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Incidence, diversity and distribution of *Fusarium wilt* pathogens of eggplant in some major growing areas of Ashanti, Eastern and Volta Regions of Ghana

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ABSTRACT

Fusarium wilt of eggplant is a major cause of losses to eggplant production globally. The disease is caused by many Fusarium species. In this study, incidence, diversity and distribution of *Fusarium wilt* pathogens of eggplants were determined in five communities each from Ashanti, Eastern and Volta Regions of Ghana during the 2017–2019 cropping seasons. Purposive sampling method was used to select 10 eggplant farms infected with *Fusarium wilt* from each community. Infected eggplants were sampled for isolation and identification of Fusarium species. *Fusarium wilt* incidence was below 10% in 57.3% of the sampled farms. Differences in disease incidence were significant ($p \leq 0.05$) between the Regions. Volta Region recorded the highest disease incidence. A total of eight Fusarium species were isolated. These included *F. accuminatum*, *F. culmorum*, *F. oxysporum*, *F. proliferatum*, *F. poae*, *F. solani*, *F. subglutinans*, and *F. verticillioides*. *Fusarium solani*, *F. oxysporum* and *F. culmorum* were the most occurring species, representing 92.79% of the isolates. Six, five and eight Fusarium species were isolated respectively in Ashanti, Eastern and Volta Regions. Inoculum density of the Fusarium species was significantly ($p \leq 0.05$) higher in the Ashanti and Eastern Regions than in the Volta Region.

Keywords: *Fusarium wilt*; eggplant; diversity; pathogens; isolation

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Introduction

Fusarium wilt and Verticillium wilt pathogens have been reported to be the major causal agents of wilt in eggplants (Kouassi *et al*., 2014). *Fusarium oxysporum* f.sp. *Melongenae* is reported to cause wilt in eggplants (Altinok, 2005). However, other *Fusarium* species such as *F. equiseti* and *F. solani* also cause severe wilt in eggplants (Mwaniki *et al*., 2016). A better understanding of *Fusarium* species diversity and distribution in the growing areas was necessary for management of Fusarium wilt of eggplants. Many of the cultivated eggplants in Africa are susceptible to *Fusarium* infection particularly, *S. melongena* (Mwaniki *et al.*, 2016). This could contribute to low performance of the crop in smallholder farms in Africa where agro inputs are minimal.

Eggplant can be considered as a target crop for poverty alleviation in many African countries including, Ghana. The crop is globally considered as an important food and nutrition security crop which yields good economic returns (FAOSTAT, 2012). Eggplant is cultivated in many communities of the middle and southern part of Ghana. Various eggplant species are used in Ghanaian dishes however, *S. aethiopicum* and *S. melongena* are produced commercially. Eggplant is cultivated between March and September in Ghana by small holder farmers on an average of 1.5 Ha of land. The Ashanti and Eastern Regions produce most of the eggplants in Ghana.

Fusarium infections of eggplant result in yield loss and quality reduction due to fruit discolouration and bad flavour (Summerell *et al*., 2003). Perhaps the most damage caused by *Fusarium* infection is the contamination of the produce by the mycotoxins produced by the *Fusarium* species. To a minor extent, temperature, soil type and relative humidity affect distribution of some *Fusarium* species. However, most of the species are cosmopolitan, an indication of high adaptability to varied climatic conditions and wide host range (Leslie & Summerell, 2006; Summerell *et al.*, 2003).

Losses in eggplant production due to Fusarium wilt disease have not received much research attention in Ghana. In this study, eggplant farms of selected eggplant producing communities of the Ashanti, Eastern and Volta Regions of Ghana were sampled and assessed for Fusarium wilt infections. The objective of the study was to compare the incidence, diversity and distribution of *Fusarium* species causing Fusarium wilt of eggplant in the Ashanti, Eastern and Volta Regions of Ghana.

Materials and Methods

Study area

This study was conducted during the 2017, 2018 and 2019 crop growing seasons in the Ashanti, Eastern and Volta Regions of Ghana. Five eggplant growing communities were selected from each Region; Offinso, Abofour, Juaso, Nsuta, and Besoro from the Ashanti Region; Kwahu-Praso, Eyerisi, Nkurakan, Huhunya and Asiakwa from the Eastern Region; and Have, Tafi, Vakpo, Aneta and Yordan communities from the Volta Region. Laboratory analyses were conducted at the Plant Pathology Laboratory of the Department of Crop and Soil Sciences, KNUST, Kumasi.

Sampling of eggplant farms for Fusarium wilt disease assessment

Eggplants farms were sampled using purposive sampling method. Eggplant farmers were identified through interaction with Agriculture Extension Agents and Heads of farmer associations in the selected communities. Eggplant farmers that had reported of wilt symptoms were selected and through snowball (respondent-driven) sampling approach, other wilt-infected eggplant farms were also identified. Ten farms were selected and visited in each eggplant growing community for disease assessment and sampling. A total of 150 farms were visited in the 15 communities in the study.

Disease incidence determination

An experimental plot of 400 m^2 which contained 420 eggplants was randomly sampled for each farm and assessed for incidence of Fusarium wilt disease. The farms were assessed at the

fruiting stage of the crop. This is the stage the disease is most noticeable. Incidence assessment was generally based on above ground observable symptoms, occasionally roots parts were examined. The symptoms that were observed included leaf and stem wilt, leaf yellowing, drooping of apical shoot, root rot and root discolouration. Presence of one or a combination of the characteristic symptoms of Fusarium wilt disease on any eggplant in a farm was termed as incidence of the disease. Disease incidence was expressed as a percentage of the number of diseased eggplants to the total number of eggplants in the farm as presented below. The incidences were grouped into three categories, namely incidence of below 10 %, 10–20% and above 20%.

$$\text{Disease incidence} = \frac{\text{Number of infected eggplants on farm}}{\text{Total number of eggplants on farm}} \times 100$$

Collection, preparation and isolation of Fusarium species from eggplant roots

10 plants were sampled in each farm visited; five plants with visible symptoms of wilt and other five with no visible wilt symptom. A systematic sampling method was used as follows; starting at the third row in the left-hand corner of the farm facing the north, a plant was collected from any other row towards the right-hand until the 10 samples were collected. Whole plants were carefully uprooted, excised at the root collar, placed in a paper envelope, separately labelled and sent to the Plant Pathology Laboratory of the Department of Crop and Soil Sciences, KNUST, Kumasi. The samples were air-dried on the laboratory bench at room temperature (25±2°C).

Air-dried eggplant roots were washed separately under running tap water to remove all soil and debris. Each root was subjectively excised to 1 cm long and 0.5 cm wide pieces for each plant. Cut pieces were surface sterilized in 0.5% NaOCl for 3 min and in 75% ethanol for another 3 min, rinsed three times in sterile-distilled water, and blotted dry with blotter paper. Six root pieces were then plated on chloramphenicol (250 mg/l) amended Potato Dextrose Agar (PDA) medium and incubated at 25±2°C for seven days at 12 hr photoperiod under fluorescent light in the incubation room (Leslie & Summerell, 2006; Watanabe, 2000). Inoculation of the eggplant root samples were done under aseptic conditions. Fungal colonies were examined and counted after seven days and then sub-cultured for identification.

Preparation of Potato Dextrose Agar (PDA)

39 grammes of powdered PDA and 250 mg Chloramphenicol were added to 500 ml of distilled water in a 1.5 litre conical flask and mixed thoroughly with a magnetic stirrer, on a hotplate until the PDA completely dissolved. Additional distilled water was used to top the solution to one liter. The conical flask was stoppered with cotton wool, wrapped with aluminium foil and sterilized in an autoclave at 121^{o}C and 0.98 kg/cm^{2} pressure for 15 minutes. The sterilized medium was allowed to cool to about 50^{o}C, poured into sterile Petri plates in the lamina flow (20 ml/plate) and allowed to solidify. Each plate was sealed with cellophane and stored for 72 hours at 25±2°C.

Identification of Fusarium species

Single conidia culture of the *Fusarium* isolates was prepared by following the protocol as follows. A culture of each Fusarium isolate was flooded with 10 ml of sterile-distilled water and sterile glass rod was used to rub the culture surface to form a conidia suspension. The suspension was filtered through a sterile double-layer cheese cloth. The conidia

concentration of the suspension was reduced by addition of sterile-distilled water until 1–10 conidia were seen in a drop of the suspension under microscope. One milliliter aliquot of the suspension was spread on three-day old chloramphenicol-sulphate amended water agar plates and incubated for three days at 25±2°C. Germinating colony was picked with a sterilized inoculating needle and transferred onto chloramphenicol-sulphate amended PDA plates and incubated at 25±2°C. All activities were done aseptically under lamina flow in the inoculation chamber. Each of the *Fusarium* isolates was examined microscopically with compound microscope and identified to species level according to the identification manuals (Ghoneem *et al*., 2009; Leslie & Summerell, 2006). Isolates were identified using the distinctive morphological characteristics of macro-conidia and micro-conidia production and colour of sporodochia, colony colour and pigment.

Experimental design and data analyses

The experiment was organized in a completely randomized design in factorial, with three blocks (regions), five treatments (communities) and ten replications (farms). Differences in mean values were analyzed with ANOVA, using Genstat 12th edition, (VSN International, UK), at confidence interval of 95%. The Duncan's multiple range test was used to separate the differences between means at 5% level of significance. The results were presented as tables and graphs and interpreted appropriately .

Results and Discussion

Incidence of Fusarium wilt disease

All the eggplant farms assessed recorded some degree of Fusarium wilt infection (Table 1). Typically diseased eggplant had lower leaf yellowing, stunting and smaller and fewer fruits. When roots were cross-sectioned, dark brown to dark colouration showed evidence of collapsed vascular tissues. Observed symptoms were similar across the farms. However, there were few eggplants with the characteristic symptoms in most of the surveyed farms.

Disease incidence by visual assessment was below 10% in most of the farms (Fig. 1). 85 farms, representing 57.3% of the total number of farms assessed in the Ashanti, Eastern and Volta Regions recorded disease incidence of below 10%. 49 farms recorded incidence of 10–20% and 16 farms recorded incidence of above 20%. Disease incidence variation was more significant between the regions than it was between the communities of the regions. The Volta region recorded significantly ($p \geq 0.05$) higher disease incidence than the Ashanti and Eastern regions. Disease incidence in the Ashanti region was not significantly different from that of the Eastern regions (Table 1).

The observed higher degree of incidence of Fusarium wilt disease of eggplant in the Volta Region could be attributed to farm practices and uniformity in cultivated eggplant variety. It was a common practice in the Volta Region to plough all the eggplant, including diseased ones back into the soil after harvest. This practice promotes the build-up of infectious propagules of the *Fusarium* pathogens over time. The extent of Fusarium wilt disease in a farm would depend on the initial inoculum concentration in the soil (Yergeau *et al*., 2006). Also in the Volta Region all the farmers cultivated the Kpando variety because of its high yield and appeal to buyers. Uniformity of planting material coupled with common tractor services in most of the farms could have amounted to rapid disease spread

and development. Different varieties of eggplant may vary in susceptibility to a disease therefore higher eggplant diversity could limit disease development and progression (Leyva-Madrigal *et al.*, 2014).

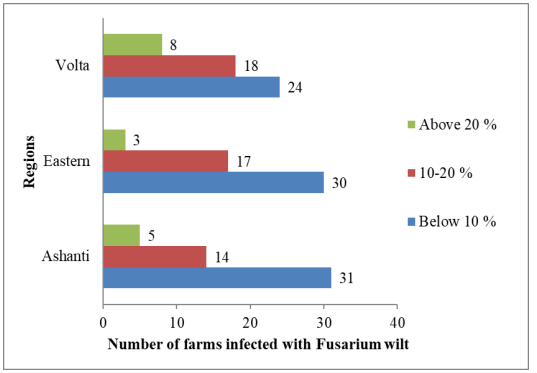

Fig. 1: Incidence of Fusarium wilt of eggplant in the selected farms of the three sampled regions of Ghana

TABLE 1
Incidences of Fusarium wilt of eggplant in the selected communities of the Ashanti, Eastern and Volta Regions

		Percent disease incidence		
Regions	**Communities**	**Below 10 %**	**10-20 %**	**Above 20 %**
Ashanti	Offinso	50d	40b	10b
	Abofour	70b	20d	10b
	Besoro	80a	20d	-
	Nsuta	50d	20d	30a
	Juaso	70b	30c	-
Eastern	Asiakwa	70b	30c	-
	Enyerisi	50d	40b	10b
	Huhunya	70b	20d	10b
	Kwahu Praso	70b	20d	10b
	Nkurakan	40e	60a	-
	Aneta	40e	60a	-
	Have	60c	30c	10b
Volta	Tafi	50d	40b	10b
	Vapko	50d	20d	30a
	Yordan	40e	30c	30a
	CV (%)	0.23	0.43	1.03

Numbers with the same letter in a column are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test

Diversity of Fusarium species and frequency of distribution in sampled regions

This study provides a report regarding diversity and frequency of distribution of *Fusarium* species associated with Fusarium wilt of eggplant among 15 growing communities in the Ashanti, Eastern and Volta Regions of Ghana. Eight *Fusarium* species namely; *F. accuminatum*, *F. culmorum*, *F. oxysporum*, *F. proliferatum*, *F. poae*, *F. solani*, *F. subglutinans*, *and F. verticillioides* were isolated from wilt infected root tissues of eggplants in different locations at different frequencies in this study. The Volta Region had the highest diversity of *Fusarium* species, where all eight *Fusarium* species were isolated (Table 2). However, regarding total infection levels as described by the mean number of colonies of *Fusarium* species in infected eggplant root samples, the region had the least total infection. The Ashanti Region had the highest total Fusarium wilt infection in the study followed by the Eastern Region (Table 3).

TABLE 2
Fusarium species isolated from eggplant in the Ashanti, Eastern and Volta Regions

Fusarium species	***Fusarium* species per Region**		
	Ashanti	**Eastern**	**Volta**
F. accuminatum	-	+	+
F. culmorum	+	+	+
F. oxysporum	+	+	+
F. proliferatum	-	-	+
F. poae	+	+	+
F. solani	+	+	+
F. subglutinans	-	-	+
F. verticillioides	+	+	+

Present (+), Absent (-)

TABLE 3
Colonies of Fusarium species per 150 eggplant root samples from the Ashanti, Eastern and Volta Regions

Fusarium Species	**% mean Colony of Fusarium species per Region**		
	Ashanti	Eastern	Volta
F. accuminatum	-	2.7c	2.2b
F. culmorum	25.2b	1.2c	8.3b
F. oxysporum	28.3b	25.4b	35.8a
F. proliferatum	-	-	2.4b
F. poae	0.4c	0.6c	0.6b
F. solani	40.8a	65.4a	43.7a
F. subglutinans	-	-	0.6b
F. verticillioides	5.3c	4.7c	6.9b
CV (%)	**1.31**	**1.84**	**1.36**
number of colonies counted	**2527**	**1841**	**492**

Numbers with the same letter in a column are not significantly different at ($p \leq 0.05$) according to Duncan's Multiple Range Test.

This observation indicates that Fusarium wilt of eggplant caused by *Fusarium* complex other than the ascribed *Fusarium oxysporum* f. sp. *melongenae* (Altınok, 2005). Mwaniki *et al*. (2016) made a similar observation where *F. oxysporum*, *F. equiseti and F. solani* were identified to cause vascular wilt in eggplants. In this study *F. solani*, *F. oxysporum* and *F. culmorum* were the most frequent species. *Fusarium solani* was however, ubiquitous and the most abundant in all the sampled communities. *Fusarium accuminatum*, *F. proliferatum*, *F. poae*, *F. subglutinans*, *and F. verticillioides* were less frequent, sporadic and found in smaller quantities (Fig. 2).

The most isolated *Fusarium* species were *F. solani*, *F. oxysporum* and *F. culmorum* which occurred in 50.4%, 28.0% and 14.4% respectively of the total *Fusarium* species CFU recovered from infected eggplant roots sampled in this study. These three species together made 92.8% of all the *Fusarium* species isolated. The less frequent species were *F. verticillioides* and *F. accuminatum* which made 5.2% and 1.3% of the isolates respectively (Fig. 2). The other *Fusarium* species occurred sporadically and in small quantities, these were *F. subglutinans* (0.1%) and *F. proliferatum* (0.3%) which were isolated only from the Volta Region.

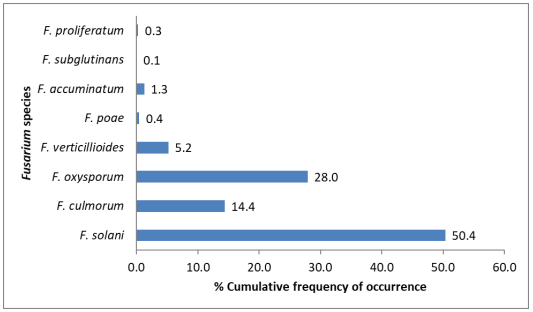

Fig. 2: Cumulative frequency of occurrence of *Fusarium* species isolated from the study areas

Fusarium infection of eggplant was widespread in the study area. Many *Fusarium* species are distributed along the geographic range of their host (Burgess *et al.,* 1994). The widespread infection observed is an indication of the vulnerability of *Solanum* species to Fusarium wilt. Mwaniki *et al.* (2016) reported that most cultivated eggplant species in Africa were susceptible to Fusarium wilt pathogens.

The Ashanti and Eastern Regions which record higher rainfall among the three regions had higher quantities of *Fusarium* isolates. However, infection was lower compared to the Volta Region which has lower amount of rainfall. According to Lester *et al*. (1988), *Fusarium*-infected plants may be symptomless in wet soils but show symptoms when the soil is moisture-stressed. Soil moisture and temperature are limiting factors for survival, activity and distribution of *Fusarium* species (Seremi & Amiri, 2010; Larkin & Fravel, 2002; Mui-Yun, 2003; Henriksen, 1999). This explains the observed differences in frequency of occurrence of *Fusarium* species in the communities and regions.

Infections caused by *F. solani* were higher than that of *F. oxysporum* and *F. culmorum*. *Fusarium solani* is prolific in dispersal due to production of abundant microconidia (Leslie & Summerell, 2006; Buxton & Perry, 2019). It is known to be well established in tropic conditions and infects a wide range of plants including pepper (Fletcher, 1994) and potato (Secor & Gudmestad, 1999). The prolific dispersion of *F. solani* was revealed in this study by the high number of root samples from which the species was isolated across the sampled communities (Table 4).

Fusarium culmorum present itself as a weak pathogen and infects roots more passively with assistance of nematode or wound. *Fusarium culmorum* may intensify its disease impact through association with other diseases (Koch & Huth, 1997). The absence of microconidia in *F. culmorum* limits the quantity of propagules or innocula produced for infection. This could therefore have affected its frequency of occurrence and distribution in the field as observed in this study. Although established as important pathogen of many crops of diverse aetiology, limited studies suggest the use of *F. culmorum* in an integrated management programme for some weeds (Shabana *et al.,* 2003a; Shabana *et al.,* 2003b).

In this study *F. oxysporum* infection was very widespread in the sampled communities, second only to *F. solani*. It was most widespread in the Eastern Region but more abundant in the Ashanti Region. The speedy production of abundant macroconidia, microconidia and chlamydospores by *F. oxysporum* enhanced its dispersion (Leslie & Summerell, 2006). Therefore spread of its infection was observed in this study, where a large number of root samples collected were infected with *F. oxysporum.*

In this study, the distribution of *Fusarium* species was distinct. *Fusarium solani* was ubiquitous, isolated in every community sampled. *Fusarium oxysporum* was more widespread in the communities of Eastern Region than the Ashanti and Volta Regions. *Fusarium culmorum* was however abundant in the Ashanti Region and sporadic in the Eastern and Volta Regions. Other isolated species; *F. accuminatum*, *F. proliferatum*, *F. poae*, *F. subglutinans*, *and F. verticillioides* were more sporadic and scanty.

TABLE 4

Frequency of occurrence of Fusarium species in infected eggplant roots sampled in communities of the Ashanti, Eastern and Volta Regions

Region	Communities	Number of root samples infected (50 root samples per community)							
		F. accuminatum	*F. culmorum*	*F. oxysporum*	*F. proliferatum*	*F. poae*	*F. solani*	*F. subglutinans*	*F. verticillioides*
Ashanti	Abofour	-	16a	-	-	-	12a	-	-
	Nsuta	-	8c	16a	-	-	8b	-	-
	Besoro	-	-	6b	-	-	8b	-	4a
	Juaso	-	-	16a	-	2	16a	-	-
	Offinso	-	12b	-	-	-	8b	-	4a
	Total	0	36	38	0	2	52	0	8
	CV (%)	0	0.99	1.06	0	0	0.34	0	1.37
Eastern	Asiakwa	-	-	20a	-	4	20a	-	-
	Huhunya	4a	-	16ab	-	-	8c	-	-
	Enyerisi	4a	-	12bc	-	-	16b	-	-
	Kwaho Praso	-	4	12bc	-	-	16b	-	12a
	Nkurakan	-	-	8c	-	-	16b	-	4b
	Total	8	4	68	0	4	76	0	16
	CV (%)	1.37	0	0.34	0	0	0.29	0	1.63
Volta	Aneta	-	-	4b	-	-	8cd	-	2a
	Have	-	4a	14a	-	-	12a	2	-
	Tafi	4	-	-	-	-	8cd	-	4a
	Vapko	-	2a	4b	-	-	10bc	-	4a
	Yordan	-	-	-	4	-	6d	-	-
	Total	4	6	22	4	0	44	2	10
	CV (%)	0	1.49	1.3	2.24	0	0.26	2.24	1

Numbers with the same letter in a column are not significantly different at ($p \geq 0.05$) according to Duncan's Multiple Range Test

TABLE 5

Frequency of colony forming units (CFU) of Fusarium species isolated from eggplant roots sampled in communities of the Ashanti, Eastern and Volta Regions

Region	Communities	Mean number of CFU							
		F. accuminatum	*F. culmorum*	*F. oxysporum*	*F. proliferatum*	*F. poae*	*F. solani*	*F. subglutinans*	*F. verticillioides*
Ashanti	Abofour	-	74a	-	-	-	59b	-	-
	Nsuta	-	23c	111a	-	-	38d	-	-
	Besoro	-	-	24c	-	-	57c	-	14b
	Juaso	-	-	44b	-	5	85a	-	-
	Offinso	-	62b	-	-	-	19e	-	20a
	Total	0	159	179	0	5	258	0	34
	CV (%)	0	1.09	1.28	0	0	0.48	0	1.4
Eastern	Asiakwa	-	-	37a	-	3	65b	-	-
	Huhunya	3b	-	23b	-	-	16b	-	-
	Enyerisi	10a	-	25b	-	-	25b	-	-
	Kwaho Praso	-	6	14b	-	-	23b	-	17a
	Nkurakan	-	-	19b	-	-	173a	-	4b
	Total	13	6	118	0	3	302	0	21
	CV (%)	1.67	0	0.36	0	0	1.09	0	1.75
Volta	Aneta	-	-	4b	-	-	8c	-	2a
	Have	-	6a	38a	-	-	12b	3	-
	Tafi	3	-	-	-	-	3d	-	4a
	Vapko	-	4a	3b	-	-	17a	-	4a
	Yordan	-	-	-	3	-	14b	-	-
	Total	3	10	45	3	0	54	3	10
	CV (%)	0	1.41	1.81	0	0	0.5	0	1

Numbers with the same letter in a column are not significantly different at ($p \geq 0.05$) according to Duncan's Multiple Range Test

Conclusion and Recommendation

The study revealed widespread of Fusarium wilt disease of eggplants in some of the major growing areas of Ghana even though percent incidence was generally low. Diverse *Fusarium* species were involved in Fusarium wilt of eggplant however *F. oxysporum*, *F. solani* and *F. culmorum* were the most dominant with high inocula density in the Ashanti and Eastern Regions. Cognisance must be given to the diversity of *Fusarium* pathogens involved with Fusarium wilt of eggplants in the development of management method for the disease.

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