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Full Length Research Paper

Effect of environmental conditions on the growth of *Cryptosporiopsis* spp. causing leaf and nut blight on cashew (*Anacardium occidentale Linn*.)

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A new disease (cashew leaf and nut blight) in Tanzania caused by a fungus related to the genus *Cryptosporiopsis* was identified in 2006. The present work investigated the effects of environmental factors on the growth of *Cryptosporiopsis* spp. causing blight on cashew. The mycelial growth, colony character and sporulation pattern of 10 fungal isolates, grown on seven different culture media namely, corn meal agar (CMA), malt extract agar (MEA), tryptone dextrose agar (TDA), potato carrot agar (PCA), water agar (WA), potato dextrose agar (PDA) and host leaf agar were observed after 10 days of incubation at 25±2°C. The colony diameter, culture characteristics and sporulation of the 10 isolates were greatly influenced by the type of growth medium used. The best mycelial extension was recorded in 12 h alternating light/dark followed by total light and total dark conditions, respectively. Seven media were evaluated for best growth of the fungi that is, *Cryptosporiopsis* spp. grew maximum on WA followed by host leaf extract media and PDA, respectively but least grew on the TDA medium. The growth of *Cryptosporiopsis* spp. was maximum in temperature range of 25 to 30°C. The most suitable pH level for growth of fungus was 7.0 and 6.0. These results will be useful for fungal taxonomic studies.

Key words: *Cryptosporiopsis* spp., culture media, light, temperature.

INTRODUCTION

Cashew (*Anacardium occidentale L*.) is a tropical nut crop that belongs to the family Anacardiaceae, which consist about 75 genera and 700 species (Nakasone and Paull, 1998). *Anacardium* contains eight species all of which are native to the coastal parts of north eastern Brazil (Azam-Ali and Judge, 2000). Cashew is an important cash crop traded worldwide that originated from South American countries like Brazil, Bolivia, Ecuador and Kenya. Cashew world production is about 400,000 tonnes. More than 50% of this production comes from South Asia and South Africa, especially India and and Peru (Behrens, 1998). Cashew major producers are India, Tanzania, Mozambique, Nigeria, Guinea-Bissau Tanzania (Opeke, 2005). It was introduced in India and Africa in the 16th century, by the Portuguese initially to protect the soil from erosion (Azam-Ali et al., 2001). The global area under cashew cultivation has risen tremendously, from about half a million hectares to four million hectares from 1961 to 2008, respectively (FAOSTAT, 2008). Cashew trees are genuinely tropical and very frost sensitive. The trees grow in a wide spectrum of climatic regions between the 25°N and S latitudes. Although the cashew can withstand high temperatures, a monthly mean of 25°C is regarded as optimal. Yearly, rainfall of 1000 mm is sufficient for production but 1500 to 2000 mm can be regarded as optimal. Diseases constitute limiting factors in production of cashew in cashew producing regions of Tanzania

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Abbreviations: CMA, Corn meal agar; **MEA,** malt extract agar; **TDA,** tryptone dextrose agar; **PCA,** potato carrot agar; **WA,** water agar; **PDA,** potato dextrose agar.

Table 1. Isolates obtained from sampled locations.

Isolate	Infected organ	Place of origin
AA1	Leaf	Naliendele
AA2	Leaf	Madangwa
AA3	Leaf	Mnazi mmoja
AA4	Pseudo fruit	Malamba
AA5	Pseudo fruit	Lyenje
AA6	Nut	Namiyonga
AA7	Nut	Chiola
AA8	Nut	Nandagala
AA9	Pseudo fruit	Nachingwea
AA10	Leaf	Newala

because the environment is conducive to the growth and multiplication of disease pathogens. In 2003, during a survey carried out by Naliendele Agricultural Research Institute in Tanzania, cashew leaves with spots were collected from cashew trees. According to Sijaona et al. (2006) the causative agent was identified as *Cryptosporiopsis* spp. Leaf and nut blight caused by *Cryptosporiopsis* spp. is a major limiting factor affecting cashew nut production in Tanzania, causing 48.4% crop loss annually (ACRR, 2006). The cashew blight samples were forwarded to Global Plant Clinic where they were deposited as Herbarium specimen (IMI 391611). The pathogen is being characterized further into its taxonomical nomenclature (GPC, 2010). An understanding of the role of environmental conditions and its effect on infection and survival of the pathogen is necessary to develop cultural disease management practices. The objective of this study was to provide information on effects of culture media and various environmental factors including temperature, pH and light on mycelial growth and conidia production of *Cryptosporiopsis* spp. causing blight disease in cashew (*Anacardium occidentale L*).

MATERIALS AND METHODS

Sample collection

The present investigations were carried out both in the field and laboratory during the period 2010-2011. All the experiments were conducted at the Agricultural Research Institute (Naliendele), Mtwara, Tanzania. One cashew leaf and nut blight sample was forwarded to the Naliendele Agricultural Research Institute Plant Pathology Herbarium, Tanzania where they were deposited as herbarium specimen *Cryptosporiopsis* spp. IMI396316 for reference. *Cryptosporiopsis* spp. was found in all of the lesions and was identified based on Sijaona et al. (2006) well illustrated and detailed description of the fungus, which is reproduced here. The typical cashew leaf blight diseased leaf and nut samples were collected from farmers' fields on commercially cultivated clones at 10 locations comprised of different agroclimatic zones in southern Tanzania (Table 1). These and brought to the laboratory for isolation of disease causing fungi.

The pathogen was isolated by direct conidial transfer method on potato dextrose agar (PDA) medium. Cashew leaves showing leaf blight symptoms were cut into small pieces of 1.2 cm, surface sterilized by sodium hypochloride for 1 min and washed in sterilized distilled water three times. The leaf bits were placed in Petri plates containing moist filter paper and incubated for four days at 25±2°C. Sporulated leaf bits were shaken onto new PDA medium to release spores thereafter the plates were incubated for four days at 25±2°C. The fungus was purified hyphal tip isolation technique (Harlapur, 2005) and the isolates were maintained on PDA slants. *Cryptosporiopsis* spp. (IMI396316) was obtained from CABI, UK for reference.

Pathogenicity test

Pathogenicity test was performed on cashew seedlings by spraying conidial suspensions (10^6 spores mL^{-1}) of the 10 isolates selected randomly on young tender leaves of nine-month-old plants. Inoculated plants were enclosed in wet plastic bags. Control plants were sprayed with sterilized deionised water. After 48 h, the plastic bags were removed and the plants observed daily for 10 days. The symptoms were observed. The inoculated cashew seedlings were observed for symptom expression on the 1st, 2nd and 3rd leaves of the seedlings. Koch's postulates were observed by consistently re-isolating the pathogen from the inoculated plants.

Maintenance of the culture

The pure cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in laboratory at 25±2°C for 10 days. Such mother culture slants were preserved at 5°C in refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies.

Effect of culture media on mycelial growth

Following the culture media, seven were used to find out the most suitable one for the mycelial growth of the fungus. Cultural characters of eleven isolates of *Cryptosporiopsis* spp. from different geographical regions were studied on seven different media. The growth characters of *Cryptosporiopsis* spp. were studied on seven solid media namely leaf extract, corn meal agar, malt extract agar, potato dextrose agar, dextrose tryptone agar, water agar and potato carrot agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm Petri plates. Such Petri plates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 25±2°C for 10 days. Each treatment was replicated thrice. Observations were taken when the fungus had completely covered the Petri plate in any one of the media. The colony diameter was recorded. The fungus colony colour, margin and sporulation were also recorded. The data on radial growth was analyzed statistically. Petri plates containing 20 ml of each of following media were inoculated with 9 mm diameter disc from 10 day old cultures of different isolates. *Cryptosporiopsis* spp. (IMI396316) was obtained from CABI, UK for reference.

i. Corn meal agar (CMA; Unipath LTD, Hampshire, England) with the following composition (g L^{-1}); corn meal extract from 50 g whole maize 2.0, agar 15 and distilled water 1000 mL.
ii. Potato Dextrose agar (PDA; Unipath LTD, Hampshire, England) – Potato infusion 200, dextrose 20.0, agar 15 and distilled water 1000 mL.
iii. Dextrose Tryptone Agar (DTA; Unipath LTD, Hampshire, England)- Tryptone 10.0, Glucose 5.0, Bromocresol purple 0.04, agar 12.0 and distilled water 1000 mL.

iv. Malt extract agar (MEA; malt extract 30.0, peptone from soymeal 3.0, agar 15.0 and distilled water 1000 mL.
v. Potato carrot agar (PCA); grated potato 20g, grated carrot 20g, agar 20g and distilled water 1000 mL prepared according to Tuite, 1969.
vi. Water agar (WA) – agar 20 g in 1000 mL distilled water and
vii. Host leaf agar (young cashew leaves 200 g, agar 20 g and distilled water 1000 mL.

Morphological studies of the pathogen

Spores of leaf and nut blight were taken from infected host tissue and mounted on a clean glass slide. To characterize isolates by colony morphology, single germinating conidia were transferred to Petri dishes containing PDA. Dishes were incubated at 25±2°C in 12 h alternate light and darkness for six days. After incubation, cultures were examined for colony color, colony margin, colony texture, and the development of pigments or crystals in the agar medium. To characterize isolates by sporulation habit, single germinating conidia were transferred to Petri dishes containing PDA. Dishes were incubated for six days. After incubation, cultures were examined at ×40 to ×100 magnifications with a dissecting microscope and sub-stage illumination for characteristics of the sporulation apparatus, presence of conidiophores, and branching of conidial chains.

Effect of different pH levels on mycelial growth

After preparation of the PDA broth, their suitable volumes were adjusted at different pH 4, 5, 5, 6, 7, 8 and 9 using 1 N HCl or 1 N NaOH. The sterilized media of different pH levels was poured in the sterilized Petri plates in about 20 ml quantities and allowed to solidify. 9 mm discs from the actively growing 10 day old cultures of different isolates were placed on the centre of the Petri plates. The plates were incubated at 25±2°C for six days after which the mycelia growth diameter was measured. Three replications were maintained for each treatment.

Effect of temperature on mycelial growth

Temperature tolerance by cultivation of the isolated fungi was determined. Petri plates containing 20 ml of PDA medium were inoculated with 9 mm mycelia disc from 10 day old culture of different isolates. Disks of mycelium were cut with a flamed cork-borer and transferred to Petri dishes containing PDA media. These plates in triplicate were incubated at 5, 10, 15, 25, 30, 35 and 40°C for 10 days. The diameter of the growing colony was measured crosswise in two directions in 10-day old cultures. The average of these two readings was taken as diameter of the colony. The experiment was conducted in completely randomized design (CRD) and the data were statistically analyzed. *Cryptosporiopsis* spp. (IMI396316) was obtained from CABI, UK for reference.

Effect of light on mycelial growth

Effects of light on mycelial extension were determined by measuring the radial growth of the colony. PDA medium was autoclaved at 121°C for 15 min, and 20 ml of it was poured into Petri dishes. Mycelial disc of 9 mm of each isolate was used to inoculate Petri plates. After cooling, a 9 mm diameter disk of actively-growing mycelia from PDA was transferred into the medium, and then incubated at 25±2°C for six days in three different light conditions in the incubator (fluorescent light). Carbon paper was used to wrap the Petri dishes for darkness. Fluorescent lamp was used for light exposure.The light conditions were: a) first group was incubated in total darkness, b) the second group was in complete light, and c) the third group was in 12 h alternating shifts of total darkness and light. Colony diameter was recorded after 10 days of incubation. *Crypto*spo*riopsis* spp. (IMI396316) was obtained from CABI, UK for reference.

Statistical analysis

All the experiments were repeated once with similar results. The data were statistically analyzed according to Gomez and Gomez (1984). The package used for analysis was SAS ver 9.2 developed by SAS Institute, (1999). “SAS/Stat user’s Guide”. SAS Institute Inc. Cary, N.C.

RESULTS AND DISCUSSION

Pathogenicity test

Cashew seedlings inoculated with *Cryptosporiopsis* spp conidial suspensions exhibited small brown spots on multiple leaves. Spots enlarged over time and closely resembled spots observed in the field, although disease severity appeared lower than for field plants. Sterile water control did not display any disease symptoms. After 72 h, leaves sprayed with *Cryptosporiopsis* spp isolate began curling thereafter to developed dark, irregularly shaped brown spots. The younger first leaves of cashew seedlings were more susceptible than the older second leaves.

Effect of temperature on mycelial growth

The temperature range indicates that the pathogen *Cryptosporiopsis* spp. causing blight disease in cashew (*A. occidentale L)* can survive and be distributed in environments that are within the range (Table 2). The 11 isolates grew well at temperatures of 30°C (88.83 mm) followed by 25°C (82.40 mm) and 35°C (72.04 mm). The temperature better suited for mycelial growth ranged from 25 to 30°C. As the temperature increased, the mycelial growth increased but at 35°C the growth started to decline. This could be attributed to increase in enzymatic activity of *Cryptosporiopsis* spp. The least growth was produced at 5°C (9.19 mm). The fungus failed to grow at 40°C probably due to the inactivation of enzymes with a resulting effect on metabolism that affects growth. There were significant differences in mycelial growth of isolates (Table 2) in different range of temperatures, $F(76.233) = 2664.7$, $P<0.0001$.

Effect of pH on mycelial growth

There were significant differences in pH levels in the 11 isolates, $F(5.197) = 3372.7$, $P<0.0001$. pH 7 was found to be ideal and produced the maximum mycelial growth of 67.79 mm followed by pH 6.0 (62.94 mm) and pH 8.0

Table 2. Effect of temperature on growth of isolates.

Isolate	Mean mycelial growth (mm) of the isolate						
	5°C	10°C	15°C	20°C	25°C	30°C	35°C
AA1	9.43^{t}	17.23^{s}	37.57^{q}	63.45^{k}	79.37^{e}	88.50^{ab}	67.53^{j}
AA2	9.87^{t}	17.53^{s}	43.43^{no}	67.73^{j}	83.57^{d}	89.37^{a}	75.93^{f}
AA3	8.55^{t}	19.03^{rs}	44.43^{mn}	70.13^{i}	87.50^{ab}	90.00^{a}	75.50^{fg}
AA4	9.00^{t}	20.10^{r}	47.37^{l}	73.50^{gh}	87.67^{ab}	89.33^{a}	74.33^{fgh}
AA5	8.53^{t}	19.20^{rs}	45.43^{m}	76.07^{f}	87.90^{ab}	89.33^{a}	68.13^{ij}
AA6	9.23^{t}	17.53^{s}	38.20^{pq}	63.63^{k}	76.40^{f}	88.10^{ab}	69.10^{ij}
AA7	10.47^{t}	18.23^{rs}	40.27^{p}	72.47^{fgh}	85.33^{c}	89.33^{a}	70.13^{j}
AA8	9.00^{t}	17.43^{s}	37.63^{q}	64.67^{k}	78.43^{e}	87.67^{ab}	68.37^{ij}
AA9	8.60^{t}	18.37^{rs}	39.17^{pq}	67.13^{j}	76.37^{f}	86.33^{bc}	75.77^{fg}
AA10	9.17^{t}	18.17r^{s}	42.33^{o}	64.43^{k}	82.10^{d}	89.67^{a}	73.47^{gh}
IMI396316	9.27^{t}	17.57^{s}	38.30^{pq}	65.40^{k}	81.77^{d}	89.47^{a}	74.13^{fgh}
Grand mean	9.19	18.21	41.28	68.06	82.4	88.83	72.04

*Means separated using least significant difference test (LSD) by the same letter are not significantly different (P<0.05) from each other.

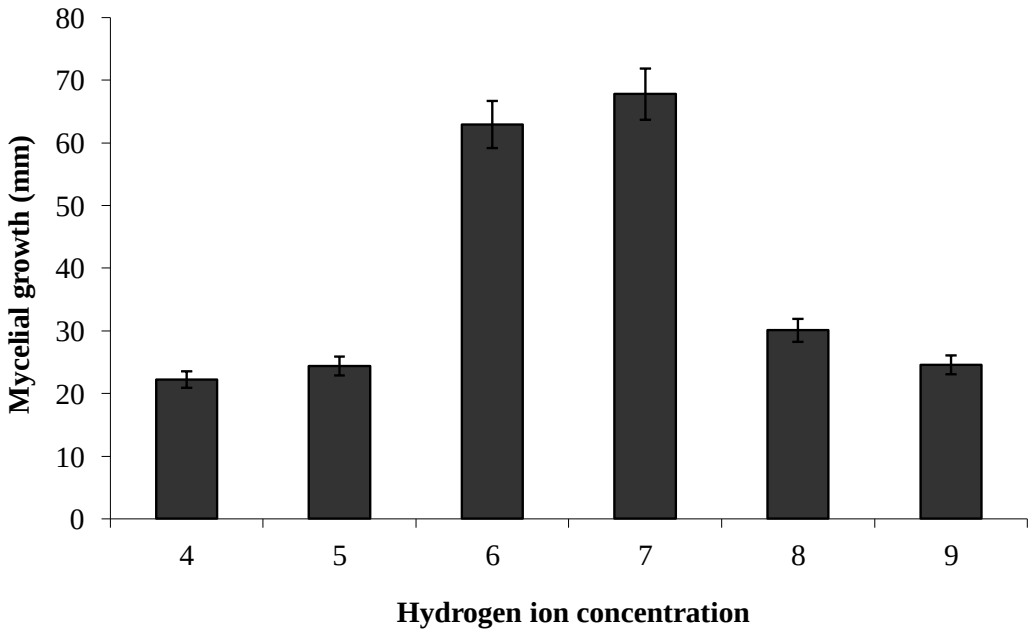

Figure 1. Effect of different pH levels on the mycelial growth of *Cryptosporiopsis* spp.

(30.09 mm). There was an observed decrease in growth when pH increased from 7.0 to 9.0. pH 4 recorded the lowest mean mycelial growth at 22.24 mm (Figure 1). The pH below 6.0 and above 7.0 produced inhibitory mycelial growth of the eleven isolates. The *Cryptosporiopsis* spp. isolates prefers pH range of 6.0 to 7.0. The fungus isolates preferred slightly acidic pH for the growth (Figure 1). The effect of pH on mycelial growth and conidial germination was not significant from pH 4 to 10.

Effects of light on mycelial growth

Photoperiod showed significant effect (F (29.89) = 750.5,

Table 3. Effect of light intensities on mycelial growth of *Cryptosporiopsis* spp. causing blight.

Isolate	Mean mycelial growth (mm) of the isolates		
	12 h photoperiod	Complete darkness	24 h photoperiod
AA1	*58.00±0.5^{d}	24.67±0.3^{n}	34.67±0.8^{hi}
AA2	52.00±0.5^{f}	25.33±0.3^{n}	32.00±0.5^{j}
AA3	48.00±0.5^{g}	29.00±0.5^{k}	31.00±0.5^{j}
AA4	52.33±0.3^{f}	27.67±0.3^{klm}	35.67±0.3^{h}
AA5	58.67±0.3^{cd}	17.33±0.3^{o}	27.00±0.5^{m}
AA6	54.00±0.5^{e}	31.00±1.0^{j}	31.00±0.5^{j}
AA7	63.67±0.3^{a}	17.67±0.3^{o}	35.67±0.3^{h}
AA8	63.67±0.3^{a}	27.33±0.6^{lm}	34.00±0.5^{i}
AA9	60.67±0.8^{b}	28.67±0.3^{kl}	34.67±0.3^{hi}
AA10	60.00±0.5^{bc}	18.67±0.3^{o}	28.33±0.6^{klm}
IM1396316	60.67±0.8^{b}	25.33±0.3^{n}	27.00±0.5^{m}
Grand mean	57.43	24.70	31.91

*Means separated using Duncan's Multiple Range Test (DMRT) by the same letter are not significantly different (P<0.05) from each other.

P<0.0001) on the growth of fungal mycelium (Table 3). The highest mean mycelial growth (57.43 mm) was observed in 12 h photoperiod, followed by 24 h photoperiod (31.91 mm). The lowest mean mycelial growth (24.70 mm) was found in complete darkness. This indicates that the fungus isolates require a period of dark followed by a light period for conidiophore formation and sporogenesis.

The fungus probably uses light to trigger the development of fruiting bodies and phototropic responses of reproductive structures. *Phycomes* and *Pilobolus* have been shown to use light in the formationP<0.0001) on the growth of fungal mycelium (Table 3). The highest mean mycelial growth (57.43 mm) was observed in 12 h photoperiod, followed by 24 h photoperiod (31.91 mm). The lowest mean mycelial growth (24.70 mm) was found in complete darkness. This indicates that the fungus isolates require a period of dark followed by a light period for conidiophore formation and sporogenesis.

The fungus probably uses light to trigger the development of fruiting bodies and phototropic responses of reproductive structures. *Phycomes* and *Pilobolus* have been shown to use light in the formation of reproductive structures (Alexopoulus et al., 1996). Most light sensitive fungi sporulate when exposed to continuous light, but some called diurnal sporulators, require a period of darkness followed by a light period (Leach, 1967). Mycelial colony was relatively dense when it was incubated in alternating shifts of dark/light conditions; this result shows that light is also an important factor in the growth of this fungus. There were significant differences in light duration among the isolates (P<0.0001). Isolates AA7 (63.67 mm) and AA8 (63.67 mm) had the highest mean mycelial growth in 12 h alternate light and dark conditions (Table 3). The lowest mean mycelial growth was recorded in complete darkness by isolates AA7 (17.67 mm) and AA5 (17.33 mm). Adeniyi et al. (2011) reported that light was found to be suitable for maximum growth of *Pestalotia* species which causes leaf spots in cashew.

Cultural study

Growth characters of *Cryptosporiopsis* spp. studied on different solid media indicated that the growth was maximum on WA followed by host leaf medium and PDA supported maximum growth of fungal colony (Table 4). Growth behavior of 11 isolates on seven different media showed significant difference in color, morphology, margin, topography and pigmentation along with sporulation in PDA (Table 5, Figure 2). There were variations among the colony characters of the isolates collected from different locations. Most of the isolates (AA2, AA1, AA4, AA6, AA7, AA9 and AA10) had white colonies whereas AA3 and AA8 produced white brown colored colonies. AA5 had dark brown colored colonies. Pale brown exudates droplets occurred in the centre. The media became brownish in all isolates with time. The substrate color for most isolates ranged from light grey to grayish in most isolates 10 days after incubation. Isolates AA3, AA5, AA7 and AA8 had grayish substrate color while the rest were light grayish. Most isolates (AA1, AA2, AA4, AA5, AA6, AA7, AA9, IMI396316 and AA10) produced smooth margins except for isolates AA8 and AA3 that showed irregular margins.

The colony topography varied from medium to raised fluffy growth in all isolates after 10 days of incubation. Isolates AA4 and AA8 showed medium raised colony topography. AA6 and AA7 produced medium fluffy growth

Table 4. Cultural characters of *Cryptosporiopsis* spp. isolates in PDA medium.

Isolate	Colony color	Substrate color	Margin	Topography	Pigmentation	*colony diameter	Sporulation
AA1	White	Light greyish	Smooth	Raised fluffy growth	White	84.33±0.3^{bcd}	++
AA2	White	Light greyish	Smooth	Raised fluffy growth	White	85.00±0.6^{bcd}	+++
AA3	White brown	Greyish	Irregular	Raised fluffy growth	Light brown	86.33±1.3^{abc}	++
AA4	White	Light greyish	Smooth	Medium raised	White	88.00±0.6^{ab}	++
AA5	Dark brown	Greyish	Smooth	Raised fluffy growth	Dark brown	90.00±0.0^{a}	+++
AA6	White	Light greyish	Smooth	Medium fluffy growth	White	88.00±0.6^{ab}	++
AA7	White	Greyish	Smooth	Medium fluffy growth	White	87.67±0.8^{ab}	++
AA8	White brown	Greyish	Irregular	Medium raised	Light brown	86.33±3.7^{abc}	++
AA9	White	Light greyish	Smooth	Raised fluffy growth	White	82.00±0.6^{d}	+++
AA10	White	Light greyish	Smooth	Raised fluffy growth	White	87.00±1.5^{ab}	+++
IMI396316	White	Light greyish	Smooth	Raised fluffy growth	White	83.00±0.6^{cd}	++

$^{+}$Poor sporulation: 1-10 spore/ microscopic field (100x); $^{++}$medium sporulation: 11-50 spores/microscopic field (100x); $^{+++}$ good sporulation: more than 100 spores/ microscopic field (100x); *mean of three replications, In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 5. Cultural characters of *Cryptosporiopsis* spp. isolates in different media.

Isolate	PDA	CMA	Host Leaf Agar	MEA	TDA	PCA	WA
AA1	White	White	White brown	White brown	Reddish purple	White	White brown
AA2	White	White	White brown	White brown	Purple	Creamish white	Light brown
AA3	White brown	Creamish white	Dark brown	brown	Reddish purple	White	White brown
AA4	White	White	White brown	White brown	Reddish purple	White	White brown
AA5	Dark brown	White brown	White brown	Brown	Purple	Creamish white	White
AA6	White	White	White brown	White brown	Reddish purple	White	White brown
AA7	White	White	White brown	White brown	Reddish purple	White	Light brown
AA8	White brown	White	Dark brown	Brown	Reddish purple	White	White brown
AA9	White	White	White brown	White brown	Reddish purple	White	White brown
AA10	White	White	White brown	White brown	Purple	Creamish white	White brown
IMI396316	White	White	Creamish brown	White brown	Reddish purple	White	White brown
Sporulation	Good	Poor	Good	Good	Medium	Medium	Poor

PDA, Potato dextrose agar; **CMA,** corn meal agar; **MEA,** malt extract agar; **TDA,** tryptone dextrose agar; **PCA,** potato dextrose agar; **WA,** water agar. $^{+}$Poor sporulation: 1-10 spore/ microscopic field (100x); $^{++}$medium sporulation: 11-50 spores/microscopic field (100x); $^{+++}$ good sporulation: more than 100 spores/ microscopic field (100x).

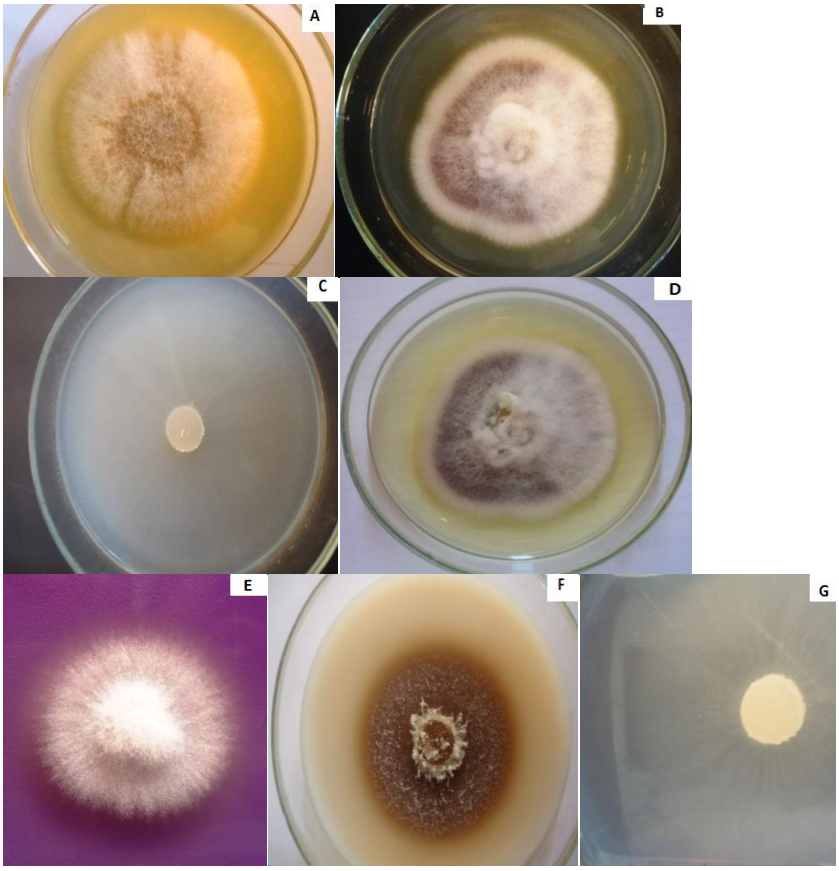

Figure 2. Growth behavior of isolate AA3 on seven different media: **A.** PDA; **B.** WA; **C.** PCA **D.** MEA; **E.** TDA; **F.** host leaf agar; **G.** CMA.

whereas the rest (AA1, AA2, AA3, AA5, AA9, AA10 and IMI396316) showed raised fluffy growth. There were variations in pigmentation of the 11 isolates. Isolates AA1, AA2, AA4, AA6, AA7, AA9, AA10 and IMI396316 produced white pigmentation. AA3 and AA8 showed light brown pigmentation whereas AA5 produced dark brown pigmentation. Good sporulation was achieved by isolates AA2, AA5 and AA10 on PDA medium. Other isolates AA1, AA3, AA4, AA6, AA7, AA8 and IMI396316 showed medium sporulation on PDA medium 10 days after inoculation. All isolates showed variations in colony color on different media (Table 5). Isolate AA1 showed white colonies on PCA, PDA and CMA while in MEA, WA and host leaf extract agar it produced white brown colonies. In the case of TDA, AA1 isolate exhibited reddish purple colored colonies. Other isolates showed variations in colony color on different media as shown in Table 5. Colonies on PDA at 25±2°C after 10 days were white to dark brown. In MEA, the colonies were white brown to brown in color. Colonies on PDA at 25±2°C after 10 days were centrally dark brown, mid zone higher brown with fine droplets of brown exudates. Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological (phenetic or phenotypic) and reproductive stages are the classic approaches and baseline of fungal taxonomy and nomenclature that are still valid (Davis, 1995; Guarro et al., 1999; Diba et al., 2007; Zain et al., 2009). Colonies of *Cryptosporiopsis* spp. were white initially becoming pale to dark brown in age. Conidiomata were numerous over the surface and they are cream colored. Macroconidia were slightly curved, aseptate, and arose from single phialides on vegetative hyphae or in conidiomata (Figure 3a, b, c and d). Spores were brown to olive brown in color, elliptical with tear shaped ends. Physical and chemical factors have a pronounced effect on diagnostic characters of fungi (Sharma and Pandey, 2010). It is necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996).

Growth of *Cryptosporiopsis* spp. on media

To check the best growth of fungi in *Cryptosporiopsis* spp., seven different media; PDA, WA, TDA, CMA, PCA, MEA and host leaf extract agar were selected and

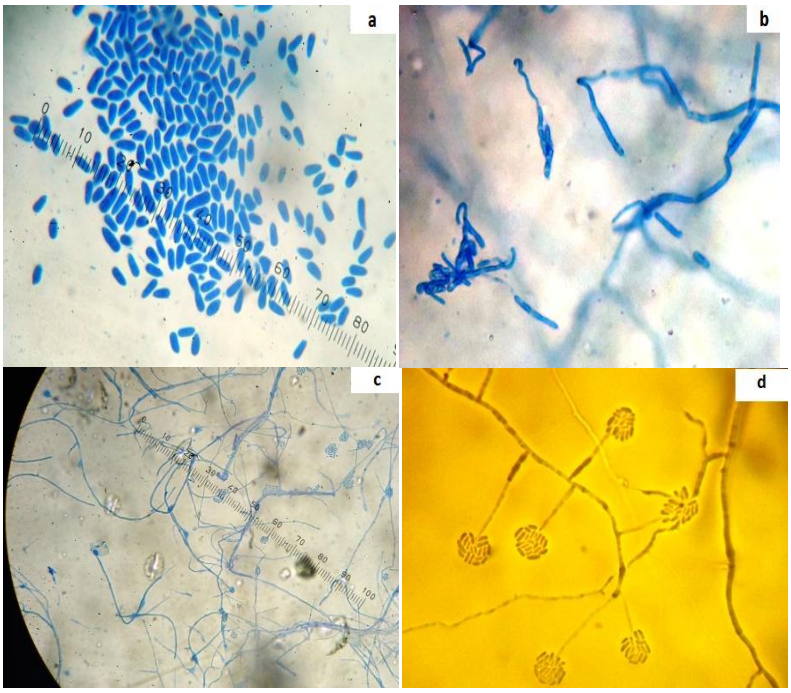

Figure 3. (a) Conidia of *Cryptosporiopsis* spp. causing leaf and nut blight; (b) Germinating Conidia (c) *Cryptosporiopsis* spp. hyphae; (d) *Cryptosporiopsis* spp. Conidiophores.

Table 6. Effect of different media on mycelial growth of *Cryptosporiopsis* spp. isolates.

Isolate	CMA	HOST LEAF	MEA	PCA	PDA	TDA	WA
AA1	*81.67±4.4^{fgh}	88.00±1.5^{abc}	73.00±1.5^{jklmn}	72.33±1.2^{klmno}	84.33±0.3^{bcdef}	69.33±1.2^{no}	84.33±4.7^{bcdef}
AA2	84.33±2.3^{bcdef}	90.00±0.0^{a}	67.67±5.9^{o}	77.33±1.5^{hijk}	85.00±0.6^{abcdef}	71.67±3.3^{lmno}	87.33±1.7^{abcd}
AA3	89.00±1.0^{ab}	86.67±3.3^{abcdef}	70.00±2.9^{mno}	89.00±0.6^{ab}	86.33±1.3^{abcdef}	75.33±2.7^{ijkl}	90.00±0.0^{a}
AA4	78.33±1.7^{ghi}	89.33±0.3^{ab}	85.00±2.9^{abcdef}	86.00±2.5^{abcdef}	88.00±0.6^{abc}	76.33±1.3^{ijkl}	90.00±0.0^{a}
AA5	86.00±3.1^{abcdef}	89.00±0.6^{ab}	87.00±2.5^{abcde}	86.00±0.6^{abcdef}	90.00±0.0^{a}	75.00±0.6^{ijklm}	89.00±0.6^{ab}
AA6	90.00±0.0^{a}	86.67±3.3^{abcdef}	88.00±0.5^{abc}	90.00±0.0^{a}	88.00±0.6^{abc}	76.33±2.3^{ijkl}	90.00±0.0^{a}
AA7	86.33±3.2^{abcdef}	89.67±0.3^{a}	88.67±1.3^{ab}	90.00±0.0^{a}	87.67±0.8^{abc}	74.00±0.6^{ijklmn}	90.00±0.0^{a}
AA8	86.67±2.8^{abcdef}	89.33±0.3^{ab}	89.00±1.0^{ab}	89.67±0.3^{a}	86.33±3.7^{abcdef}	75.67±0.8^{ijkl}	90.00±0.0^{a}
AA9	83.00±0.6^{cdefg}	89.67±0.3^{a}	89.00±0.6^{ab}	87.67±0.3^{abc}	82.00±0.6^{efgh}	73.33±2.7^{ijklmn}	89.00±0.6^{ab}
AA10	86.00±0.6^{abcdef}	89.67±0.3^{a}	85.00±2.9^{abcdef}	90.00±0.0^{a}	87.00±1.5^{abcde}	71.67±0.8^{lmno}	89.67±0.3^{a}
IMI396316	82.33±0.9^{defgh}	90.00±0.3^{a}	90.00±0.0^{a}	89.00±0.6^{ab}	83.00±0.6^{cdefg}	78.00±0.6^{ghij}	90.00±0.0^{a}
Grand Mean	84.8±0.8	88.9±0.4	82.9±1.5	86±1.0	86.2±0.5	74.2±0.6	89.0±0.5

*In a column, mean mycelial diameter (mm) followed by a common letter is not significantly different at the 5% level by DMRT. **PDA,** Potato dextrose agar; **CMA,** corn meal agar; **MEA,** malt extract agar; **TDA,** tryptone dextrose agar; **PCA,** potato dextrose agar; **WA,** water agar.

incubated for 10 days (Figure 3, Table 6). There were significant differences in radial growth of the isolates used in different solid media, F (6, 231) = 841.17, P<0.0001. The results of the cultural studies of *Cryptosporiopsis* spp. on solid media indicated that the radial growth was maximum on water agar (89 mm) which was significantly superior over all other media. This was followed by host leaf medium (88.9 mm) and PDA (86.2 mm) which were on par. The least radial growth was obtained in TDA (74.2 mm). Several workers recognized the importance of reproductive structures for inoculums production and studies have been conducted on the effects of various media components along with important physiological parameters that lead to maximum sporulation (Kim et al., 2005; Saxena et al., 2001; Saha et al., 2008). Type of culture media and their chemical

compositions significantly affected the mycelia growth rate and conidial production of *Phoma exigua* (Zhae and Simon, 2006).

Conclusion

Our findings reveal that culture media differentially influenced the growth, colony character and sporulation of the test fungi. Out of seven test media employed in the present study, PDA, MEA and host leaf agar were foundto be the most suitable for good sporulation while PDA reproduced most visible colony morphology. Colonies grew rapidly on a variety of media. It is concluded that instead of using any single culture medium, a combination of two or more media will be more appropriate for routine cultural and morphological characterization of fungi to observe different colony features.This is the first report on physiological studies of cashew leaf and nut blight associated with *Cryptosporiopsis* spp. and this has implications for the epidemiology of the disease. Further studies will create bases for the morphological and molecular characterization of these organisms for better understanding of their biology, identification and classification.

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