SHORT COMMUNICATION

Report on outbreak and *in vitro* management of leaf spots disease caused by *Pestalotiopsis* sp. on oil palm seedlings in nurseries in Ghana

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ABSTRACT

Aims: Oil palm (*Elaeis guineensis*) is the most productive and the highest yielding edible oil crop in the world and economic crop cultivated in Ghana. In September 2017, an outbreak of leaf spot caused by *Pestalotiopsis* sp. on oil palm seedlings was reported for the first time in Ghana. The disease incidence reached 85%, assuming an epidemic situation. This study is geared towards developing appropriate management strategies by identifying phytopathogenic fungi that caused leaf spot on oil palm seedlings.

Methodology and results: Ten symptomatic leaves were picked per plot into sterilized plastic Ziploc bags and brought to laboratory. The leaves were washed under running tap water, cut into 1 cm pieces each, surface-sterilized with 10% sodium hypochlorite solution, rinsed three times in sterile distilled water and blotted on tissue paper (Gonthier *et al.*, 2001). The sterilized samples were transferred aseptically onto potato dextrose agar (PDA) plate containing 0.5 mg/L of chloramphenicol and subcultured till pure culture was obtained. The result showed pure white colony which was concentric, cottony and velvety with slimy black dots of conidia mass on the tip of aerial mycelia. The fungus isolated and identified from the lesions on the leaf was *Pestalotiopsis* sp. and its pathogenicity confirmed.

Conclusion, significance and impact of study: The result from the study concludes that *Pestalotiopsis* sp. could infect *E. guineensis*, which developed the same symptoms observed naturally in the field after inoculation. The fungus was identified based on morphological characteristics.

Keywords: *Pestalotiopsis*, *Elaeis guineensis*, leafspot, pathogenicity, carbendazim.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a tropical perennial native to West and Southwest Africa, and is economically important because of its multiple uses, including food, for cosmetics and fuel. Global production of palm and palm kernel oils were about 60.9 million metric tonnes and 6.67 million metric tonnes, respectively in 2016 (Oil World Annual, 2016), with African countries producing about 5.4 percent. Oil palm production area in Ghana is approximately 352,800 ha, with small-scale producers and processors producing about 71% of the country's total (MASDAR, 2011).

One factor limiting the attainment of food security in Africa is high crop loss through insect pests and diseases. The oil palm industry is thus threatened by these biological factors. Among the oil palm diseases that occur in oil palm plantations is Leaf spot disease (LSD). Leaf spot disease of oil palm seedlings is caused by various

phytopathogenic fungi from several genera: *Annellophora*, *Bipolaris*, *Cercospora*, *Curvularia*, *Colletotrichum*, *Calonectria* (*Cylindrocladium*), *Exserohilum*, *Gliocladium*, *Pestalotiopsis*, *Pestalotia*, *Phaeotrichoconis*, *Phyllachora*, *Pseudocercospora*, and *Stigmata* (Kittimorakul *et al.*, 2013; Nakarin *et al.*, 2013; Sunpapao *et al.*, 2014). The disease first manifests as yellowish-brown necrotic spots, which later spread across the leaf vein in patches (spots) and later assume pleomorphic shapes. The pathogen infects mainly oil palm leaves, at any growth stage, thus affecting their quality and market values. Leaf spot caused by *Pestalotiopsis* sp. is assuming importance in Ghana and as a new emerging disease, not much work has been done on it. The objective of this study was to develop appropriate management strategies by identifying the causal agent of the disease on oil palm seedlings.

MATERIALS AND METHODS

Disease assessment

Activities involved include survey and field examination to determine disease prevalence and severity using disease index sheets following the methods described by Anonymous (2017) and Kittimorakul *et al.* (2013), with slight modifications. The study was done in 120 nursery plots. Random sampling technique was used to select nurseries. Seventy thousand oil palm seedlings were assessed for disease incidence and severity.

Personal observations were also made on the disease symptoms on the palms and other suspected hosts of the pathogen(s) in the field. Plot maintenance and disease spread were also studied. One hundred rows of seedlings per plot were randomly selected with 10 seedlings selected within each row. Samples of infected leaves were collected from all the plots examined. The infected samples collected were put into 229 mm x 324 mm sterilized Ziploc bags, labeled and brought to the Plant Pathology laboratory of CSIR-Oil Palm Research Institute (OPRI), Kusi for pathological analysis.

Disease incidence

A total of 70 000 seedlings were examined at the nursery. Disease Incidence per selected plot was computed as follows (Lekete, 2014):

$$\% \text{ Leaf spot disease incidence on the field} = \frac{\text{Number of infected seedlings per plot}}{\text{Total number of selected seedlings per plot}} \times 100$$

Isolation and identification of causal agent of leaf spot disease on oil palm seedlings

Under this activity, disease diagnosis and laboratory analysis were carried out in Plant Pathology laboratory at CSIR-Oil Palm Research Institute, Kusi, Ghana.

Disease sampling

Diseased plant parts of oil palm seedlings showing typical leaf spot symptoms were collected from all the nursery plots examined in Ghana. Disease sampling was done in January and February, 2018.

Isolation and identification of leaf spot pathogen

Isolation of the pathogen was done using Standard isolation techniques prescribed by Kumar and Soodan (2006). Ten symptomatic leaves from each plot examined were collected into sterile Ziploc bag (229 mm x 324 mm) and brought to the Plant Pathology laboratory. About 5 mm pieces of each of diseased leaves were submerged in 10% of Sodium-Hypo-Chloride (NaOCl) for 1 min and transferred into 15% Hydrogen Peroxide solution (H₂O₂) and serially washed in four changes of sterile distilled water and blotted dry. The sterilized samples were

aseptically transferred onto 2% (w/v) water agar containing 0.5 mg/L of chloramphenicol and incubated at 28 ± 1 °C for three days. The individual mycelia growth that appeared were transferred aseptically onto potato dextrose agar (PDA) plate, according to procedures by Lekete (2014) and subcultured on PDA till pure cultures obtained. Pure cultures were obtained through sub-culturing and isolated fungi were identified.

Growth conditions and observations

Mycelial disc agar plug was taken from one week old actively growing colony of pure culture of each isolated fungus using 5 mm diameter cork borers. The set-ups were placed in incubation room at 28 ± 1 °C. Colony characteristics of each isolate were observed and recorded every two days for one week. Colony surface coloration was identified using the description by Shrestha *et al.* (2006). Cultures from various plots were grouped based on their morphological resemblances. There were three replications per each isolate. The conidia mass and conidiophores were later identified on glass slides by mounting a small portion of black, slimy conidial masses with blue stain and observed under Laborlux S Leitz Compound microscope of lens magnification of 40X.

Pathogenicity test

To confirm pathogenicity, 50 bags of six-month old healthy seedlings (*Dura* x *Pisifera*) were used. Twenty seedlings placed under main nursery and 20 under pre-nursery conditions with five as control per setup. This was to assess the symptoms variations observed on the field. Surfaces of three leaflets of selected seedlings each were disinfected with 70% ethanol (Sezer and Dolar, 2012). 10-µl conidia suspension (10⁵ spores/mL) of 10-day old culture of the isolated fungus was dropped on the cleaned leaves. The entire setups were covered with transparent polythene bags to artificially induce symptoms.

Fungicidal trials

This involved *in vitro* evaluation of some selected fungicides at OPRI Plant Pathology laboratory, Kusi.

The bioassay technique of Rahman (2013) was modified and employed to evaluate the effects of three recommended fungicides on mycelial growth of the leaf spot isolates *in vitro*. The fungicides were; Suncozeb 80WP, (Mancozeb 80WP ai.), Goldazim 500SC (CARBENDAZIM ai. at 500 g/L), (systemic and broad-spectrum) and Hepridion (Hepridion 70WP ai) (contact and broad spectrum).

Evaluation of fungicides bioassay in vitro

Radial growth of the test organisms was determined in various concentrations of the fungicides. The fungicides used in the experiment are listed in Table 1.

Table 1: Fungicides with their concentration.

Chemical name	Mode of action	Trade name	Concentration (ppm)
Mancozeb	Contact	Suncozeb 80 WP	25, 50, 75, 100 and 125
Carbendazim	Systemic	Goldazim 500 SC.	25, 50, 75, 100 and 125
Hepridion	Contact	Hepridion 70 WP	25, 50, 75, 100 and 125

Preparation of fungicides

Different fungicides were evaluated *in vitro* against *Pestalotiopsis* sp. following the poison food technique Rahman (2013). Concentrations of the fungicides were selected based on the recommended dosage. Recommended weights and volume of individual fungicides were used. A volume of 0.5 mL of Goldazim 500 SC. and a weight of 0.5 g each of Suncozeb 80WP and Hepridion 70WP were suspended in 100 mL of sterile distilled water to give stock solution (absolute concentration). Serial dilutions of 25, 50, 75, 100 and 125 ppm concentrations of each fungicide were used. Required amount of fungicides were mixed with 100 mL of PDA, and later shaken to allow it to mix well. Then, 20 mL each of mixture was poured into sterile Petri plates each and allowed to solidify. Agar plates were later inoculated with 5 mm-diameter discs of mycelia taken with flame-sterile cork's borer from one-week old culture of leaf spot isolate(s). Inoculated plates were incubated for six days at 28 ± 1 °C in the incubation chamber. Four plates were used for each treatment and each plate was considered as a replication. *Pestalotiopsis* sp. pathogen grown on PDA without any fungicides served as control. Each fungicide concentration was computed (Lekete 2014) as follows:

$$AD(g) = \frac{RD(g)}{RV(ml)} \times AV(ml)$$

Where AD=Application dosage weight (g/mL), RD=Recommended dosage weight (g/mL), AV=Constant application volume (mL), RV=Recommended volume (mL)

Fungicides dilutions were calculated as: $C_1V_1=C_2V_2$
Where:

C1 is the stock solution, V1 is volume of stock solution required, C2 is the final solution, and V2 is the final volume.

Effects of fungicides on radial growth of leaf spot pathogen(s)

The radial growth of *Pestalotiopsis* sp. mycelium in each plate was recorded as an average of two diameters measured at right angles to one another. Average mean

of all replications was also recorded when maximum growth was observed in control plates. Percentage inhibition of growth was calculated using 'Vincent's formula' by Jamadar and Lingaraju (2011) shown below:

$$I = \frac{C-T}{C} \times 100 \%$$

Where: I = Average percentage inhibition

C= Average Radial growth in control plate

T= Average Radial growth in fungicide plates

Radial growth was measured to assess the toxicity of each fungicide concentration.

Experimental design and data analysis

The treatments were analysed with complete randomized design (CRD) with four replications and treatment means separated using Least Significant Difference (LSD) at 1 %. The data were analyzed statistically using R Stat 2015.

RESULTS AND DISCUSSION

Field observation

Field observation showed that leaf spot disease incidence in the selected nurseries reached 85%, assuming an epidemic situation. The disease assessment was done during dry season, hence about 98% of the total plant assessed were found to be water stressed and nutrient deficient. According to Elliott (2015), water stress and nutritionally deficient seedlings are often problematic, and in many nurseries, nutrient deficiency causes chlorosis and necrosis (death) of the leaf tissue, thereby creating the wound necessary for *Pestalotiopsis* infection. This was observed on all the studied plots visited making disease severity score range as high as 2-4.

Field examination

The disease usually appears as dark brown/black and sunken spots on leaves, but symptoms varied based on location. The disease initially starts as small, water-soaked spots and later manifests as yellow-brown to black spots, and gradually increases from 5 mm to 30 mm in diameter, changing from circular to irregular lesions and expanding along and across the leaf vein in patches (spots). Spots on infected leaves at the main nursery were surrounded with yellow haloing (Figure 1); whilst that in pre-nursery were without yellow haloing (Figure 2). Acervuli were visible on the leaf surface lesions, 150-200 mm diameter.

Generally, Oil palm leaf spot is believed to be caused by 14 different causal agents (genera), but the one caused by *Pestalotiopsis* sp. at the nursery begins as small water-soaked spots. The symptoms progressed from scattered light-yellow spots, changing to dark brown/black spots covered almost all parts of the infected leaves (Figure 1). The infected areas expand into circular

yellow to light brown patches with brown to black centres (Figure 2). As the spots expand, lesion centres can turn deep brown with some spots developing yellow to light brown margins around the centres (Figure 1). During favourable weather conditions the size of the spot-on infected leaf could expand averagely between 5 mm to 25 mm in diameter within a week and in severe cases, the infected leaf die-off.

Disease incidence and sampling

A total of 360 samples of symptomatic leaf spot were collected from 120 nursery plots in Ashanti and Eastern Region of Ghana.

Disease diagnosis

The most common fungal disease isolates found in this study were *Pestalotiopsis* leaf spot 299 isolates (90.30%), *Marasmiellus* Dieback leaf blight 27 isolates (5.60%), and *Fusariums* wilt 19 (2.20%) (Table 2).

Table 2: Fungal Isolates identified.

Diseases	No. of Isolates	Percentage incidence (%)
<i>Pestalotiopsis</i> (Leaf spot)	299	90.3
Dieback leaf blight	27	5.6
<i>Fusariums</i> wilt	19	2.2
Others (Minor)	15	1.2
Total	320	

The major pathogens which caused leaf spot on oil palm seedling belonged to genus *Pestalotiopsis*. The obvious symptom of *Pestalotiopsis* leaf spot lesions appear black and sunken on leaves. Some of the old lesions have somewhat light brown borders and may contain black slimy or dry masses of spore clusters in the centre and could be best described as small black spots on the leaves (Figures 1 and 2).

Figure 1: Symptoms of leaf spot in Main nursery.

Figure 2: Leaf spot infected seedlings at Pre-nursery.

Morphological characterization

Fungal colonies on PDA grew to 25-50 mm diameter in one week at room temperature (28 ± 1 °C). The colony of *Pestalotiopsis* was pure white with concentric zonations, cottony and velvety with slimy dots of conidia mass produced on the tip of aerial mycelia (Figure 3). Characteristics of conidiophores and conidia were observed under compound microscope. The conidiophore was single with one conidium, simple or branched, straight or flexuous, plain, transparent, hollo with no septations.

Figure 3: Concentric pure white colony depicting black conidia mass of *Pestalotiopsis* sp. of leafspot.

Conidia were fusiform, five-celled (5-distoseptate), straight or slightly curved with constrictions at the septa (Figure 4) and measured 12.8 to 25.6×6.1 to 9.4 μm . The cells comprised three coloured median cells. The three median cells were light brown to dark brown, and two end cells were colourless or hyaline. Apical and basal cells are with appendages. Apical cells had 2 to 4 appendages ranging from 8.2 to 24.6 μm long. Basal cells had 1 appendage ranging from 3.4 to 6.2 μm long (Figure 4), thus showing the exact features of *Pestalotiopsis* sp. and based on morphological characteristics described above following principles of disease diagnosis, the fungal

isolate was identified as *Pestalotiopsis* sp. Although, similar fungal pathogen was identified in Thailand by Nakarin *et al.* (2013) as *Pestalotiopsis thea* causing leaf spot on oil palm seedlings, the species name of *Pestalotiopsis* identified in Ghana is yet to be confirmed using molecular tools.

Figure 4: Conidia of *Pestalotiopsis* sp.

Proof of pathogenicity

In all a total of 50 seedlings were inoculated. After two weeks, typical symptoms were observed on all inoculated palm seedlings leaves (Figure 5). The same fungus was re-isolated from the lesions, confirming Principles of Koch's postulates.

The fungus *Pestalotiopsis* sp. was known to infect monocot and dicot plants causing array of symptoms including grey blight, twig dieback, leaf spot on palm trees in Florida and some Asian countries including Thailand, but this is the first report on the fungus causing leaf spot on oil palm seedlings in Ghana. The disease occurred sporadically, but very severe, at the sampling sites. Although, it is too early to assess the potential importance of the disease, given its ability to produce severe outbreaks, it may become a threat to the oil palm industry in Ghana and Africa.

Figure 5: Results of pathogenicity under Main and Pre-nursery conditions.

Effect of fungicides against *Pestalotiopsis* sp.

Three fungicides namely, Suncozeb (Mancozeb 80WP), Hepridion and Carbendazim 500SC at five concentrations each were tested against leaf spot pathogen (*Pestalotiopsis*). The results are presented in Table 2. Suncozeb (Mancozeb 80WP), Hepridion and Carbendazim inhibited the growth of *Pestalotiopsis* sp. to various degrees (Table 3).

Table 3: Inhibition percentage of *Pestalotiopsis* sp. at different fungicides concentration

Fungicides	Concentration (ppm)	Inhibition Percentage (%)
Suncozeb (Mancozeb 80WP)	25	30.82
	50	39.38
	75	40.93
	100	44.45
	125	60.79
Control	0	0.00
LSD at 1 %		1.10
Hepridion 70WP	25	31.16
	50	34.93
	75	61.47
	100	80.31
	125	85.45
Control	0	0.00
LSD at 1 %		1.40
Carbendazim 500SC	25	97.7
	50	98.23
	75	99.47
	100	100.0
	125	100.0
Control	0	0.00
LSD at 1 %		2.20
Co-efficient of Variation		2.31

Field examination revealed Mancozeb based fungicides used by most of oil palm farmers on their farms/nurseries, unfortunately, the two promising fungicides (Carbendazim and Hepridion) evaluated *in vitro* in this study were the least patronized by these farmers. This may be the reason why *Pestalotiopsis* sp. was not effectively controlled on the field.

The significance differences observed in the inhibition of mycelia growth of the test organism showed differential effectiveness of the fungicides tested. The, causal agent of leaf spot disease on oil palm appears to be more adversely affected by systemic fungicide Goldazim 500 SC than the two contact fungicides (Mancozeb 80 WP and Hepridion). This may explain why more than 50% of the fungicides used *in vitro* in this study, which are mostly used by the farmers, could not suppress the growth and spread of the leaf spot disease on oil palm seedlings in the field. Carbendazim was very effective even at low concentration. Mancozeb was found not to be very effective even at the highest concentration.

CONCLUSION

The study revealed that leaf spot disease is emerging as one of the major diseases of oil palm in Ghana. Current disease incidence in Ghana is 85%, assuming an epidemic situation. Morphological characterization and pathogenicity test showed that, *Pestalotiopsis* sp is the causal agent of leaf spot disease on oil palm seedlings in Ghana and that the disease could infect as young as one-month old seedling. The disease can be managed through application of systemic fungicide (Goldazim 500SC (CARBENDAZIM ai. at 500 g/L) and effective cultural and Agronomic practices.

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