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CHARACTERISATION AND CONTROL OF *Curvularia lunata* INFECTING FARMER-SAVED RICE SEEDS IN GHANA

C. OPPONG, C. AMOATEY[1] and J.O. HONGER[2]

West African Centre for Crop Improvement, College of Basic and Applied Sciences, University of Ghana, Ghana
[1]Department of Crop Science, College of Basic and Applied Sciences, School of Agriculture, University of Ghana, Ghana
[2]Soil and Irrigation Research Centre, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Ghana
Corresponding author: johonger@yahoo.com

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ABSTRACT

Curvularia species are increasingly important seed-borne fungi, whose identification has been done based solely on cultural and morphological features in Ghana. To confirm the identity of the fungus in Ghana, isolates of the fungus obtained from farmer-saved rice seeds were identified using cultural and morphological features, complemented with phylogenetic analysis of the internal transcribed spacer regions of isolates. The susceptibility of isolates to three plant based fungicides, Levo, Kobe and Ex-icute was determined with *in-vitro* and *in-vivo*. All isolates produced curved shaped spores with several septations. In the phylogram, they all clustered in the *C. lunata* clade, confirming their *C. lunata* species status. All isolates were susceptible to the three organic fungicides and seeds treated with the three fungicides reduced fungal infection percentage. For safer environmental practices, these plant based fungicides are recommended for rice seed treatment.

Key Words: *Curvularia*, internal transcribed spacer region, phylogram

RÉSUMÉ

Les espèces de *Curvularia* sont des champignons séminicoles de plus en plus importants, dont l'identification a été effectuée uniquement sur la base de caractéristiques culturelles et morphologiques au Ghana. Pour confirmer l'identité du champignon au Ghana, des isolats du champignon obtenus à partir de semences de riz conservées par les agriculteurs ont été identifiés à l'aide de caractéristiques culturelles et morphologiques, complétées par une analyse phylogénétique des régions d'espacement internes transcrites des isolats. La sensibilité des isolats à trois fongicides à base de plantes, Levo, Kobe et Ex-icute a été déterminée *in vitro* et *in vivo*. Tous les isolats ont produit des spores de forme courbe avec plusieurs septations. Dans le phylogramme, ils se sont tous regroupés dans le clade *C.*

lunata, confirmant leur statut d'espèce *C. lunata*. Tous les isolats étaient sensibles aux trois fongicides organiques et les semences traitées avec les trois fongicides ont réduit le pourcentage d'infection fongique. Pour des pratiques environnementales plus sûres, ces fongicides à base de plantes sont recommandés pour le traitement des semences de riz.

Mots Clés : *Curvularia*, région d'espacement interne transcrite, phylogramme

INTRODUCTION

Rice (*Oryza* sp.) is currently one of the major food staples consumed in Ghana (Essabrah-Mensah, 2018). Demands for the crop outstrip production (Ragasa *et al*., 2014), making the country, food insecure, in terms of this crop. Estimates show that local production of the crop lags behind demand by between 66-70%, a consequence of the growing preference for rice among Ghanaian households, especially as consumers become wealthier and more urbanised (IFPRI, 2020).

To bridge the widening demand-supply gap, huge sums of foreign currency are spent on rice imports to supplement local production in the country. Between 2007 and 2015, the amount spent on imported rice rose from United States dollars 151 m to 1.2 bn. It is, however, clear that if local production of rice is improved, the import bill of rice in the country will reduce, thereby freeing cash for other sectors of the economy. However, one major problem confronting the local production of rice in Ghana, is the lack of good quality seeds.

In Ghana, majority of rice farmers save part of their harvest of a particular season, as seeds for rice production for the following season. Studies have shown that farmer-saved rice seeds in Ghana are infected with fungi of different taxonomic groupings. Osumanu (2012), identified 15 fungi from farmer-saved seeds from the major agroecological zones of Ghana. Asamoah (2012), also identified 20 fungal species from farmer-saved rice seeds collected from inland valleys of the Ashanti region of Ghana.

Among the major fungi frequently isolated from farmer-saved rice seeds worldwide are members of the genus *Curvularia*. *Curvularia* species which include both pathogens and saprophytes (Sanchez-Marquez *et al*., 2008). They have been associated with foliar and sheath diseases of rice in the field and have been demonstrated to be seed-borne (Mew and Gozales, 2002). *Curvularia protuberata* has been associated with germination failure of rice seeds (Sisterna and Dal Bello, 1998). Twelve species of *Curvularia,* namely, *C. eragrostidis, C. intermedia, C. siddiquii, C. oryzae*, *C. lunata, C. pallescens, C. trifolii, C. clavata, C. geniculata, C. inequalis, C. uncinata and C. cymbopogonis,* have been detected from rice seeds (Benoit and Mathur, 1970; Rashid, 2001). In Ghana, *C. lunata*, *C. pallescence* and *Curvularia* sp have been reported on rice seeds (Asamoah, 2012; Osumanu, 2012). *Curvularia lunata* is one of the few species of the genus *Curvularia*, widely reported on rice seeds in Ghana (Asamoah, 2012; Osumanu, 2012). Since the year 2010, the fungus has been associated with brown leaf spots of rice, making it one of the fungi of interest in the rice production system in Ghana (Honger; unpublished data). However, the presence of the fungus in the country is still contestable considering the crude cultural and morphological methods used for its identification. Although it is not entirely true, the influence of the environment on these features often leads to wrong inferences about fungal diseases (Nur *et al.,* 2015). There is, therefore, need more accurate methods to be deployed for the identification of the fungus in Ghana.

Although chemical treatment of seeds is the common method for controlling seed borne fungi, the inability of most rice farmers in Ghana to separate their seeds from the grains,

means that measures must be taken to avoid the use of inorganic fungicides in the treatment of rice seeds. On the other hand, there are organic fungicides available on the markets, which could be used for the treatment of rice seeds against these fungal species. These plant derived fungicides, however, have not been evaluated for the purpose under Ghana conditions. The objective of this study was to confirm more precisely the identity of this pathological fungus and its response to selected plant-derived fungicides available on the market in Ghana.

MATERIALS AND METHODS

Study area. Farmer-saved rice seeds of different varieties were obtained from the irrigated rice production areas of the Coastal savannah zone comprising of the Greater Accra (Ashiaman and Asutuare), Eastern (Akuse and Kpong) and Volta (Afife) regions of Ghana. Irrigation schemes have been set up in these areas and farmers have access to fresh water from the Volta Lake that meanders from the Northern part to the coastal areas of the country, to the South, where it enters the sea.

Assessment of the health status of farmer-saved rice seeds. Farmer-saved rice seeds collected from 75 farmers were used in the study. Four hundred seeds from each farmer were assessed for their health status, using the standard blotter method (ISTA, 2015). Petri plates, lined with a double layer of moistened filter paper were set up and 25 seeds were placed in each plate and incubated under alternating light and darkness of 12 hours of light and 12 hours of darkness, for 7 days. Seeds were examined under a stereomicroscope and fungi showing the characteristic of curved and septated spores of *Curvularia,* were plated on PDA (39 g l^{-1}) for 7 days at 28 °C. Colony diameter was measured daily for 7 days and growth rate was calculated as the seven-day average of mean daily growth (millimetres per day). Mycelia and spores were fixed on slides and the shape, length and breadth of 20 conidia harvested from the cultures were recorded.

DNA extraction and polymerase chain reaction. Deoxyribonucleic acid (DNA) was extracted from *Curvularia* species, using the CTAB method. Polymerase chain reaction (PCR) was carried out with the DNA extracted from isolates as templates. The primer pair ITS1/ITS4, (ITS1: 5'-TCCGTAGGTGAAC CTGCGG-3'and ITS4: 5'-TCCTCCGCTTAT TGATATGC-3') designed to amplify the internally transcribed spacer (ITS) region of isolates (White *et al.,* 1990), was used in the PCR. The mixture was made up of 5 µl of template DNA, 2.5 µL each of forward and reverse primers, 1.25 µl of 2 m M MgCl2, 25 µl of master mix (10 m M Tris-HCl, 50 mM KCl, 1.5 m M MgCl2, 0.2 m M dNTPs, 5% Glycerol, 0.08% IGEPAL® CA-630, 0.05% Tween® 20, 25 units ml^{-1} Taq DNA Polymerase, pH 8.6@25°C) (New England Biolabs, UK) and 13.75 µl of deionised autoclaved water. A negative control was included in the amplification. The PCR cycles were as follows: initial denaturation at 95 ^{O}C for 30 s, followed by 35 cycles of denaturing, annealing and extension at 95 ^{O}C for 10 s, 59 ^{O}C for 15 s and 72 ^{O}C for 30 s, with a final extension at 72 ^{O}C for 5 min. The Amplification products were separated by 1.5% w/v agarose gel (Invitrogen, Carlsbad, CA), stained with Ethidium bromide alongside 1.0 kb marker at 100 V for about 1.5 hours. Bands were observed under UV light.

Purification and sequencing of amplified products. The PCR amplified product of the ITS region of isolates were sent to Inqaba Biotech in South Africa for purification and sequencing. Ten picomole of each primer was used to sequence the product directly from both directions. Sequences were entered into the BIOEDIT software and edited and consensus strand for each isolated generated from the forward and reverse strands

Phylogenetic analysis of sequences. Sequences of 4 isolates of the suspected *C. lunata* obtained in this study (Table 1), were aligned to sequences of 28 *Curvularia* species, downloaded from the EMBL data base (Table 2). The sequences included that of *C. siamense*, which was used as an out-group. The sequences of the 32 isolates were aligned by Clustal W and the alignment generated was used to draw a phylogram.

The phylogram was drawn using MEGA 5 software (Tamura *et al.,* 2011). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap test was used to indicate the percentage of replicate trees in which the associated taxa clustered together (1000 replicates). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.,* 2004). All positions containing gaps and missing data were eliminated.

Efficacy of organic fungicides. The effect of organic fungicides on the mycelia growth of *C. lunata* was evaluated using five treatments, including single rate of four different fungicides, a reference fungicide and a non-treatment control (Table 3). One hundred milliliters of molten potato dextrose agar (39 g l^{-1}) in 150 ml conical flasks, were amended with the required rates of the different fungicides, and poured into sterilised Petri dishes to set. Plugs of mycelia of a 7 day old culture of *C. lunata* on PDA, were taken using an 8 mm cockborer and placed singly in the middle of the Petri dishes containing the fungicidal-amended PDA. The plates were covered and tied in transparent polyethene bags; and incubated in the laboratory at temperature of 22-25ºC. The control was PDA without fungicide treatments.

Each treatment consisted of 5 petri dishes, and the entire experiment was repeated once. The plates were monitored daily and the diameter of the mycelial growth measured along diagonal lines drawn at the reverse side of the plates, using a marker pen and a ruler. Data collection commenced at 3 days after incubation, when substantial growth was observed in the control plate; up to the 8th day after incubation, when the growth has covered the entire plate in the control plates. Data collected were used to calculate percentage inhibition, using the formula:

$$\text{Percentage inhibition} = \frac{C\text{-}T \times 100}{C}$$

Where:

C = radial mycelial growth in control plates; and T = radial mycelial growth in treated plates.

The percentage inhibition was arcsine transformed and subjected to analysis of variance, using GenStats software, version 12. Means were separated using LSD at P< 5%.

Effect of organic fungicides on Curvularia-infected rice seeds. Clean and healthy rice seeds of the Legon I variety, were procured from the Soil and Irrigation Research Centre. The health status of the sample was first determined by placing 100 seeds in 4 petri dishes (25 each) lined with moistened filter

TABLE 1. Isolates of *Curvularia species* obtained from this study and their GenBank accession numbers

Strain identification	Host	Source	GenBank accession numbers (ITS)
CLCRS-01	*Oryza sativa*	Seeds	MW600259
CLCRS-02	*Oryza sativa*	Seeds	MW600260
CLCRS-03	*Oryza sativa*	Seeds	MW600261
CLCRS-04	*Oryza sativa*	Seeds	MW600262

TABLE 2. Sequences of *Curvularia* species downloaded from the EMBL nucleotide data base and their accession numbers

Species	Strain identification	Host	Country	Accession numbers
C. aeria	CBS 294.61	Air	Brazil	HE861850
C. aeria	FMR 11667	Blood	USA	KP131931.1
C. americana	UTHSC	Homo sapien	Spain	NR_146239.1
C. americana	UTHSC 10-1276	Homo sapien	Spain	HG779020.1
C. americana	UTHSC 09-2863	Homo sapien	Spain	HG779019.1
C. australiensis	BRIP 19588a	*Chloris gayana*	Australia	KC424613
C. australiensis	IMI 53994	*Oryza sativa*	Australia	KC424595
C. australiensis	FC2AP	*Aegle marmelos*	India	KR363626
C. coicis	CBS 192.29	-	Japan	NR_147457.1
C. coicis	ZJ13	Coix Lachryma-jobi	China	KJ572136.1
C. coicis	CBS 126978	-	Netherlands	MH864368.1
C. geniculata	CDKVR02	*Cynodon dactylon*	India	KP666183.1
C. geniculata	EAN403	-	Malaysia	MK518444.1
C. lunata	CBS 730.96	*Bouteloua dactyloides*	Ex-type	NR_138223.1
C. lunata	B2836	Soil	Malaysia	MK204512.1
C. oryzae	CBS 169.53	Ex-type	-	NR_138221.1
C. oryzae	2715	*Espeletia sp.*	Colombia	EU272519.1
C. oryzae	C104	*Glycine max*	Brazil	JQ936251.1
C. pseudobrach.	CPC 28808	*Eleucine indica*	Thailand	NR_164423.1
C. pseudobrach.	HNWN001	*Arecha catechu*	China	MH516132.1
C. senegalensis	JUF0019	*Aloe Vera*	Bangladesh	MH368101.1
C. senegalensis	SC5.2	*Saccharum sp.*	India	MH087110.1
C. siamense	MAN-GH19	*Mangifera indica*	Ghana	KJ019351.1

TABLE 3. Treatments evaluated for their effect on the mycelial growth of *C. lunata*

Name	Active ingredient	Use	Rate of application
Ex-icute	51% plant oils	Organic fungcide	5 ml l^{-1}
Kobe	Chrysophenol parietin	Bio-fungicide	15 ml l^{-1}
Levo	50% Sophora flavescent plant extract	Botanical insecticide/fungicide	15 ml l^{-1}
Mancozeb	Mancozeb	Inorganic fungicide	4 g l^{-1}
Water	-	Control	-

paper and incubated for 7 days. Results showed that seeds were free from the major fungal species infecting rice. Spore suspension of *C. lunata* was prepared by scrapping mycelia of an 8 day old culture of the fungus on PDA and mashing it in sterile distilled water. The slurry was then filtered through double cheese cloth and the spore suspension adjusted to 1.5 x 10^7 spores/ml.

Five hundred seeds of the rice sample obtained, were soaked in 200 ml of the spore suspension of *C. lunata* for 5 minutes; after which the seeds were retrieved and kept on moistened filter paper in Petri dishes, to maintain high humidity. Twenty four hours later, the five hundred seeds were divided into 5 lots of 100 seeds per lot. Each lot was soaked in one of the treatments stated in Table 3, for 15 minutes; followed by air drying. The control was seeds soaked in sterile distilled water only. Treated and the control samples were placed on moistened filter papers in Petri dishes (4 petri dishes of 25 seeds per dish for each treatment), and incubated at 22-25 ºC and 65% RH. Seven days after incubation, data on the number of seeds infected and number of seeds that germinated, were collected and used to calculate the following:

$$\text{Percentage infection} = \frac{\text{Number of seeds infected}}{\text{Number of seeds incubated}} \times 100$$

$$\text{Percentage germination} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds incubated}} \times 100$$

Seedlings vigour = (length of radicle + length of plumule) x percentage germination

The experiment was repeated once and the mean of the data obtained were calculated prior to analysis**.** Data on percentage inhibition of infection and percentage germination of seeds were arcsine transformed and together with the seedlings vigour, were subjected to analysis of variance using GenStat statistical package (12^{th} edition). Means were separated with Least Significant Difference at 5% probability level.

RESULTS

Cultural and morphological characteristics of *Curvularia* species. Cultural and morphological characteristics of isolates did not differ significantly among the *Curvularia* species obtained from the farmer-saved rice seeds (Table 4). Isolates of the fungus produced suede-like to downy, black colonies of mycelium which took 7 days to fill an entire 8 mm Petri dish, containing PDA (Fig. 1 A). The average growth rate was 1.1 mm day^{-1} (Table 4). The hyphae were septate and dark in colour. The conidiophore was erect, septate, unbranched and dark reddish brown. The conidia were ellipsoidal, smooth-walled, rounded at the end or became thinner toward the end. Its olive brown end cells were paler, while it was large, dark and curved at the subterminal cell (Fig. 1 B). The subterminal cell bulge and was larger than the rest of the cells. Conidial were of dimensions 15.3 to 22 × 8.7 to 10.9 (average 18.6 × 9.8 µm) (Table 4). The characteristics of the isolates showed they were *C. lunata.*

TABLE 4. Cultural and morphological characteristics of isolates of *Curvularia* species obtained from farmers-saved rice seeds

Isolate designation	Colour of mycelia	Growth rate (mm day^{-1})	*Conidial dimensions (µm)
CLCRS-01	Suede-like to downy, black	1.10	18.5 x 9.9
CLCRS-02	Suede-like to downy, black	1.10	18.5 x 9.8
CLCRS-03	Suede-like to downy, black	1.10	18.6 x 9.8
CLCRS-04	Suede-like to downy, black	1.10	18.6 x 9.7

*n=20

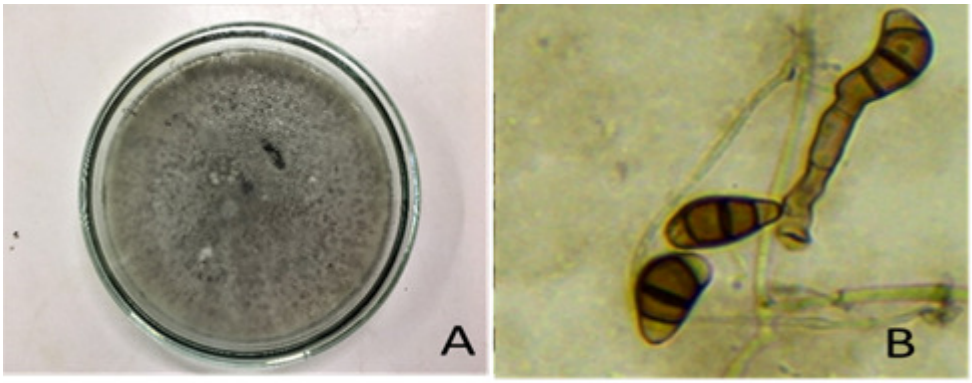

Figure 1. Cultural and morphological characteristics of *C. lunata* isolated from rice seeds. A = mycelial growth on PDA; B = ellipsoidal condia and conidiophore. Mg. x400.

Molecular characterisation (Phylogenetic analysis of ITS region of *C. lunata*). An approximately 600 bp product of the ITS region was amplified using the primer pair ITS1/ITS4 from the isolates. The assembled sequences were 568 bp long. Approximately, 555 bp product of the ITS region from isolates collected in the study and those retrieved from the GenBank (Table 3), were aligned and used in phylogenetic analysis. The optimal tree with the sum of branch length of 0.59480979 is shown in Figure 2. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches (Felsenstein, 1985). There were a total of 422 positions in the final dataset. Out of the 32 *Curvularia* species used in the study, all 4 isolates obtained from the rice seeds in Ghana clustered together in the *Curvularia lunata* clade. The clade includes the strain type of *C. lunata* and another *C. lunata* strain of confirmed identity. The clade was supported by a high bootstrap of 98% (Fig. 2). This shows that the isolates obtained from rice seeds belongs to the *Curvularia lunata* species.

***In-vitro* evaluation of fungicides.** There was a significant difference ($P<0.05$) in inhibition of the mycelial growth of *C. lunata,* among the different fungicides from day 3 to day 7 after incubation (Table 5). In each of these days, the highest inhibition of 100% was obtained on media amended with Mancozeb; while the lowest was obtained on media amended with Levo or Kobe. From day 3 to 6, there was no significant difference in inhibition obtained on media amended with Mancozeb and Ex-icute. However, the difference in inhibition on these two media was significant at day 7 after incubation, with Ex-icute amended media resulting in a lower inhibition of 80% (Table 5). Also, the inhibition of the mycelial growth of the fungus on Levo and Kobe was significantly lower than on Mancozeb and Ex-icute, amended media at day 7 after incubation (Table 5).

Seed germination and seedlings vigour. There was a significant difference ($P<0.05$) in the control of *C. lunata* on infected seeds (Table 6). The highest value was obtained on seeds treated with Mancozeb; while the lowest was obtained on seeds treated with Kobe. There was no significant difference in control of *C. lunata* on seeds treated with Ex-icute, Levo and Kobe (Table 6).

Also, there was a significant difference ($P<0.05$) in percentage germination of seeds treated with the different fungicides and the non-treated seeds (Table 6). The highest of 88.0% was obtained on seeds treated with Mancozeb and Kobe; while the lowest was obtained on seeds that were not treated with any fungicide. The percentage germination obtained on seeds treated with Levo was,

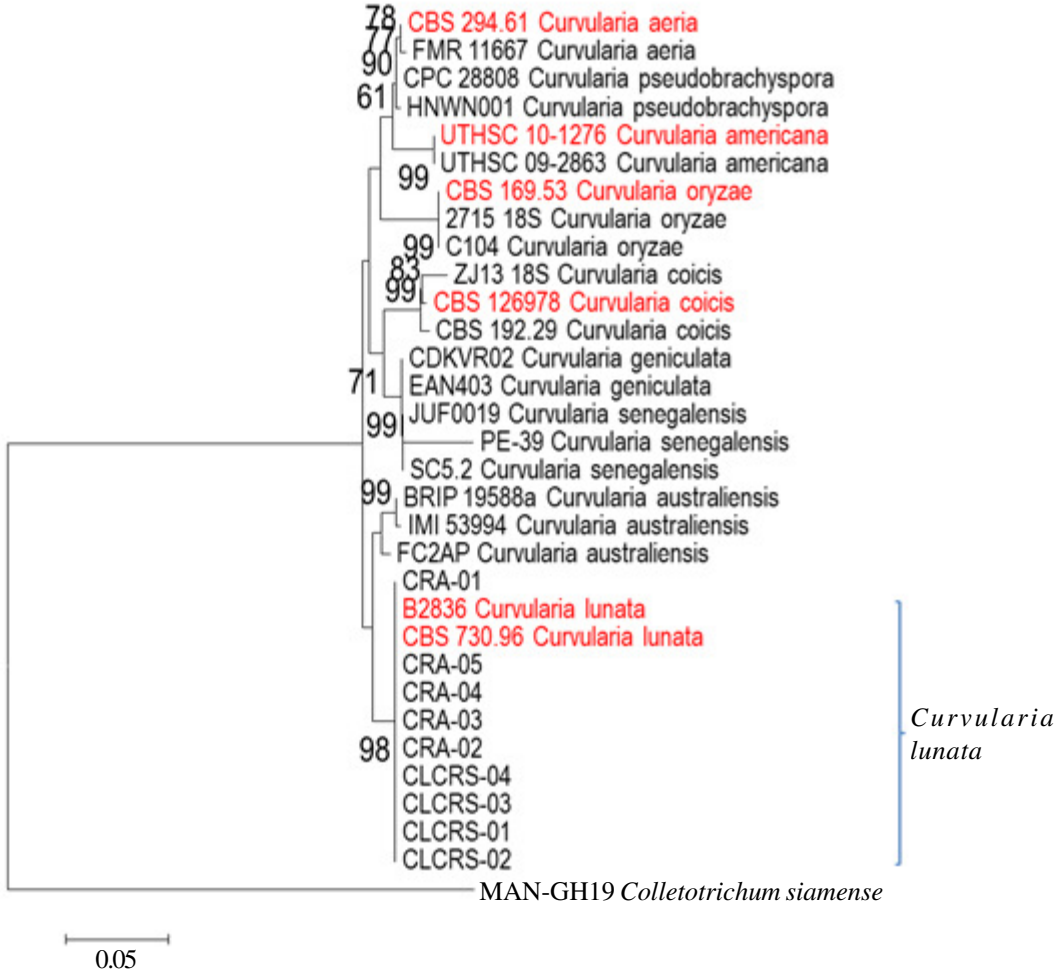

Figure 2. A phylogram drawn with the multiple sequence alignment generated with the rDNA-ITS sequences of the 32 isolates of *Curvularia* species used in the study. *C. siamense* was used as an out group. Isolate designation and species name have been listed. Strain types are indicated in red.

TABLE 5. Inhibitory effect of different fungicides (%) on the radial mycelial growth of *C. lunata* obtained from rice seeds

Treatment (Fungicide/15 l of water)	Number of days after incubation				
	3	4	5	6	7
Ex-icute (5 ml)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	80.2 (63.6)	80.0 (63.5)
Kobe (15 ml)	53.9 (47.2)	53.3 (46.9)	56.2 (48.5)	44.0 (41.5)	41.0 (39.8)
Levo (15 ml)	56.7 (48.9)	73.3 (68.9)	49.0 (42.8)	41.0 (39.8)	53.0 (46.7)
Mancozeb (60 g)	100.0 (90.0)	100. (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
L.S.D (5%)	1.9	36.2	9.6	10.5	7.7

Transformed data in parenthesis

TABLE 6. Effect of different fungicidal treatments on the percentage control of *C. lunata* on infected seeds, percentage germination of seeds and seedlings vigour of treated rice seeds

Treatment	Percentage control	Germination	Seedlings vigour index
Control	0.0	39.0 (38.4)	375.0
Ex-icute (5 ml l^{-1})	88.0 (70.6)	50.0 (45.1)	556.0
Kobe (15 ml l^{-1})	80.0 (63.5)	88.0 (70.6)	1156.0
Levo (15 ml l^{-1})	88.0 (69.9)	78.0 (62.1)	840.0
Mancozeb (4 g l^{-1})	99.0 (87.1)	88.0 (70.0)	875.0
LSD (P>0.05)	7.9	10.8	287.0

Transformed data in parenthesis

however, not significantly different from what was obtained on seeds treated with Mancozeb and Kobe. Among the fungicidal treated seeds, the lowest percentage germination was obtained on seeds treated with Ex-icute (Table 6).

Seedling vigour index was significantly different (P<0.05) among treatments (Table 6). The highest index of 1156.0 was obtained from seeds treated with Kobe; while the lowest was obtained from untreated seeds. The difference in vigour index from seeds treated with Kobe and Mancozeb was not significant. Also, the difference in vigour index between untreated seeds and Ex-icute treated seeds was not significant (Table 6).

DISCUSSION

All the isolates of the fungus obtained in this study, produced curved shaped conidia, characteristic of the members of the *Curvularia* genus (Fig. 1B). Based on the traditional methods alone, these isolates could be identified as either *C. lunata* of *C. pallescence,* which have been reported previously on rice seeds in Ghana (Asamoah, 2012; Osumanu, 2012) or any of the *C. eragrostidis*, *C. intermedia*, *C. siddiquii*, *C. oryzae*, *C. pallescens*, *C. trifolii*, *C. clavata*, *C. geniculata*, *C. inequalis*, *C. uncinata and C. cymbopogonis*, which have been reported on rice seeds in different parts of the world (Benoit and Mathur, 1970; Rashid, 2001). This shows that while all the isolates can be placed in the *Curvularia* genus, their species status cannot be easily resolved with the traditional methods alone. There was, therefore, need for the methods to be complemented with molecular characterisation, for proper identification of the isolates.

Molecular phylogenetics is one of the reliable methods available for delineating among fungal species (Damm *et al*., 2000; Honger *et al*., 2015). In the present study, isolates with confirmed identities clustered in clades corresponding to their original species in the phylogram (Fig. 2). The clades were well supported with high bootstrap values, an indication that the method was quite robust. In the same phylogram, all the isolates obtained from the rice seeds in Ghana, clustered in the *C. lunata* clade, confirming their species status. Identification of previously unknown fungi by means of phylogenetic studies has been achieved by several authors worldwide (Damm *et al*., 2000; Honger *et al*., 2014). Though in most cases, there was a combination of several genes or gene regions, others have been done with reliance on the ITS region alone (Nur *et. al*., 2015). The high bootstrap support of the *C. lunata* clade in the phylogram drawn in this study, was a further confirmation of the reliability of the method employed in the present study. Therefore, combining the cultural and

morphological features of the fungi obtained from the rice seeds in this study, with the sequence analysis of the ITS region, has confirmed with minimum doubt, that the isolates were *C. lunata*. *Curvularia lunata* has been properly identified on rice seeds in India (Yuvarani *et al*., 2021) and this, therefore supports its identification in this study.

Curvularia lunata has been associated with leaf spot disease of rice in Ghana (Honger; unpublished data) and is, therefore, fast becoming a pathogen of interest in rice growing areas of the country. Therefore, it has been suggested that control measures against the pathogen should start with the seeds, since the pathogen is seed-borne (Mew and Gonzales, 2002). Seed treatment using fungicides may be the fastest way to achieve this. Currently, chemical control of fungal pathogens is gradually shifting from the use of synthetic fungicides, which leave harmful residues in food and damage the environment, to less harmful substances derived from plant sources (Sati and Josh, 2011). In the present study, the three products, Levo, Kobe and Ex-icute, derived from plant sources, were inhibitory enough to mycelial growth of *C. lunata* and could, therefore, be used to control the fungus. All three products ably inhibited growth of *C. lunata* isolated from the rice seeds, with Ex-icute matching the performance of Mancozeb. Several studies have shown that products derived from plant sources can be detrimental to growth of fungal species. For example, Awurum and Ucheagwu (2013), reported that *X. aethiopica* extracts reduced the frequency of *Mucor* spp., *Fusarium* spp., *Aspergillus* spp., *Colletotrichum* spp. and *Curvularia* spp. isolated from cowpea seeds in Nigeria. Rongai *et al*. (2015), also reported that *Salvia guaranitica and Punica granatum* were effective against *Fusarium oxysporum*. Ozcan and Chalchat (2008) reported that Rosemary essential oil was effective against *Fusarium oxysporum, Botrytis cinerea* and *Alternaria alternata*. Crude extracts of *Agapanthus africanus* showed antifungal action against phytopathogens *in vitro* and in *vivo* (Tegegne *et al.,* 2007).

The two products, Ex-icute and Levo, effectively reduced the infection of rice seeds by the *C. lunata* (Table 6). Seeds treated with these plant products resulted in improved seed germination and seedlings vigour. Similar findings have been reported by Ahmed *et al.* (2013). Hassan *et al.* (2015) also reported that local plant based extracts effectively manage seed borne fungal species resulting in an increase in seedling germination and vigour. Since in most African countries farmers may not distinguish between grains and seeds; and when under pressure, may mill and consume the seeds as grains (Nambiro *et al*., 2004), it is safer for them to treat their seeds with these plant based substances, rather than the synthetic inorganic toxic fungicides.

CONCLUSION

Some isolates of fungi suspected to be *C. lunata* were obtained from farmer-saved rice seeds in Ghana and were identified using their cultural, morphological features and sequence analysis of the ITS region. This clears all doubts about the identity of the fungus in Ghana, which was previously identified based on the traditional methods alone. To the best of our knowledge, this is the first report of identification of *C. lunata* on rice seeds by means of phylogenetic studies and morphological features. The isolates are highly sensitive to some plant derived products, namely, Levo, Ex-icute and Kobe. These fungicides were able to reduce the infection of rice seeds by the fungus, *C. lunata* and in most cases, improved the seeds germination and seedlings vigour of infected rice. For safety purposes, these plant based fungicides are recommended for the control of *C. lunata* (and possibly other fungi) on rice seeds.

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