*Ghana J. Sci.* **55** (2015), 51 – 65

# IMPORTANCE OF THE ONION LEAF TWISTER DISEASE IN GHANA AND THE EFFECT OF *TRICHODERMA ASPERELLIUM* ON THE MYCELIAL GROWTH AND SPORULATION OF THE CAUSAL AGENT

N. Gyempeh, S. K. Offei, E. W. Cornelius and J. O. Honger*

*(N.G. & E.W.C.: Department of Crop Science, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon; S.K.O.: Biotechnology Centre, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon; J.O.H.: Soil and Irrigation Research Centre, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon)*

**Corresponding author's email: johonger@yahoo.com*

## Abstract

Studies were carried out to determine the disease incidence and severity of the onion leaf twister disease in the Eastern Region of Ghana to confirm the aetiology of the disease in the country. Field survey was carried out in two districts of the Eastern Region and the disease incidence and severity were measured. The causal agent was isolated on media and identified using morphological characteristics, and polymerase chain reaction with the species specific primer CgInt combined with ITS4 primer. The effect of *Trichoderma asperellium* on the mycelial growth and sporulation of the causal agent of the disease were determined. The results showed that the disease incidence was 43.6 per cent and 62.5 per cent in the Kwahu South and Fanteakwa districts, respectively, with severity of 0.8 and 2.7 respectively, on a scale of 1 – 5. The causal agent was confirmed as *Colletotrichum gloeosporioides*. The bio-agent, *T. asperellium* was able to significantly suppress the mycelial growth and sporulation of the pathogen infecting the onions.

## Introduction

Onions (*Allium cepa* L.), are plants of economic importance and have world-wide importance (Griffiths *et al*., 2002). The crop ranks fourth in world production of vegetables, with a volume of 64,101t (FAO, 2005). It is an important vegetable in Ghana (Sinnadurai, 1992) which is mainly cultivated on commercial basis in the Northern and Upper East regions. Production in southern Ghana is concentrated in Fanteakwa and Kwahu South districts (Eastern region), Akatsi (Volta region), Ashaiman (Greater Accra Region), Berekum (Brong Ahafo Region) and Mankessim (Central Region) (Awuah *et al*., 2009). The most common varieties grown in Ghana are Bawku red and Malavi. Other imported cultivars include Crystal white, Red creole and Texas grano (Abbey *et al*., 2000). Onions are usually grown for use as a flavourer in foods, and are also of medicinal importance in Ghana and West Africa, where they are used in the treatment of chicken pox, common cold, influenza, measles, and rheumatism (Schwartz & Mohan, 1995).

Despite its importance, onion is susceptible to a number of diseases in Ghana including *Botrytis* leaf blight, bulb rot, downy mildew, onion twister, purple blotch, pink

rot, soft rot and white rot (Oduro, 2000; Offei *et al*., 2008). In 2009, a leaf twisting disease which was occasionally accompanied by curling, twisting, and chlorosis of leaves, elongated neck and sunken lesions occurring on leaf sheaths was reported by farmers in the Fanteakwa (Diedeso, Papramantam, Mpaemu) and Kwahu South (Adawoso, Amartey, Kwahu Amanfrom) districts of the Eastern region. The disease was locally called by farmers as 'akyimkyimakyimkyim' literally meaning twisting which is different from *Fusarium* bulb rot disease of onion caused by *Fusarium oxysporum* f.sp. *cepae* reported in the New Oworobong area of the Eastern Region in the 1980's (Awuah *et al*., 2009). The leaf twisting disease was reported to spread fast, and farmers estimated that the disease could wipe between 50 per cent to entire crops on an infected field.

All the varieties of onion grown in the area were reported to be susceptible to the disease, and this resulted in a situation where some farmers abandoned onion cultivation. Making matters worse, the agricultural extension agents (AEAs) in the affected areas were not able to diagnose the disease and, therefore, could not recommend appropriate control measures (Azidoku & Gyemfi, verbal communication, 2010). Offei *et al*. (2008) reported of a leaf twisting disease that was attributed to *Colletotrichum gloeosporioides*, however, the scale of destruction caused by the disease raised questions as to whether the two diseases were the same. Due to the scanty information about the outbreak of the disease in these areas, there was the need for a study that would generate important information for the formulation of control measures against the disease.

Currently in Ghana, application of fungicides for the control of diseases caused by fungi is the commonest form of disease control being practiced. However, problems associated with the method such as harmful side effects to the environment and the farmer, have stimulated research into ways of exploring more environmentally friendly methods for the management of plant disease worldwide. Among these novel methods is the use of biological agents that are antagonistic to the disease causing pathogen.

Species within the genus *Trichoderma*, have been evaluated in several areas and found to be very potent in the control of target pathogens (Verma *et al*., 2007). Elsewhere, *Trichoderma harzianum* has been used successfully to control witches broom on cocoa (De Marco *et al*., 2003), and *Trichoderma lignorum* and *Trichoderma virens* have been very effective in the control of damping off of beans (Verma *et al*., 2007). *Trichoderma asperellium* has also been found to be very effective in reducing the epidemics caused by *Rhizoctonia solani* on tomato (Cotxarrera *et al*., 2002). The bioagent, *T. asperellium* has been imported into Ghana for efficacy trials on some selected plant pests and pathogens, and it would be worthwhile to determine its efficacy on the causal agent of onion leaf twister disease in Ghana for a possible adoption to control the disease in the country.

The study was, therefore, carried out to determine the incidence and severity of the onion leaf twisting disease in the Kwahu South and Fanteakwa districts in the Eastern Region of Ghana, confirm the aetiology of the disease using morphological, molecular and pathogenicty studies and evaluate the

effect of *T. asperellium* on the growth and development of the causal agent of the disease.

## Experimental

*Field survey*

Two districts namely Fanteakwa and Kwahu South where leaf twisting disease of onion had been reported were selected. In each district, three sites where the disease was prevalent were randomly selected for the study. The selection was done by first obtaining a list of onion farmers from the AEA's working in the two districts. Focus group discussions were also held with the AEA's to determine the communities in their districts where the disease was prevalent. Five onion farmers in such communities were then randomly selected from the list of onion farmers provided by the AEA's.

*Determination of incidence and severity of leaf twisting disease of onion in the Fanteakwa and Kwahu South districts*

The incidence of leaf twisting of onion in the three experimental sites in each district where the disease was known to be endemic was determined by first selecting five onion farms at random. On each farm, five quadrants of 1 $m^2$ were demarcated on the field (four quadrants placed at the corners and one at the center of the field), and onion plants found in these marked areas were counted. Disease incidence (DI) (Vidhyasekaran, 2004) was calculated using the formula:

$$DI\ (\%) = \frac{NIP}{TNP} \times 100$$

where NIP is the number of infected plants, and TNP the total number of plants (infected and uninfected). Individual plants were then scored for disease severity using a modified 1 – 5 disease assessment key (Table 1) (Alberto, Duca & Miller, 2003). The data obtained were compared for the two districts using *t*–test. Genstat software 9.2 version was used to perform the analysis.

Table 1

*Assessment key for severity of onion twister disease*

| *Disease score/index* | *Meaning* |
|---|---|
| 1 | No symptom on plant/healthy plants |
| 2 | Curling, twisting/wilting of leaves |
| 3 | Yellowing, chlorosis of leaves |
| 4 | Elongation of neck region |
| 5 | Rotted bulbs +/- appearance of whitish, oval, sunken lesions, pinkish acervuli |

*Isolation and morphological identification of fungus associated with leaf twisting disease of onion*

Samples of diseased and healthy onion plant parts were collected and transported to the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, and kept in a refrigerator at approximately 5 °C, and used for the isolation and identification of the causal agent. The isolation of microorganisms was first done on water agar (Oxoid Ltd, Hampshire, England) and sub-cultured onto potato dextrose agar (Oxoid Ltd, Hampshire, England). Plates were incubated at 23 to 31 °C and 60 per cent to 70 per cent relative humidity (RH) for 7 days. After that, single spore cultures were produced (Choi, Hyde & Ho, 1999) and used for the following parts of the study.

*Identification of Isolated fungus: Cultural and morphological characteristics*

Identification of the isolates of the fungi was based on growth rate, colour, morphology of mycelia, conidia and sporulating structures as described by Arauz (2000),Agrios (2005) and Barnett & Hunter (2006). Slides for microscopic examination were prepared by mounting bits of mycelia and conidia in lactophenol cotton blue. Micrographs were then taken using a Canon Powershot SX120 IS digital camera

*Polymerase chain reaction (PCR)*

Deoxyribonucleic acid (DNA) was extracted from isolates using the Genomic DNA isolation protocol (CTAB) (McGarvey & Kaper, 1991). The extracted DNA was used as a template in a PCR, using species-specific primer for *Colletotrichum gloeosporioides* (CgInt; 5′-GGCCTCCCGCCTCCGGGCGG-3′) (Mills *et al*., 1992) in combination with the conserved primer ITS4 (5′-TCCTCCGCTTATTGATATGG-3′) (White *et al*., 1990). PCR Bead (~2.5 units of PuReTaq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2 200 µM dATP, dCTP, dGTP and dTTP and stabilizers including BSA) was used in a 25 µl reaction mixture. PCR was performed in a Thermal Cycler iCycler (BIORAD®, USA) using the following programme: Initial denaturation at 94 °C for 5 min followed by 30 cycles at 94 °C for 1 min, annealing at 55 °C for 2 min and extension at 72 °C for 3 min, and then a final extension at 72 °C for 10 min. PCR products (7 µl) were separated by horizontal gel electrophoresis in one per cent agarose in 1X TAE buffer at 80 V for 60 min. Gels were stained in dilute ethidium bromide (0.5 µl), visualised under UV light, and photographed using the gel documentation system.

*Pathogenicity test*

Pathogenicity test was based on the method described by Ebenebe (1980) and involved both foliar and soil inoculations. Sandy loam soil was dug, sieved (0.2 mm sieve) to remove debris and poured into 1m × 1m nursery seed box. The soil was sterilized by drenching with Mancozeb (2.7 $gl^{-1}$) and left overnight for excess water to drain off. Malavi onion seeds taken from a stock previously confirmed to be free from either *C. gloeosporioides* or *G. moniliformis* were drilled in 1 cm deep rows in the seed box and covered with a thin layer of soil. The seeds were watered and covered with a black polyethylene shade to reduce sunlight. Watering was done twice a day till 6 weeks after sowing. Cultural practices such as pricking out and hand weeding were done to ensure good seedling health. Seedlings were transplanted after 6 weeks into 9 cm plastic pots. Twenty-five plants were used for foliar spray inoculation treatment and another 25 plants for soil inoculation treatment. Each set of controls consisted of 15 plants.

Conidial suspension of the suspected causal agent was produced by comminuting samples of acervuli of the fungal isolate with 500 ml of sterile distilled water in a sterilised JBJ - 103 Blender for 60s, after which the slurry was filtered through two layers of cheesecloth. The conidial concentration of the filtrate was then adjusted to about 2.5 × 106 conidia $ml^{-1}$ using sterile distilled water with the aid of a haemocytomer. Plants se-

lected for foliar inoculation treatments were sprayed to wetness with the conidia suspensions. For soil inoculation treatments, 10 ml of spore suspension was pipetted into each 5 cm hole made at the root zone of the onion seedling. The control plants received only sterile distilled water. Treated plants and their controls were incubated in a humidity chamber (26 °C and 95% RH) for 4 days at the Biotechnology Centre, University of Ghana, Legon and then transferred to a greenhouse at the Department of Crop Science, University of Ghana, Legon, where the environmental conditions were maintained at 28 °C and 72 per cent RH for 28 days. The duration of sunlight was about 12 h per day. Infected plants were taken to the laboratory for re-isolation of causative organism.

*Effect of* T. asperellium *(a bio-control fungus) on mycelial growth and sporulation of* C. gloeosporioides

*T. asperellium* employed for the *in-vitro* antimicrobial assay was obtained from The Real Integrated Pest Management Company, Kenya. The assay for antagonism was performed on PDA in 9 cm petri dishes by dual culture method (Fokkeme, 1978). The mycelial plugs (5 mm diameter) of *C. gloeosporioides* and antagonist *T. asperellium* were placed on PDA in the same Petri dish 6 cm from each other. The paired cultures were incubated at 25 °C. Petri dishes inoculated only with the test pathogen served as control (Zivkovic *et al*., 2010). Each treatment was replicated three times and the set up was kept in the laboratory using a completely randomized design (CRD).

The percentage growth inhibition (PGI) of *C. gloeosporioides* was calculated using the formula:

$$PGI\ (\%) = \frac{KR\text{-}RI}{KR} \times 100$$

where PGI = percentage growth inhibition, KR = distance (measured in mm) from the point of inoculation to the colony margin on the control dishes and RI = distance of fungal growth from the point of inoculation to the colony margin on the treated dishes in the direction of the antagonist (Korsten & De Jager, 1995).

On the 3rd and 9th days after incubation, mycelial plugs of *C. gloeosporioides* were taken from plates containing the two fungi species and those containing the fungus alone. The plugs were taken from four symmetrical positions equidistant from the centre of each petri dish using a sterile cockborer. The plugs were introduced into a 10 ml distilled water in 50 ml bottles, and a drop of tween 20 solution was added to each bottle. The spores were detached by manual shaking of the bottles after which their numbers were counted with the compound microscope and a haemocytometer.

## Results

*Symptoms of leaf twisting disease of onion*

The most common field symptoms of the disease observed during the survey were curling, twisting, chlorosis of leaves, and abnormal elongation of the neck, rotted bulbs and appearance of whitish oval sunken lesions on leaf sheaths. In the advanced stage of the disease, some bulbs rot before harvest whilst others decay rapidly in storage. Some of the diseased plants had no visible acervuli or lesions on their leaf sheaths. In other instances, acervuli of *Colletotrichum* sp. were found on the leaf sheaths or on well-defined

sunken lesions on the leaf blades (Fig. 1).

*Incidence and severity of leaf twisting disease of onion in the Fanteakwa and Kwahu South districts*

Onion farms in Fanteakwa and Kwahu South districts recorded 62.5 per cent and 46.3 per cent mean incidence of the disease, respectively. There was no significant difference ($P < 0.05$, t = 1.64, t prob. = 0.113, df = 28) in the incidence of leaf twisting disease between infected farms in Fanteakwa and Kwahu South districts. The severity of the disease was 2.7 in Fanteakwa which was significantly ($P < 0.05$, t = 4.47, t-prob. < 0.001, df = 28) higher than that at Kwahu South District which was 0.8.

*Identification of the causal agent of the disease*

*C. gloeosporioides* was consistently isolated from diseased onion plants sampled from nurseries and farms in the two districts. Culture on PDA consisted of whitish fluffy aerial mycelia covering the entire 9 cm plate within 7 days at 23 °C to 31 °C and 60 per cent to 70 per cent RH. The culture later developed pinkish acervuli at the center as the fungus grew (Fig. 2A). The hyaline conidia were short rods with rounded ends. Some of these were slightly narrower at the middle than at the ends whilst others were slightly curved (Fig. 2B). The average conidium measured 16.1 µm in length and 5.6 µm in width (data not shown). In the PCR, the species-specific primer CgInt in conjunction with ITS4 primer amplified approximately 500 bp fragment from genomic DNA of on-


Fig. 1. Onion plants selected from the field. A = Healthy plant with well-formed bulb; B = diseased plant showing twisted leaves

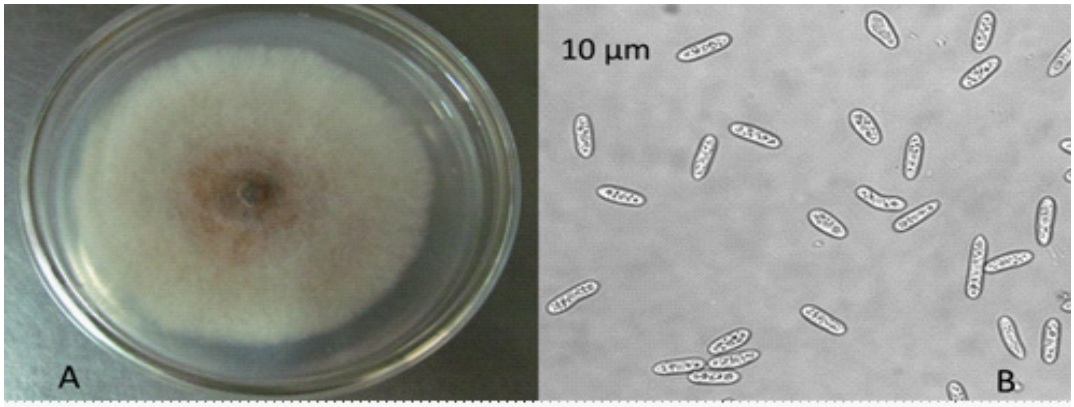


Fig. 2. Cultural and morphological characteristics of *C. gloeosporioides* isolated from diseased onion plants collected from the field. A = Mycelial growth on PDA after culturing for 7 days. B = Spores produced by the fungus (Note the rounded edges)

ion isolate and the reference mango isolate of *C. gloeosporioides*. No PCR product was amplified with water control in the reaction (Fig. 3).

*Pathogenicity test*

Similar symptoms were observed on the foliar spray-inoculated or soil-inoculated 7-week old onion seedlings. Curling and twisting symptoms appeared 2 days after inoculation on 25 per cent of foliar spray-inoculated and soil-inoculated seedlings in the growth chamber. On the 4th day after inoculation, 50 per cent each of the foliar spray-inoculated and soil-inoculated showed chlorosis of leaves. Within 12 days of inoculation, 80 per cent of foliar spray-inoculated and soil-inoculated plants had developed onion twister disease symptoms including curling, twisting, yellowing and well-defined sunken lesions on the leaf sheaths at the necks. These lesions contained clusters of acervuli of *C. gloeosporioides*. The control plants had no symptoms of the disease (Fig. 4). *C. gloeosporioides* was re-isolated from the artificially inoculated plants.

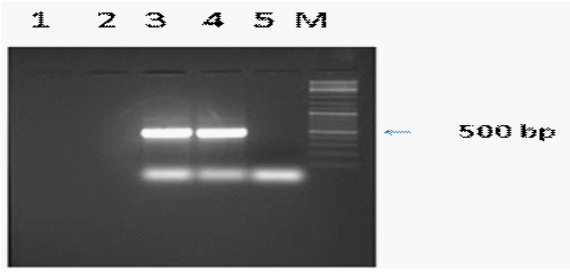


Fig. 3. A gel showing an approximately 500 bp PCR product amplified from DNA of isolates used in the study. Lane 5 = negative control (water), lane 4 = isolate from onion, lane 3 = reference isolate from mango, lanes 2 and 1 = empty wells. M = 1kb marker. (The primer pair CgInt/ITS4 was used in the PCR).

*The inhibitory effect of* T. asperellium *on mycelial growth and sporulation of* C. gloeosporioides

Initial colour of the young culture of *T. asperellium* was white. This changed to green and covered the 9 cm plate by the 4th day of incubation. There was no inhibition during the first 2 days of incubation since mycelium of the two fungi had in the first couple of days not inter-

Fig. 4. Onion twister disease on artificially inoculated onion seedlings. (From r-l; seedling inoculated with water through the foliage, seedling inoculated with water through soil, seedling inoculated with *C. gloeosporioides* through the foliage, seedling inoculated with *C. gloeosporioides* through soil).

acted. However, the interaction between the two was observed on the 3rd day of incubation, when the mycelial diameter growth of test *C. gloeosporioides* was 34 mm compared to the control (40 mm). Mycelium of *T. asperellium* grew rapidly to enmersh *C. gloeosporioides* and almost filled the 9 cm diameter petri dish. Mycelial growth of test *C. gloeosporioides* was inhibited by the *T. asperellium* unlike the control which continued to grow and filled the plate by the 9th day of incubation (Fig. 5). The percentage inhibition from the 3rd to the 7th day were 15 per cent, 21 per cent, 38 per cent, 49 per cent and 58 per cent, respectively (Fig. 6). Similar results were obtained after repeating the experiments.

*Inhibitory effect of* T. asperellium *on sporulation of* C. gloeosporioides

The antibiosis effect of *T. asperellium* on sporulation of *C. gloeosporioides* are shown in Fig. 7. There was 60 per cent inhibition of sporulation of *C. gloeosporioides* on the 3rd day of incubation. However, on the 9th day, there was a further increase in percentage inhibition of sporulation of *C. gloeosporioides* from 60 per cent to about 93 per cent when the control had totally covered the plate (Fig. 7).

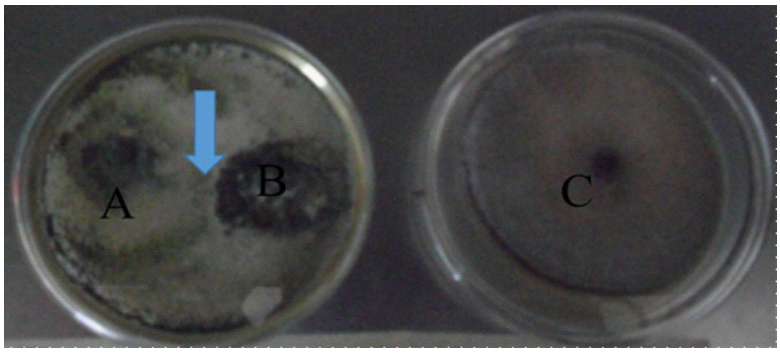


Fig. 5. Zone of inhibition (arrowed) between *T. asperellium* (A) and *C. gloeosporioides* (B) in dual culture assay on PDA after 9 days of incubation at 23ºC to 31°C and 60 per cent to 70 per cent RH. C = *C. gloeosporioides* growing on PDA without the *T. asperellium* (note the larger mycelial diameter compared to growth on the plate containing both species).

## Discussion

Symptoms associated with onion twister disease include leaf curling, twisting, yellowing, abnormal elongation of the neck region and rot of bulbs (Sikirou *et al.*, 2011; Ebenebe, 1980). These symptoms were all found associated with the diseased onion plants observed on the

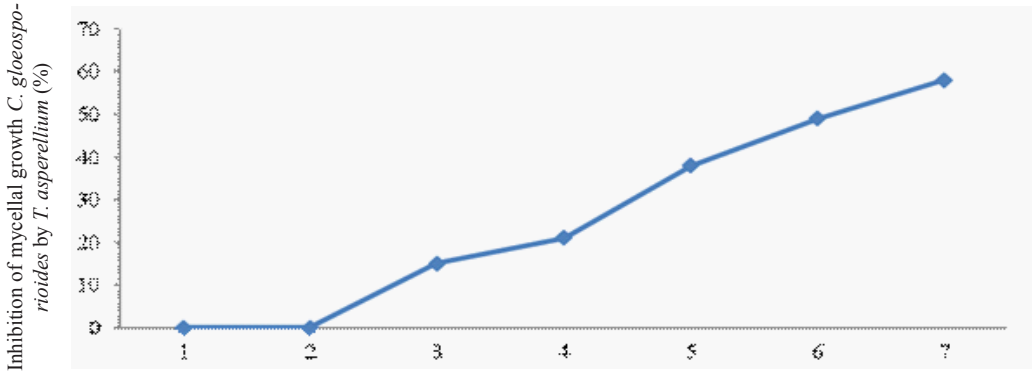


Fig. 6. Antibiosis effect of *T. asperellium* on mycelial growth of *C. gloeosporioides* cultured on PDA and incubated at 23º to 31ºC and 60 per cent to 70 per cent RH for the indicated period in days.

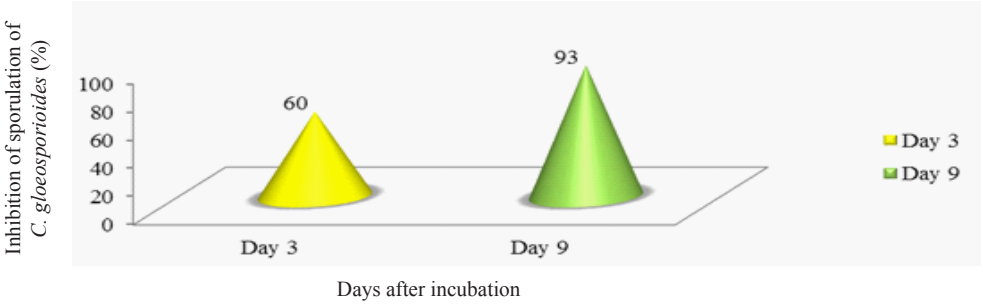


Fig. 7. Antibiosis effect of *T. asperellium* against sporulation of *C. gloeosporioides* cultured on PDA at 23º to 31°C and 60 to 70 per cent RH.

fields confirming that the disease is present in Ghana as reported by Offei *et al*., (2008). This also underscores the fact that the same disease is what farmers in the study area were calling 'akyimkyimakyimkyim' which locally means twisting.

The onion leaf twister disease could be very destructive. The study showed that affected plants may eventually die before reaching bulb formation stage if left uncontrolled. This was evident in the artificial inoculation studies carried out with the causative organism. Disease incidence of 46.3 per cent and 62.5 per cent recorded in the Kwahu South and Fanteakwa districts, respectively, during the field survey implies that farmers were losing a high proportion of the crop to the disease. Indeed, when farmers were interviewed on losses, estimates given ranged from 25 per cent to 100 per cent in a season (Gyempeh, 2013).

The damaging effect of the disease was not restricted to the two districts. It has been reported that the disease was able to wipe out an entire onion farm in an organic experimental field in the Lower Manya District in the same Eastern Region. The reports also stated that onion farmers in the Lower

Manya District, who mainly supply the crop to the Ashiaman market, are now shifting from all-year-round production of onions to include other vegetables in a rotational system with the hope of reducing the impact of the disease. This has resulted in the reduced flow of onions to the markets (Honger, unpublished). The disease had been estimated to cause yield loss of 50 per cent to 100 per cent in several fields in Northern Nigeria, where practically all the onions in that country are grown (Ebenebe, 1980) and also in Brazil (Schwartz & Mohan, 1995). These reports from elsewhere give further confirmation to the findings of the current research work. Therefore, understanding the epidemiology of the disease, and the nature of the causal agent is critical for formulation of control measures, to forestall the huge destructions being caused by the disease, in the two districts in general and the entire region as a whole.

The high disease incidence in the Kwahu South and Fanteakwa districts could be attributed to several factors. During the survey, it was observed that infected onion debris produced after harvesting were heaped and left on the field. This practice retains the inoculum in the soil and as crop rotation is not practiced, it may result in the persistence of the inoculum of the causal agent of the onion twister disease on the field (Agrios, 2005). In the case of Fanteakwa, where the disease severity was higher, onion farmers shared boundaries with each other making it easier from contaminated materials to be moved from one farm to the other. This could contribute immensely to the retention of the causal agent of the disease in the area. Again, the farms were situated close to the Volta lake, where occasionally, the fields were flooded when the lake overflows it banks. The humidity around such water bodies are also expected to be higher all year round. These conditions are favourable for the growth and development of fungi pathogens and, hence, the disease they cause. The wide spread of the farms in the Kwahu South and their locations far from major water bodies, which would likely increase humidity all year round may be contributing to the comparatively lower severity of the disease in the area.

Also of epidemiological importance to the disease is the type of onion cultivars being cultivated in the study area. Bawku red was the predominant variety cultivated since its seeds were readily available in Ghana. Being a red variety, it was expected to have a higher concentration of allyl-propyl disulphide in the leaves and, therefore, more resistant to infection by *C. gloeosporioides* (Alberto *et al*., 2003; Delahaut & Newenhouse, 2003). However, a comparison of how susceptible different onion varieties are to the causal agent of the onion twister disease in Ghana, showed that Bawku red was more susceptible to the pathogen than other varieties such as Malavi, red Creole and Texas grano (Gyempeh, 2003). This may indicate that the Bawku red produced fewer levels of the alkyl disulphide compound than originally expected. The cultivar also has a longer maturity period of about 90 days which increases its exposure to the pathogen in the field. The predominant cultivation of Bawku red variety may explain the high losses (50 to 75%) encountered by farmers in the two districts.

Malavi, the other variety used in the two

districts, proved tolerant to the disease as it had a short maturity period of about 70 days, and may have a higher concentration of the antifungal compounds in the leaves. It is however, cultivated on a lower scale compared to Bawku red. The yellow variety (Texas grano) which was moderately susceptible to onion twister disease was cultivated on a small scale by few farmers usually upon request from customers (Abdulahi & Brimah, verbal communication, 2011).

Onion twister disease has been attributed to *C. gloeosporioides* in Ghana and elsewhere including Nigeria and The Philippines (Offei *et al*., 2008; Alberto *et al*., 2003; Ebenebe, 1980). However, recent views about the aetiology of the disease have become diverse. It has been suggested that the twisting and neck elongation that accompany the disease could not be induced by *C. gloeosporioides*. This is because such symptoms connote a systemic infection which is uncharacteristic of *C. gloeosporioides* and, therefore, may be caused by another fungus, *Gibberella moniliformis*, which has also been isolated from diseased plants (Alberto *et al*., 2003). In the study, in which all of these disease symptoms were recorded, it was imperative that the pathogen isolated from these diseased plants was identified using modern techniques and practices to establish the aetiology of the disease without doubt. The methods used were cultural, morphological, molecular and pathogenicity test. The cultural characteristics of the pathogen, which was the production of white fluffy mycelia with abundance production of acervuli, was an indication that the fungus was of the genus *Colletotrichum*. The production of round-edged spores in the acervuli showed that the pathogen was *C. gloeosporioides* (Agrios, 2005; Arauz, 2000).

However, environment is known to have an effect on stability of morphological traits, whilst intermediate forms of these traits can also be associated with frequent sub-culturing. Due to these, it has been reported that morphological features alone are inadequate in delineating among members of the genus *Colletotrichum* (Honger *et al*., 2004a; Freeman, 2009; Bailey *et al*., 1996; Freeman & Rodriguez, 1995). Therefore, morphological characterisation was complemented with the PCR technique for the identification of the pathogen in the study. The technique has been used by several workers to identify *C. gloeosporioides* on several crops worldwide using the species specific primer, CgInt in combination with the universal primer, ITS4 (Honger *et al*., 2004b; Liu *et al*., 2010; Mills *et al*., 1992). In the study, the approximately 500 bp PCR product was amplified from DNA extracted from the fungus as prescribed for the identification of *C. gloeosporioides*. Data from the study (Fig. 3) showed without doubt that the fungus was *C. gloeosporioides*.

Inoculation studies also showed that the fungus was able to induce all the symptoms associated with onion twister disease on the seedlings including anthracnose and leaf twisting. The symptoms were induced on plants that were inoculated via foliar spraying or through the soil. This is consistent with reports by Ebenebe (1980), that *Glomerella cingulata* (teleomorph of *C. gloeosporioides*) can cause onion twister disease via soil infection or aboveground inoculum. The findings in the study, therefore, confirm without doubt that the pathogen was respon-

sible for the onion twister disease in Ghana. It also gives credence to earlier reports of the pathogen being the cause of the disease in several countries in the world including Nigeria, Benin and Georgia (Sikirou *et al*., 2011; Nischwitz *et al*., 2008; Ebenebe, 1980).

Several control measures have been proposed for the management of the onion leaf twisting disease. These include planting of disease free bulbs, adoption of proper nursery practices and fungicide applications. In recent times, biological control agents are being employed for the control of several plant pathogenic fungi, both as foliar spray or as seed treatment (Verma *et al*., 2007). Species of the genus *Trichoderma* are gradually gaining importance as one of the most important and widely used bio-control agents (Verma *et al.*, 2007). These organisms have been used to control most of the known fungi species that affect fruits and vegetables worldwide. *T. viride* has been used to successfully control rot of yams caused by *Erwinia* species, brown blotch of cowpea caused by *C. truncatum* and white rot of onions caused by *Sclerotium cepivorum* (Bankole & Adebanjo, 1996; Clarkson, Payne & Whipps, 2002; Okigbo & Oikediugwu, 2000).

Other species such as *T. lignorum, T. virens, T. hamatum, T. harzianum* and *T. pseudokoningii* have been used to successfully control plant pathogenic fungi such as *Aspergillus flavus, Fusarium moniliforme, Sclerotium rolfsii, Alternaria alternata* and *Rhizoctonia solani* (Calistru, Mclean & Berjak, 1997; Mukherjee & Raghu, 1997; Verma *et al.*, 2007). These bio-agents are reported to use methods such as faster metabolic rates, production of antimicrobial metabolites, and physiological conformation as key factors to antagonize their hosts (Verma *et al.*, 2007). In the study, *T. asperellium* was evaluated for its antagonistic activity against *C. gloeosporioides*, the causal agent of onion twister disease in Ghana. The bio-agent was able to inhibit the mycelial growth of the pathogen by more than 60 per cent and nearly totally inhibited spore formation by the pathogen. Similar studies have shown that *T. asperellium* was very effective against *Fusarium oxysporum*, and was able to control the wilt of tomato caused by the bio-agent (Cotxarrera *et al*., 2002). The bio-agent has been reported to use antibiosis, mycoparasitism and competition for nutrients to control its target fungi (Verma *et al*., 2007). There is, therefore, the potential for the use of the bio-agent to control the onion twister disease in Ghana.

The results of the study showed that the mycelial growth and sporulation of the pathogen, *C. gloeosporioides* was not inhibited totally and may, therefore, mean that the bio-agent may not be as effective as most chemical fungicides in Ghana, which gave 100 per cent inhibition of mycelial growth and sporulation of *C. gloeosporioides* (Honger *et al*., 2004; Gyempeh, 2013). However, it has been argued that the nature of the formulation of bio-control agents for field applications greatly enhances their biological control activities (Verma, 2007). It is, therefore, possible that the bio-agent could match the performance of these chemical agents on the field. There is the need for further studies to verify the extent of control that can be achieved with the bio-agent on the field.

## Conclusion

The study have shown that onion twister disease is prevalent in most onion farms in the Fanteakwa and Kwahu South districts of the Eastern Region. Factors such as continuous cultivation of very susceptible onion cultivars, retention of plant debris in the field, proximity of farms to each other and high humidity in the production areas are possible factors contributing to the spread and severity of the disease. *C. gloeosporioides* was confirmed as the causal agent of the onion twister disease in the study area, using both traditional and molecular methods and pathogenicity test. *T. asperellium* was able to inhibit the mycelial growth and sporulation of the causal agent, an indication that it has the potential of being used as a biocontrol agent against the disease on the field. The findings in the study would be useful in the formulation of control measures against the disease in future studies.

## Acknowledgement

The authors thank Food and Agriculture Budgetary Support (FABS) Onion Project for funding the study.

## References

Abbey, L., Danquah, O. A., Kanton, R.A.L. & Olympio, N.S. (2000) Characteristics and storage performance of eight onion varieties. *Ghana Journal of Agricultural Science* **40**, 9 – 13.

Agrios, G.N. (2005) *Plant pathology. 5th edn.* Academic Press, Inc. San Diego. 922 pp.

Alberto, R.T., Duca, M.S.V. & Miller, S.A. (2003) Integrated management of anthracnose (*Colletotrichum gloeosporioides* Penzig) Penzig and Sacc.: A disease of increasing importance in onion. *Tropical Plant Pathology Journal* **39**, 43 – 48.

Arauz, L.F. (2000) Mango Anthracnose: Economic impact and current options for integrated management. *Plant Diseases* **84**, 600 – 611.

Awuah, R.T., Kwoseh, C., Koranteng, S.L., Okpala, R.O.C. & Amoako-Attah, I. (2009) Appearance of *Fusarium* basal rot of onion in the Kwahu South District of Ghana. *Ghana Journal of Horticulture* **7**, 84 – 88.

Bailey, J.A., Nash, C., Morgan, L. W., O'Connell, R. J. & TeBeest, D. O. (1996) Molecular taxonomy of *Colletotrichum* species causing anthracnose of Malvaceae. *Phytopathology* **86**, 1076 – 1083

Bankole, S. A. & Adebanjo, A. (1996) Biocontrol of brown blotch of cowpea caused by *Colletotrichum truncatum* with *Trichoderma viride*. *Crop Protection* **15**, 633 – 636

Barnett, H.L. & Hunter, B.B. (2006) *Illustrated genera of imperfect fungi.* The American Phytopathological Society, St. Paul, Minnesota, USA. 218 pp.

Calistru, C., McLean, & M. Berjak, P. (1997) *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species. A study of the production of extracellular metabolites by *Trichoderma* species. *Mycopathologia* **137**, 115 – 124.

Choi, Y.W., Hyde, K. D. & Ho, W. H. (1999) Single spore isolation of fungi. *Fungal Diversity* **3**, 29 – 38.

Clarkson, J. P., Payne, T. M. & Whipps, J. M. (2002) Selection of fungal biological control agents of *Sclerotium cepivorum* A for control of white rot by sclerotial degradation in a UK soil. *Plant Pathology* **51,** 735 – 745.

Cotxarrera, L., Trillas-Gay, M. I., Steinberg, C. & Alabouvette, C. (2002) Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress *Fusarium* wilt of tomato. *Soil Biology and Biochemistry* **34**, 467 – 476.

Delahaut, K.A. & Newenhouse, A.C. (2003)

*Growing onions, garlics, leeks, and other alliums in Wisconsin. A guide for fresh-markets growers.* University of Wisconsin System Board of Regents and University of Wisconsin-Extension, Cooperative Extension. 24 pp.

De Marco, J. L., Valadares-Inglis, M.C. & Felix, C.R. (2003) Production of hydrolytic enzymes by *Trichoderma* isolates with antagonistic activity against *Crinipellis perniciosa*, the causal agent of witches' broom of cocoa. *Brazilian Journal of Microbiology* **34**, 33 – 38.

Ebenebe, A. C. (1980) Onion twister disease caused by *Glomerella cingulata* in northern Nigeria. *Plant Disease* **64**, 1030 – 1032.

FAO (2005) UN Food and Agriculture Organisation, 2005 (Onions). *http//www.answers.com/topic/onion#cite_note-25 (1/11/10)*.

Fokkeme, N. J. (1987) Fungal antagonism in the phylosphere. *Annals of Applied Biology Journal* **89**, 115 – 117.

Freeman, S. (2009) Genetic diversity and host specification of *Colletotrichum* sp. on various fruits. In *colletotrichum: Host specificity, pathology and control* (J.A. Bailey and M.J. Jeger eds), pp 131 – 144. CAB International, Wallingford, UK.

Freeman, S. & Rodriguez, R. J. (1995) Differentiation of *Colletotrichum* species responsible for anthracnose of strawberry by arbitrarily primed PCR. *Mycological Research* **99**, 901 – 905.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B. & Smith B. (2002) Onions: A global benefit to health. *Phytotherapy Research Journal* **16**, 603 – 615.

Gyempeh, N. (2013) *Aetiology and control of the onion twister disease in the Eastern Region of Ghana* (MPhil Thesis). University of Ghana, Legon. 93 pp.

Honger, J.O., Offei, S. K., Oduro, K. A., Odamtten, G.T. & Tatu, S. N. (2014a) Identification and species status of the mango-biotype of *Colletotrichum gloeosporioides* in Ghana. *European Journal of Plant Pathology* **140**, 455 – 467.

Honger, J.O., Offei, S. K., Oduro, K. A., Odamtten, G.T & Tatu, S. N. (2014b) Phenotypic and molecular characterisation of *Colletotrichum gloeosporioides*, causal agent of mango anthracnose in Ghana. *Ghana Journal of Science*. **54**, 71 – 82.

Honger, J. O., Offei, S. K., Oduro, K. A. & Odamtten, G.T (2015) Chemical control of mango anthracnose disease in Ghana. *Ghana Journal of Agricultural Science* **49**, 15 – 28.

Korsten, L. & De Jager, E. S. (1995) Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. *South African Avocado Growers Association Yearbook* **18**, 124 – 130.

Liu, B., Louws, F.J., Sutton, T.B. & Correl, L, J. (2010) A rapid qualitative molecular method for the identification of *Colletotrichum acutatum* and *Colletotrichum gloeosporioides. European Journal of Plant Pathology.* DOI10-1007/s10658 – 011.

McGarvey, P. & Kaper, J. M. (1991) A simple and rapid method for screening transgenic plants using the PCR. *Biotechniques* **11**, 428 – 432.

Mills, P. R., Sreenivasaprasad, S. & Brown, A. E. (1992) Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. *European Microbiological Society Microbiology Letter* **98**, 137 – 143.

Mukherjee, P. K. & Raghu, K. (1997) *Trichoderma* sp. as a microbial suppressive agent of *Sclerotium rolfsii* on vegetables. *World Journal of Microbiology and Biotechnology* **13**, 497 – 499.

Nischwitz, C., Langston, D., Sanders, H. F., Torrance, R., Lewis, K. J. & Gitaitis, R. D. (2008) First report of *Colletotrichum gloeosporioides* causing twister disease of onion (*Allium cepa*) in Georgia. *Plant Disease* **92**, 974.

Oduro, K. A. (2000) *Checklist of plant pests in*

*Ghana*. Plant protection and regulatory services directorate. MoFA, Ghana. 105 pp.

Offei, S. K., Cornelius, E.W. & Sakyi-Dawson, O. (2008) *Diseases of crops in Ghana and their management*. Smartline Publishing Ltd., Accra, Ghana. 104 pp.

Okigbo, R.N. & Oikediugwu, F. E. (2000) Studies on biological control of postharvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride. Journal of Phytopathology* **148**, 351 – 355.

Schwartz, H. F. & Mohan, S. K. (1995) *Compendium of onion and garlic diseases*. APS Press, St. Paul, Minnesota. 55121 – 2097, USA. 54 pp.

Sikirou, R. F., Beed, J., Hotegni, S., Winter, F., Assogba-Komla, R. & Miller, S.A. (2011) First report of anthracnose caused by *Colletotrichum gloeosporioides* on onion (*Allium cepa*) in Benin. *New Disease Reports*. 23 pp.

Sinnadurai, S. (1992) *Vegetable cultivation*. Asempa Publishers, Ghana.

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R.Y. & Val´ero, J. R. (2007) Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Engineering Journal* **37**, 1 – 20.

Vidhyasekaran, P. (2004) *Concise Encyclopedia of Plant Pathology.* The Haworth Press, 10 Alice Street, Binghamton, New York. 619 pp.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) *Amplification and direct sequencing of fungal ribosomal RNA Genes for Phylogenetics.* 322 pp.

Zivkovic, S., Stojanovic, S., Ivanovic, Z., Gavrilovic, V., Popovic, T. & Balaz, J. (2010) Screening of antogonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Archives of Biological Science, Belgrade* **62**(3), 611 – 623.

Received 27 Aug 15; revised 22 Feb 16.