosporiopsis spp* colony growth on MEA amended with Chlorothalonil (720g/L), Tebuconazole (4.5%) + sulphur (70%), Triadimefon 25% + Metalaxyl 20% and Tebuconazole (0.75 lbs), was probably due to the effect of toxic metabolites that were released during their breakdown during the course of the experiment.

Table 3
Effect of different fungicides and adjuvants on the growth of *Cryptosporiopsis spp.*

Fungicides and adjuvants	Mycelial growth inhibition (%)
Picoxystrobin	* (42)40.36±2.9[f]
Mancozeb 80%	(63)52.22±0.4c[d]
Mancozeb 480g/kg + Metalaxyl 100g/kg	(42)40.39±1.7[f]
Triadimefan 25% + Metalaxyl 20%	(82)65.03±0.6[b]
Tebuconazole (0.75 lbs)	(83)65.30±0.3[b]
Mancozeb 640g/kg + Metalaxyl 80g/kg	(67)55.18±0.6[c]
KCL	(58)49.78±0.2[d]
Chlorothalonil	(89)70.97±0.3[a]
NaOCl 3.85% m/v	(7)15.22±0.2[g]
NaOH	(52)46.07±0.6[e]
Sulphur 80% w/w	(38)38.19±0.4[f]
Tebuconazole (4.5%) + sulfur (70%)	(89)70.97±0.3[a]
Mancozeb 680g + Metalaxyl 40g/kg	(52)45.85±0.4[e]
LSD	3.3523
CV%	4.28

* Mean followed by the same alphabets in a column is not significantly different according to Least Significant Difference (LSD, P < 0.05) test; CV% =4.28).

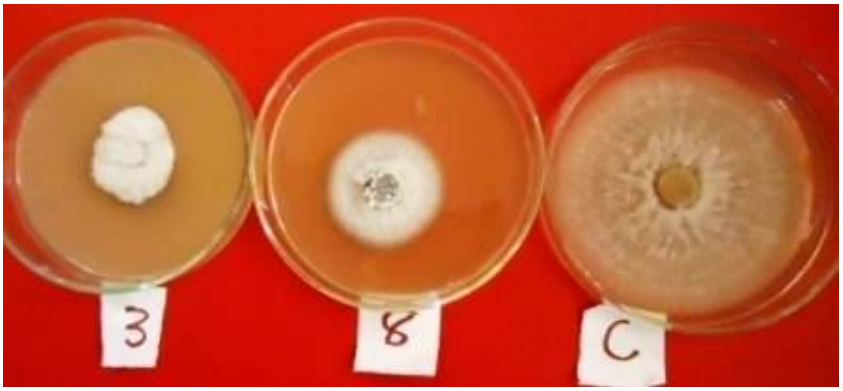

Fig. 1. Effect of various pesticides on germination of *Cryptosporiopsis* spp mycelia growth: (*3*) Mancozeb 640g/kg + Metalaxyl 80g/kg *(8)* Mancozeb 680g + Metalaxil 40g/kg *(c)* Control.

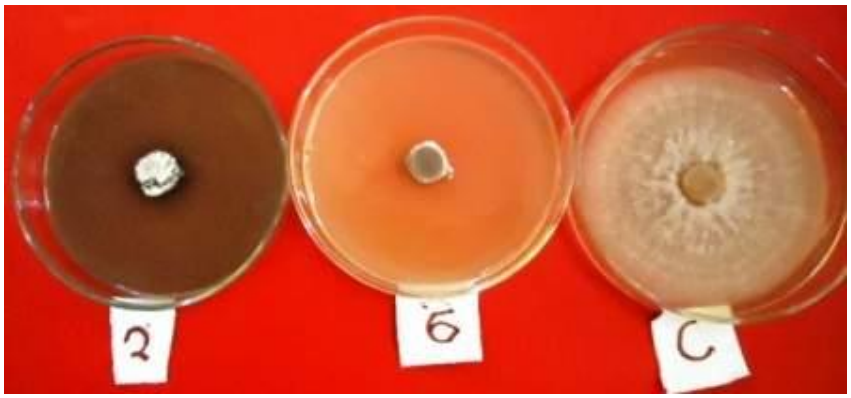

Fig. 2. Effect of various pesticides on germination of *Cryptosporiopsis* spp mycelia growth: *(2)* Chlorothalonil 720g/L *(6)* Tebuconazole (4.5%) + sulphur (70%) *(c)* Control.

3.3. Host range

The host range study was conducted to know the ability of *Cryptosporiopsis* spp to survive on different hosts in the absence of main host. Eight host plants as mentioned in the material and methods were artificially inoculated with *Cryptosporiopsis* spp. The results of disease reaction on different test hosts are presented in Table 4 and it is confirmed that out of eight hosts, five hosts, *viz*., *Manihot esculenta, Sorghum bicolor, Ipomoea batatas, Vigna radiata* and Eucalpytus spp were found to be infected by *E Cryptosporiopsis* spp.

Table 4
Status of crops as hosts of *Cryptosporiopsis* spp other than cashew.

S. No.	Scientific name	Family	status
1.	*Mangifera indica* L. (Mango)	Anacardiaceae	-
2.	*Manihot esculenta* Crantz (Cassava)	Euphorbiaceae	+
3.	*Sorghum bicolor* (L.) Moench (Sorghum)	Poaceae	+
4.	*Cajanus cajan* (L.) Millsp (Pigeon Pea)	Fabaceae	-
5.	*Ipomoea batatas* (L.) Lam (Sweet potato)	Convolvulaceae	+
6.	*Citrus limonum* Risso (Lemon)	Lamiaceae	-
7.	*Vigna radiata* (L.) Wilczek (Green gram)	Fabaceae	+
8.	*Eucalpytus spp*	Myrtaceae	+

- = No infection; + = infection

The pathogen *Cryptosporiopsis* spp was re-isolated from these infected hosts and cross inoculated to cashew plants. Similarly, cashew isolates were found to infect above hosts. The cross inoculations attempted was successful. The present study established that four hosts, *viz*., *Manihot esculenta, Sorghum bicolor, Ipomoea batatas, Vigna radiata* and Eucalpytus spp served as the alternative hosts to *Cryptosporiopsis* spp. The pathogen could perpetuate during off-season on these alternative hosts and serve as source of secondary infection. The typical brown spot symptoms were produced on the leaves of 5 hosts including *Manihot esculenta* Crantz (Cassava), *Sorghum bicolor* (L.) Moench (Sorghum), *Ipomoea batatas* (L.) Lam (Sweet potato), *Vigna radiata* (L.) Wilczek **(**Green gram) and *Eucalpytus spp* under greenhouse conditions (Fig 3 & 4). The pathogen was re- isolated and found alike with mother culture upon microscopy.

Older tissues were more resistant to penetration by *Cryptosporiopsis* spp in plants that were infected while young tissues were more susceptible. Few lesions were observed in *Manihot esculenta* Crantz as compared to other plants. Lesions were observed on *Vigna radiata* were intially brown in color. These often increased in size rapidily, causing most of the plant shoot, stem to wither, and sometime resulting in death of plants. In *Ipomoea batatas*, lesions appeared as large yellow patches that were accompained by leaf chlorosis. There were light brown lesions on *Manihot esculenta* Crantz curled leaves. Brown spots developed quickly within two days in *Eucalpytus spp* extending in both the Lamina and midrib. Disease symptoms begin as small brown spots on leaves. Infection begins with the appearance of irregularly shaped small spots. In addition to causing leaf spots and defoliation, stem lesions were associated with *Cryptosporiopsis spp.* Symptoms of *Cryptosporiopsis* spp infection developed on *Sorghum bicolor* leaves. Brown leaf spots occur on both sides of the glasslike and flat leaves. The results of the studies revealed that the plants of *Mangifera indica*, *Cajanus cajan*, *Citrus limonum* were non host of *Cryptosporiopsis spp*.

4. Discussion

4.1. Effect of Selected Pesticides and adjuvants on Conidial Germination and Mycelial Growth of *Cryptosporiopsis spp*, a cashew blight pathogen

Chlorothalonil (720g/L) inhibited *Cryptosporiopsis spp* mycelial growth and conidial germination probably because it reduces fungal intracellular glutathione molecules to alternate forms which cannot participate in essential enzymatic reactions, ultimately leading to cell death. Tofoli *et al (*2003) also showed efficacy of chlorothalonil against *A. alternata*. Tebuconazole (0.75 lbs) and Tebuconazole (4.5%) + sulphur (70%) showed relatively good efficacy against *Cryptosporiopsis spp* mycelial growth. Early studies have demonstrated that tebuconazole [(*RS)*-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1methyl)pentan-3-ol] is an effective

systemic fungicide to control diseases like Fusarium head blight of wheat (Zhensheng *et al.,* 2001).The two combinations Mancozeb 480g/kg + Metalaxyl 100g/kg and Mancozeb 680g + Metalaxyl 40g/kg had differences in mycelial and conidia efficacy. The effectiveness of Metalaxyl results from inhibition of uridine incorporation into RNA and specific inhibition of RNA polymerase-1. Differential sensitivity of mycelia and conidia to the same compound has been reported for another hyphomycetous fungus, *Metarrhizium anisopliae* (Metschnikoff) Sorokin strain ESC-1, a biocontrol agent for the German cockroach, *Blatella germanica* (L.). Pachamuthu *et al.* (1999) observed that *M. anisopliae* mycelium was more sensitive to insecticides than the conidia. This phenomenon is believed to be due, to some extent, to the dissimilar mechanisms involved in mycelial development and conidial germination. This study confirmed an early report by Bruck *et al.,* 1981 that mancozeb had a small effect on *Cryptosporiopsis spp* mycelium growth relative to its large effects on inhibition of spore germination. Germination of conidia may be less affected by exogenous factors because the process primarily involves the utilization of nutrient reserves and a minimal amount of carbon from the environment or substrate, whereas mycelial growth requires the utilization of exogenous carbon and nitrogen sources (Gottlieb 1976; Smith and Grula 1981; St. Leger et al. 1989). In the course of its growth, mycelia may take up other materials that are present in the substrate, including pesticides. Sodium hydroxide and potassium chloride could be used as adjuvants with pesticides to increase the inhibition levels. Copper hydroxide reportedly killed conidia of *Phomopsis amaranthicola* (Wyss *et al.* 2004). The effect of different combinations of pesticides and adjuvants on the fungus was not studied here. Further studies should be carried out with selected combinations of pesticides and adjuvants used in crop production. Prasad (1994) also reported that metalaxyl did not reduce the mycelial growth of *C. purpureum*. Fungi differ in their sensitivity to pesticides of the same chemical class or same mode of action. Therefore, a generalization cannot be made as to what types of fungi are more or less sensitive to a certain pesticide. Long-term exposure of *Cryptosporiopsis spp* mycelia and short-term exposure of conidia to the same pesticides produced different results. The feasibility of mixing fungicides with adjuvants having different formulations for the control of cashew blight needs to be studied. According to data presented by Tixier et al. (2000), microorganisms are able to break down diuron, resulting in the release of monodemethylated and didemethylated metabolites, which are more toxic than diuron itself. A possible explanation for the nontoxic effects of imazapyr is its tendency to be photodegraded; because imazapyr has a half-life of 2 d in aqueous solution (Mallipudi et al. 1991), it is possible that exposure of the plates that contained imazapyr to light (12 h/d) degraded it before it could have an effect on the colony growth of *Dactylaria higginsii*. On the other hand, the negative effect of cyromazine may be due to its ability to inhibit chitin synthesis, a process that occurs in insects as well as in fungi. Nikkomycin Z, a fungal chitin synthase competitive inhibitor, was able to block chitin synthesis in sheep blowfly (*Lucilia cuprina* [Wiedemann]) (Diptera: Calliphoridae) (Tellam *et al.* 2000). *In vitro* evaluation of fungicides provides useful and preliminary information regarding efficacy of fungicides against pathogen within a shortest period of time and therefore, serves as a guide for field testing.

4.2. Host range

Results of artificial inoculation showed that different plants in the test reacted differently to *Cryptosporiopsis spp*. In addition, the cashew blight pathogen may vary considerably in pathogenicity. The findings are in accordance with Ullstrup (1966) and Mazzani *et al*. (1997) who reported that the maize leaf blight pathogen *Exserohilum turcicum* also attacked sorghum, Sudan grass, Johnson grass and teosinte. Tsai *et al*. (2001) observed that *Erucastrum rostratum* causing leaf spot of maize in Taiwan was capable of causing leaf spots on weed hosts, *viz*. *Chenopodium album, Panicum maximum* and also cultivated crops, namely sugarcane, sorghum and wheat. When penetration of *Cryptosporiopsis* spp occurred, a series of host defence responses have been elicited in *Manihot esculenta* Crantz (Cassava). After the initial penetration into the sub-stomatal cavity, adjacent cell walls thicken. Although haustoria were produced in some species such as *Cajanus cajan* and *Mangifera indica*, a host defence response might have isolated them and prevented further development.

The present study established that *Manihot esculenta, Sorghum bicolor, Ipomoea batatas, Vigna radiata* and *Eucalpytus* spp are potential alternative hosts to *Cryptosporiopsis* spp and may play a role in the epidemiology of cashew blight disease. These alterative hosts may serve as secondary foci of inoculum, thus establishing their role in epidemiology of the disease. Most species of plant pathogens can attack a broad diversity of plant species (Farr *et al.,* 2004), but the number of plant species with which a pathogen interacts in a local community is generally much lower. The studies revealed that *Mangifera indica*, *Cajanus cajan* and *Citrus limonum* were non-hosts of *Cryptosporiopsis spp*.

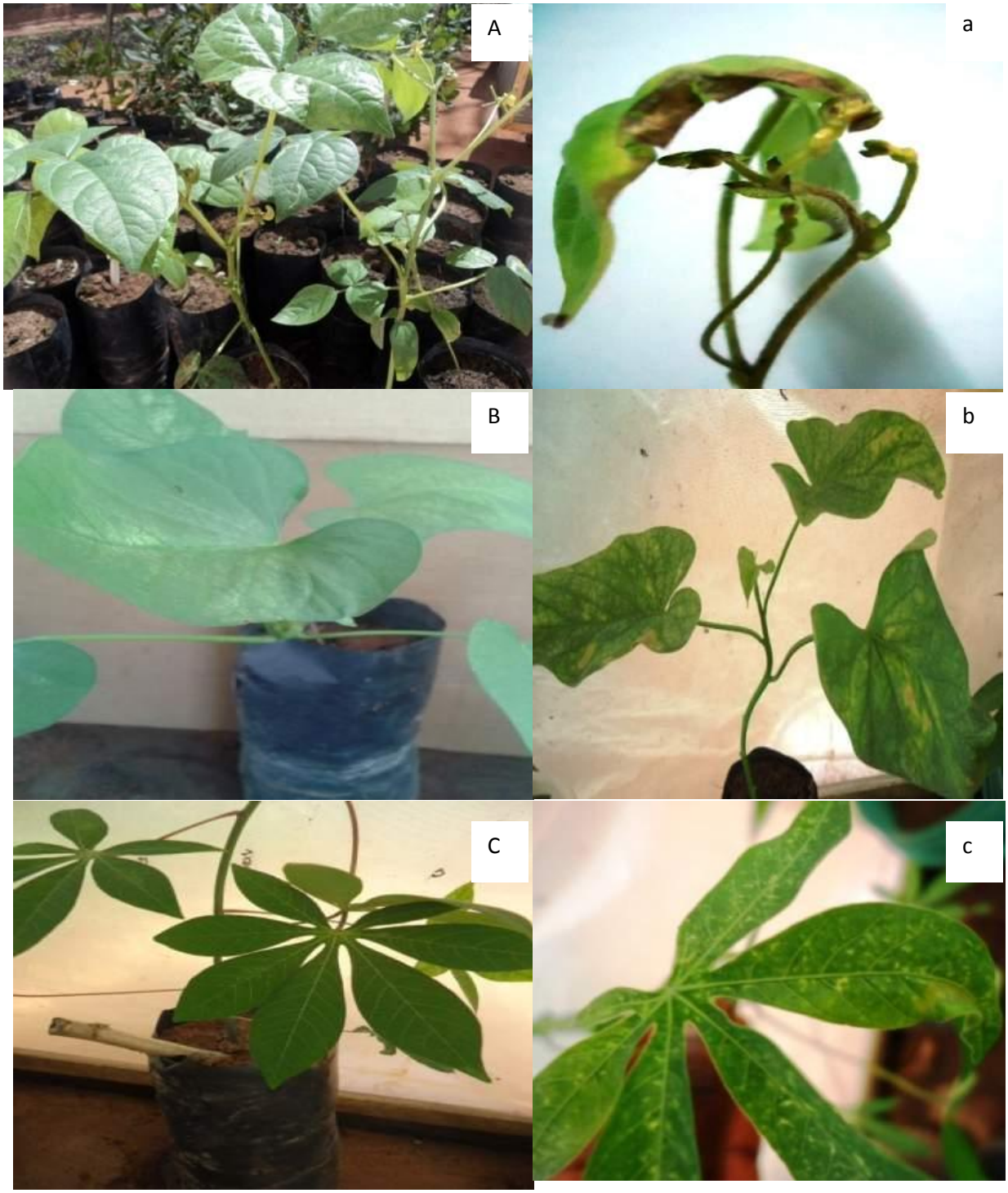

Fig. 3. Healthy and infected Pigeon Pea (A,a), Sweet potato (B,b) and Cassava (C,c) plants in the greenhouse.

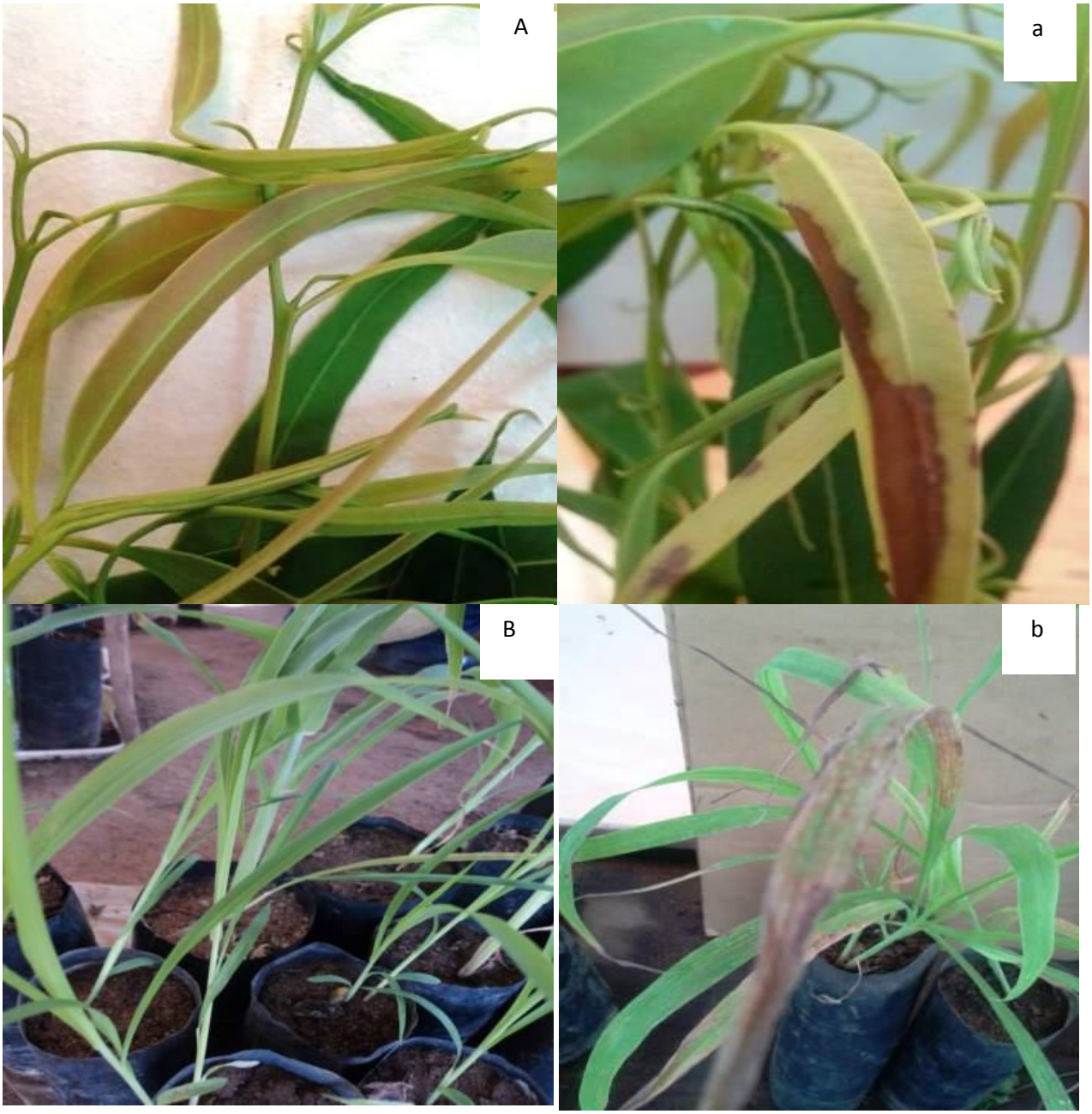

Fig. 4. Healthy and infected *E*ucalyptus (A,a) and sorghum bicolor (B, b).

The study will help in studying *Cryptosporiopsis spp* disease epidemics. Host selectivity is important to understand the epidemic development of plant diseases (Altizer *et al.,* 2003), and enables us to estimate fungal biodiversity (Hawksworth, 2001), to manage agriculture and forestry systems (Fininsa and Yuen *et al.*, 2001), and to analyse the risk for global movement of plants and pathogens (Animal and Plant Health Inspection Service, 2005).

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