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Rice false smut and its management in major rice growing areas in Ashanti region of Ghana

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***Ustilaginoidea virens*, the pathogen that causes rice false smut (RFS) disease induces infertility in infected spikelets, reduces grain weight and caused up to 75% yield loss. Surveys on RFS disease were conducted from September to December, 2015 in 60 rice farms within three districts: Ejisu-Juaben, Ejura-Sekyedumase and Asante Akim-North, in the Ashanti region of Ghana. Incidence and severity, as well as farmers' perception of the disease were obtained using questionnaires. Symptomatic rice panicles were collected from farms for identification of associated pathogen in the laboratory. Effect of four inorganic fungicides: Mancozan super, Suncozeb, Nordox and Sidalco Defender on mycelial growth and sporulation of the RFS pathogen were studied using the food poisoning method. Fourteen rice varieties were evaluated for their response to the disease. The surveys revealed that most (60%) of the farmers had no knowledge of the disease. The highest incidence of RFS disease was observed at Duampompo (23.3%) in Ejisu-Juaben Municipal/District, Ntemaso (23.3%) in Ejura-Sekyedumase District, and Habitat (38.0%) in Asante-Akim North District. The highest severity was observed at Ntemaso (5.0) in Ejura-Sekyedumase and Habitat (7.6) in Asante-Akim North District, with Bomfa recording the highest severity (5.0) in the Ejisu-Juaben Municipal. *U. virens* was isolated from diseased rice samples collected from the surveyed fields. The four fungicides evaluated exhibited 100% inhibition on the growth of *U. virens* mycelium eight days after inoculation *in vitro* however, Nordox was the most efficient after 23 days inoculation and the Mancozan super was the least efficient. Evaluation on rice varieties response to *U. virens* should be repeated and the effectiveness of fungicides should be tested under the field-infected plants in order to sustain the present results.**

Key words: *Ustilaginoidea virens*, rice false smut, disease.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the dominant staple food and cash crop in Ghana (Danso-Abbeam, 2014). The crop is consumed daily and is the sole most essential basis of human calories. It is the third most cultivated crop worldwide, after maize and sugarcane (FAOSTAT, 2014). MoFA (2009) reported that urbanization,

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population increase and change in consumer preference account for the continuous increase in rice consumption in Ghana over the years. The annual per capita consumption of rice in Ghana have been increasing; from 17.5 kg during 1999 to 2001 to 24 kg during 2010 to 2011 (MoFA, 2012). The per capita/consumption of rice in 2012 to 2014 was estimated at 32 to 35 kg. Rice consumption in Ghana is projected to reach a milestone of about 63 kg in the year 2015 (MoFA, 2014).

Inspite of the technological advances in its production, diseases remain a major cause of yield losses and lower profits worldwide, as they cause reduction in quantity and quality and increase production cost of the crop (Ghini et al., 2008). According to ISSER (2008), Ghana's agricultural sector receives aid from several organizations (Farmer-Based Organizations, Non-Governmental) to increase rice production (yield), reduce poverty and increase farmers' income. Farmers receive training as well as high yielding crop varieties for production. Despite all these inputs received by the farmers, production remains low (Faltermeir, 2007).

Rice false smut (RFS) disease is caused by an Ascomycete, *U. virens* (Cooke) Takahashi. The chlamydospores of *U*. *virens* survive in the rice seeds as well as the soil throughout the cropping season and they serve as principal sources of inoculum for the disease (Ashizawa et al., 2010). The life cycle of the pathogen, infection and disease development are unclear (Fu et al., 2014). Also, characterization of the pathogen-host interaction and pathogen history is difficult (Fu et al., 2012). During infection, *U. virens* produces mycotoxins such as Ustilotoxins A and B which contaminate rice seeds and straw. These mycotoxins are harmful to man and livestock (Shan et al., 2013). According to Tanaka et al. (2008), RFS disease has become an important disease of rice, causing significant yield losses globally. The disease reduces yield, affects grain quality and imposes health hazards significantly in all rice producing areas. Rani (2014) reported that yield losses caused by RFS disease is attributed to both smut balls as well as chaffiness, reduction in grain weight and infertility of the spikelet near the smut balls. Reduction of yield by the disease is alarming in some major rice growing regions. Upadhyay and Singh (2013) reported that yield loss due to RFS disease from many rice growing areas ranges from 1 to 75%. The fungus enters the plant through the small opening between the lemma and palea and initial infection occurs in the grain which reduces seed germination (Ladhalakshmi et al., 2012). The RFS pathogen grows and contaminates the infected plant tissues, including the stamen and filament. Thus, this is regarded as the plant stamen-filament disease (Tang et al., 2013). Previously, the disease was considered a minor one due to its rare occurrence. However, it has now become a serious concern for rice production (Zhou et al., 2008).

Inorganic fungicides such as Simeconazole (Fluorophenyl) and 2.5% Wenquning (*Bacillus subtilis*) have been used to control the disease (Ashizawa et al., 2010). However, the use of some partial resistant plant genotypes such as Neixiangyou 8156, Zhongyou 177, Heyou 6, Nei5you 317, Suaiyoulianhe 2 and Nongfengyou 256 have proved more effective (Liang et al., 2014). The research seeks to identify intervention(s) for sustainable rice production and also study the RFS disease for effective management in order to minimize yield losses and thus, improving the livelihood of farmers.

MATERIALS AND METHODS

Survey and sample collections were conducted from September to December, 2015 to obtain information on RFS disease in three districts/municipalities and also to ascertain farmers' perception of the disease on 60 rice farms within three districts in the Ashanti region of Ghana namely Ejisu-Juaben, Asante-Akim North and Ejura-Sekyedumase (Table 1).

The Ejisu-Juaben District is located in the central part of the Ashanti region. It lies between latitude 1°15' N and 1°45' N and longitude 6°15' W and 7°00' W. It records very high relative humidity during rainy season and early mornings. The relative humidity is not stable; it records lowest during midday when weather is hot despite rainy seasons unlike other districts in the Ashanti region. The district has the major rainfall season starting from March to July with an average rainfall of 1200 to 1500 mm annually. Its shorter rainfall season starts in September and ends in November with an average rainfall of 900 to 1120 mm (Ghana Statistical Service, 2010).

Ejura-Sekyedumase District is situated within longitude 1°5' W and 1°39' W and latitudes 7°36' N and 7°9' N. It is located at the north of the Ashanti region. The district has two rainfall patterns: The bimodal pattern in the south and the unimodal pattern in the north. The major rainfall season starts from April to November. Annual rainfall ranges from 1200 to 1500 mm. Relative humidity is extremely high throughout the rainy season, recording 90% in June and 55% in February (International Telecommunication Union (ITU), 2012, Tacoli, 2012). Asante-Akim North District is situated at the eastern part of the Ashanti region and lies between latitude 6° 30' N and 7° 30' N and longitude 0° 15' W and 1°20' W. The district has a total annual rainfall ranging from125 to175 mm, with the major rainy season occurring from May to July and the minor season occurring from September to November. Relative humidity is generally high throughout the year in this district, ranging from 70 to 80% in the dry season and 75 and 85% in the wet season (Ghana Statistical Service, 2012).

Purposive and stratified sampling methods (Miles and Humber, 1994) were used to select five rice farmers in 12 communities within the three districts/municipality. Farmers were interviewed with the aid of a questionnaire to obtain information such as their knowledge of the RFS disease, sources of seeds, preferred varieties, types of fertilizer used, major diseases they encountered, as well as some management practices they employed. The selected fields were then assessed for incidence and severity of rice false smut disease. In each farm, five 5 m x 5 m plots were demarcated in four corners and one in the middle for assessment. Percentage disease incidence per plot was determined as a ratio of the number of diseased hills over the total number of hills in the plot. Visual scoring for severity in each plot was done using the International Rice Research Institute's Standard Evaluation System (IRRI, 1996) for rice on a scale of 0 to 9, where 0 (no disease), 1 (symptom on less than 1% of plant), 3 (symptom on 1-5% of plant), 5 (symptom on 6-25% of plant), 7 (symptom on 26-50% of plant) and 9

Table 1. Districts/Municipal surveyed with their communitie.

District/Municipal	Communities			
Ejisu-Juaben	Nobewam	Bomfa	Besease	Duampompo
Asante-Akim-North	Ohene-Nkwanta	Juansa	Habitat	Nyantokro
Ejura-Sekyedumase	Aframso	Kyere-adireagya	Ntemaso	Mawabamumum

Table 2. Inorganic fungicides with their corresponding active ingredients and sources.

Inorganic fungicide	Active ingredient	Company/country
Mancozan Super	Mancozeb 640 g/kg+Metalaxyl 80 g/kg WP	Louis Dreyfus Commodities/France
Nordox 75 WG	Copper fungicide	Nordox Industries/Norway
Suncozeb	80% WP Mancozeb	Zhejiang Xinan Co. Ltd./China
Sidalco Defender	435 g/l Copper Oxychloride	Sidalco Limited/UK

(symptom on 51-100% of plant).

In total 60 samples of rice panicles showing typical symptoms of RFS disease (smut balls) were collected from the fields, using an improved tissue segment method (Rangaswami, 1972). Diseased panicles identified in each plot were detached using a knife, and the total sample from each farm was carefully packaged in a plastic bag for further identification. Causal organism of the RFS disease was identified and the effects of different fungicides on the mycelium growth of the causal organism were evaluated using the food poisoning method in the Plant Pathology laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST). Inorganic fungicides were obtained from Chinese Agro-chemical Shop, Kumasi, Ghana. The cultures were grouped, based on the district/municipality where infected rice panicles were collected. Isolates from one district/municipality but different communities were grouped. To clarify the particular identity shared by isolates from one district and communities, the cultures were mounted on the microscope for examination and identification. The cultures were then grouped, based on morphological characteristics such as nature of mycelium growth, colour and spore size. Mycelium growth and colour were characterized by visual assessments while spore sizes were characterized through the aid of micrometrics SE premium software mounted on the Leica (Leica Microsystem Company Ltd. Germany). Thus, the isolates were grouped into three separate groups. Table 2 shows the list of inorganic fungicides used in this study (evaluation against *U. virens*).

250 ml of potato dextrose agar (PDA) prepared in five separate Erlenmeyer flasks were amended with 0.8 ml of Sidalco Defender, 1.25 g of Nordox 75 WG, 1.2 g of Mancozan Super or 1 g of Suncozeb. Each rate was based on manufacturer's recommended rates. The PDA with no amendment served as control. Completely randomized design (CRD) with five replications was used.

Fungal growth from both amended and control plates were observed daily for growth and fungus characteristics. Measurement of mycelium growth was done when the growth of fungi in the control plate attained maximum growth at eight days after inoculation. Persistence of the four fungicides in terms of inhibition of mycelium growth, as well as sporulation of *U. virens* were determined. Growth inhibition of the isolates by the inorganic fungicides was determined using the radial growth method (Soad and Samir, 2005). This method involves measuring the diameter of mycelia in both the control and amended plates, when growth in the control plates had achieved maximum diameter. The corresponding percentage growth inhibition was calculated using the formula (Sundar et al., 1995):

$$\text{Inhibition (\%)} = \frac{Y-A}{Y}\ x\ 100$$

Where, Y is mean diameter of *U. virens* growth in control plate and A is the mean diameter of growth in amended plates.

Data were subjected to analysis of variance (ANOVA) using two-way analysis with Genstat Statistical Package (Genstat, 2007), 9th edition. Treatment means were separated by least significant difference test at 5% probability. Square root √(x + 1) transformation and Arc sine square root transformation were applied to data as many biological variables do not meet the assumption of parametric statistical test. Square root was used for count data while Arcsine was used for data in percentages.

RESULTS AND DISCUSSION

Farmers' perception of rice false smut disease in the surveyed areas

During the survey, it was observed that 80% of rice farmers were males; 33% of them had basic education, 13% had tertiary education while 27% had no formal education. About 45% of the farmers were between 41-50 years-old while 2% were below 20 years-old. About 33.3% of the farmers observed RFS disease on their fields during the harvesting stage while 13.3% observed the disease at the maturity stage of the crop. Some symptoms of the disease observed by farmers were yellowish (48.3%), greenish (20%) and black (31.7%) smut balls. The disease occurred mostly (70%) in the major growing season. About 32% of farmers reported RFS as the major disease affecting rice production in the surveyed areas. Other diseases of rice were identified during the study. Since most farmers had at least some basic education, they practiced good agronomic techniques resulting in high yields. The result agrees with findings of Hussain and Byerlee (1995) who reported that farmers with some level of formal education adopt new technologies such as application of fertilizers, disease and pest management, and use of proper planting distance for rice cultivation faster thereby increasing yield. Diseases affecting rice production in the surveyed areas were RFS, rice blast, sheath rot, rice bacterial blight, and yellow mosaic virus. According to farmers, inadequate management of these diseases resulted in production failure. These results supports those by Nelson et al. (2001) where farmers from developing countries have significant difficulties in the management of crop diseases which often results in crop losses.

Incidence and severity of rice false smut disease in the surveyed areas

The incidence and severity of RFS disease in the Ejisu-Juaben municipality, Duampompo had the highest mean incidence whereas Nobewam recorded the lowest. The mean incidence of RFS disease recorded at the four communities were not significantly different at P>0.05 from each other.

Habitat recorded the highest (38.0) mean incidence of RFS disease while Juansa and Nyantokro recorded the lowest (15.6) mean incidence of the disease in Asante-Akim North District. No significant differences were found among other locations. In the Ejisu-Juaben Municipal, Bomfa recorded the highest (5.0) mean severity of RFS disease with Besease and Nobewam recording the lowest (3.5). In the Ejura-Sekyedumase District, Ntemaso recorded the highest (5.0) mean severity while Mawabamumu recorded the lowest (3.3). Also, mean severities of RFS disease recorded in Aframso, Ntemaso and Kyeredieagya were not significantly different (P>0.05) from each other.

Habitat recorded the highest (7.6) mean severity of RFS disease in the Asante-Akim North District with Juansa recording the least (1.7). There was no significant differences (P>0.05) between the mean severities recorded in Habitat and Ohene-Nkwanta.

Comparing the districts, Asante-Akim North District recorded the highest incidence (38.0) while Ejisu-Juaben Municipal and Ejura-Sekyedumase Districts recorded the lowest (23.2).

The presence of the alternative host of *U. virens, cylindrica* L in the district could be the possible reason for high incidence. According to Rani (2014), *U. virens* survives on the weed and cause disease thus, producing plenty chlamydospores as inocula for RFS disease on rice. It could also be attributed to environmental conditions such as rainfall, temperature and relative humidity experienced during production and exchange of planting materials (seeds). This is supported by Rao and Raju (1955) that environmental promote incidence and severity of RFS disease. There was no significant difference (P>0.05) between disease incidences recorded in at the four communities. Since these communities are within the same district, there was a higher possibility of exchange of infected rice varieties for planting among farmers in the communities. This may have resulted in the same incidences of disease recorded in the communities as reported by Fan et al. (2015) that exchange of planting materials are a possible cause of high incidence. Ntemaso recorded the highest (23.3) incidence of RFS disease in the Ejura-Sekyedumase District. The presence of weed such as *Dracaena marginata* Lam was observed on farmers' fields in the Ntemaso community. This weed has been reported as an alternative host of the RFS pathogen. High incidence of RFS disease in the community could be attributed to the presence of this weed on the fields. This is in support of the findings of Gohel et al. (2014) that RFS pathogen, *U. virens*, lives on alternative hosts in paddy fields to produce plenty of chlamydospores of which might act as the basis of infection and later infect the rice after planting. The lowest (21.9) incidence recorded at Aframso may be due to the use of moderately susceptible or tolerant rice varieties used by farmers. Farmers in this community may have used tolerant rice varieties. Tebeest and Jecmen (2012) reported that the number of smut balls or incidence of infection of rice false smut disease depends on the level of susceptibility of the varieties involved. Habitat community had the highest incidence of rice false smut disease in the Asante-Akim North District with Juansa recording the least (14.2). The weed *cylindrica* L was observed in most of the fields surveyed in habitat, and relatively fewer fields in Juansa. The presence of this weed in Habitat community might have caused the high incidence of the disease in the community as was reported by Rani (2014) who reported that *U. virens* causes disease in *I. cylindrical.* The higher severity of disease recorded in Ntemaso may be attributed to the application of higher dosage of nitrogen fertilizers by the farmers in the community. Though soil samples were not taken to determine the total nitrogen per site, some communities used nitrogenous fertilizers while other used manure and some used none. This result supports that of Mohiddin et al. (2012) that excess nitrogen makes the plant less fibrous and less resistant to RFS disease as observed in Habitat community. According to Fan et al. (2015), exchange of susceptible rice varieties to RFS and growing of diseased rice seeds could result in to high incidence and severity of RFS disease in farmers' fields. This is in agreement with report of Nessa et al. (2015) that severity of rice false smut disease depends on soil fertility and flood water depth.

Isolation and identification of the causal organism of rice false smut disease

The growth of the colonies from all the three districts/municipality were creamy-white flat or raised with

Plate 1. Front view of mycelia plated *U. virens* on PDA.

Plate 2. Back view of mycelia plated *U. virens* on PDA.

slight undulations, with the mycelia fluffy, compact and leathery (Plates 1 and 2 and Table 3). The spore sizes of fungal isolates varied from 33.90 to 35.13 µm. These characters identified through laboratory analysis from the diseased plant tissues collected from the surveys proved that the pathogen is *U. virens* shown in Table 4. In The description of *U. virens* by Joshi and Sharma (1975) and Verma and Singh (1988), the mycelia of the fungus appear whitish to creamy, with the spore size ranging from 30 to 40 µm in diameter.

Effect of four inorganic fungicides on growth and sporulation of *U. virens in vitro* using the food poisoning method

There was no mycelial growth of *U. virens* in plates amended with Mancozan super, Nordox, Sidalco defender and Suncozeb fungicides, while there was a mean of 8.5 cm growth in the control plates eight days after plating (Table 4). This treatment was effective in eight days after which some fungicides lost their potential. Nordox effectively controlled the mycelial growth of *U. virens* at 100% until 23 days while growth was observed on the three fungicides from the 10^{th} day after inoculation. There was significant difference ($P<0.05$) between the control and the remaining treatments.

This indicates that all four fungicides used in the test were effective against *U. virens* since they inhibited the growth of fungus (Plate 3). The result supports that by Wagbe et al. (2015) who evaluated six different fungicides; SAA (Mancozeb 63% + Arbendazin 12%) 75 WP, Azoxystrobin 25 SC, Mancozeb 75 WP, Propiconazole 25 EC, Chlothalonil 75 and Heconazole 5 EC for their efficacy *in vitro* against *A. helianti.* Mancozeb at 2500 ppm showed the highest percent inhibition of 88.88% and is an active ingredient in Suncozeb.

Determination of persistence of the fungicides on *U. virens in vitro*

The persistence of four fungicides was assessed until 23 days of plating. All fungicides exhibited varying degrees of inhibition of *U. virens* mycelial growth. Even though some of them lost their potential eight days after, Nordox maintained its efficacy in controlling *U. virens (*mycelial growth) of by 100% for the entire 23 days, until the end of the experimental period (Table 5).

Nordox, with the most effective active ingredient (copper fungicide) completely controlled mycelial growth at 23 days. The results showed that Nordox had the highest persistence, followed by Sidalco defender (435 g/l copperoxycholoride) and Suncozeb (80% WP Mancozeb). Mancozan super (Mancozeb 640 g/kg and Metalaxyl 80 g/kg WP) had the lowest persistence. The result is in conformity with that of Hafiz et al. (2016) who evaluated six different fungicides namely Captan, Carbendaim, Tilt, Copperoxychloride, Mancozeb and Topsin against four strains of *Colletotrichum falcatum* and the highest inhibition percent was recorded in plate amended with Mancozeb (95, 90, 85 and 80% at 30% concentration) while the least inhibition percent was recorded in plate amended with Captan at 1% concentration (37.31, 32.31, 27.3 and 22.31 concentration).

Table 3. Morphological characteristics of fungal isolates from survey areas.

District	Colour of mycelia	Mean spore size(µm)
Ejisu-Juaben	Creamy-white	33.90
Ejura-Sekyedumase	Creamy-white	35.13
Asante-Akim North	Creamy-white	34.26

Table 4. Mean radial growth of fungal colonies on fungicide amended PDA plates, eight days after plating.

Treatment	Mean radial growth (cm)
Mancozan super	0.0[b]
Suncozeb	0.0[b]
Nordox	0.0[b]
Sidalco Defender	0.0[b]
Control	8.5[a]
CV (%)	7.0

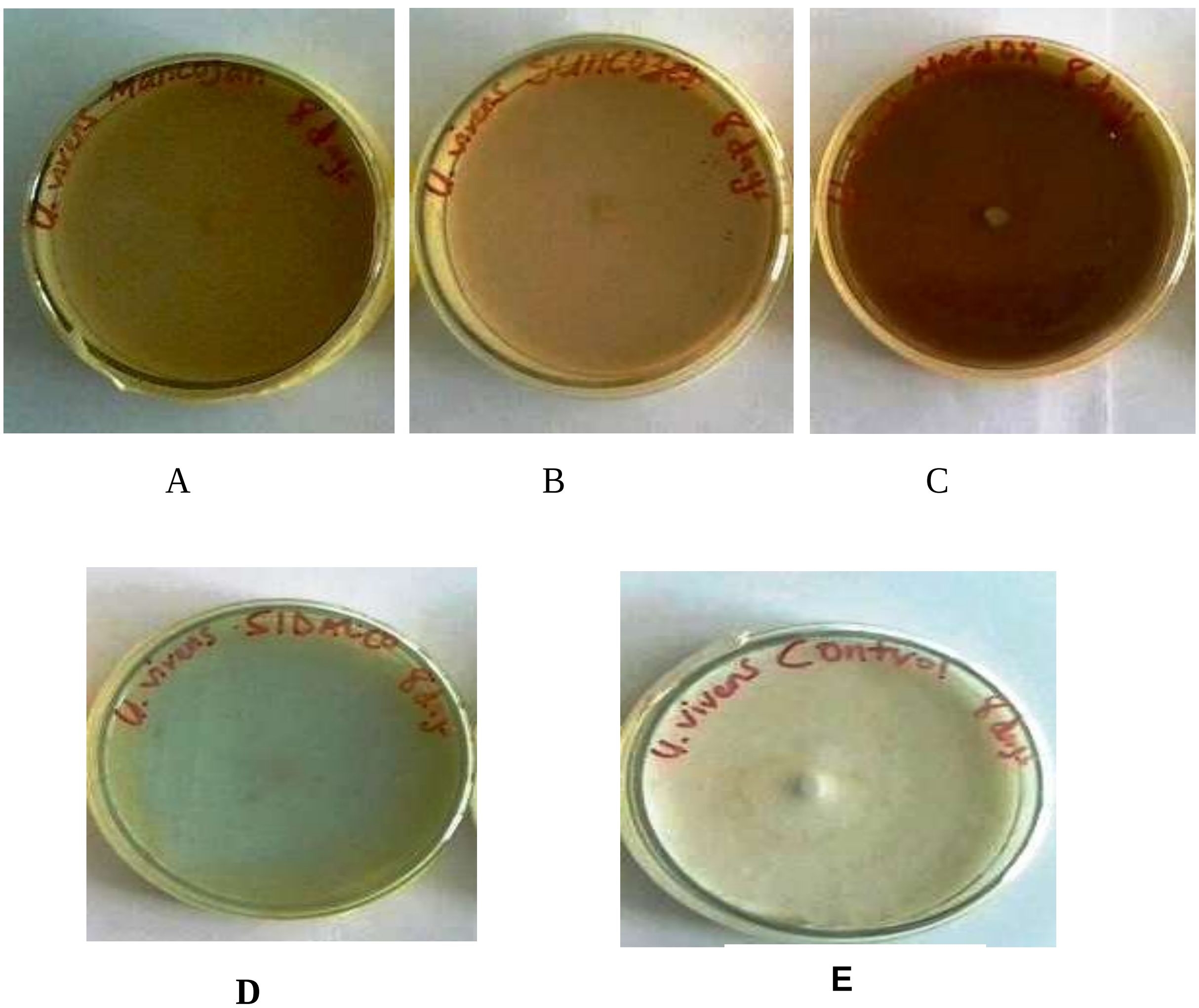

Plate 4. Growth of *U. virens* on Mancozan super (A), Suncozeb (B), Nordox (C) and Sidalco defender (D) fungicides, and the Control (E), eight days after plating.

Table 5. Mean radial growth of fungal colonies at 2 to 23 days after plating.

Treatment	Mean radial growth of mycelia (cm) at				
	2 days	8 days	14 days	20 days	23 days
Mancozan super	0.00	0.00	0.93	2.20	3.04
Suncozeb	0.00	0.80	1.15	2.10	2.50
Nordox	0.00	0.00	0.00	0.00	0.00
Sidalco defender	0.00	0.00	0.00	1.10	2.10
Control	1.85	8.49	9.00	9.00	9.00

Conclusion

The causal agent of rice false smut disease, *U. virens,* was identified from diseased rice panicles obtained from the surveyed districts/municipality in the Ashanti Region of Ghana. The present study revealed that farmers' knowledge of rice false smut disease was generally low. The fungicides evaluated exhibited 100% inhibition on the growth of *U. virens* eight days after inoculation *in vitro* in Nordox (copper fungicide), Mancozan super (Mancozeb 640 g /kg + Metalaxyl 80 g/kg), Suncozeb (80% WP Mancozeb) and Sidalco defender (435 g/l copper oxycholoride), however, after 23 days only Nordox inhibited mycelium growth. The highest (23.3) incidences of RFS disease were observed in Duampompo in Ejisu-Juaben Municipal and Ntemaso in Ejura-Sekyedumase District and Habitat in Asante-Akim North District. The highest severities of RFS were recorded in Ntemaso in Ejura-Sekyedumase, Bomfa community in the Ejisu-Juaben Municipal and Habitat in Asante-Akim North District.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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