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Incidence and severity of Fusarium wilt on Bambara nut (*Vigna subterranea* L.) landraces in Western Kenya

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Abstract

Bambara nut, *Vigna subterranea* (L.) Verdc. is one of the indigenous legume crops grown in Kenya. The crop is highly nutritious and drought tolerant with the ability to produce higher yields than other legumes such as common groundnuts and beans produced under the same conditions. Bambara nut demand is on the increase due to the current focus on neglected underutilized crop species. The crop yield is however low because of biotic stresses among others. *Fusarium oxysporum* Schlechtend f.sp. *voandzeia* is one of the most destructive fungal pathogens affecting Bambara nut in Kenya. A greenhouse experiment was therefore carried out to determine the incidence and severity of the disease on local landraces of Bambara nut. The experiment was laid out in a completely randomized design replicated three times. Disease incidence and severity varied significantly with landrace. The maroon speckled and brown dark eyed landraces had the highest disease incidences (80.5% and 80.0%). Followed by brown light eyed, maroon and black landraces with diseases incidences of 79.5%, 78.9% and 78.6% respectively and lastly the red landrace with least disease incidence of 76.2%. Disease severity also varied with landrace. The maroon followed by the brown dark eyed landraces had the highest disease severities of 45.5% and 44.3% then the red, maroon speckled and black landraces with severities of 43.5%, 43.1% and 42.8% respectively. The Brown light eyed landrace had the least disease severity of 42.6%. There was a significant interaction between landrace and days after inoculation with respect to disease severity. At 75 days after inoculation disease severity was similar for all the landraces with approximately 40% severity. The area under disease progress curves (AUDPC) varied with landrace. The Maroon landrace had the highest AUDPC while the Brown landrace had the least area. The study confirmed the virulence of the pathogen on the crop and the need to manage the disease for improved yield performances.

Keywords: *Bambara nut, Fusarium oxysporum, Incidence, Severity*

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1. Introduction

Bambara nut, *Vigna subterranea* (L.) Verdc. is one of the indigenous legume crops grown in sub Saharan Africa. The crop ranks third after ground nuts (*Arachis hypogaea* L.) and cowpeas (*Vigna unguiculata* L.) in most parts

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of Africa (Mkandawire, 2007). Bambara nut seed is highly nutritious containing about 49-63.5% carbohydrates, 15-25% protein, 4.5-7.4% lipids and 5.2-6.4% fiber (Bamshaiye *et al.*, 2011). The crop is drought tolerant with the ability to produce higher yields than other legumes such as groundnuts and beans produced under the same conditions (Masindeni, 2006). Bambara nut is important in low input agricultural production systems because of its ability to fix atmospheric nitrogen into the soil with the aid of *Bradyrhizobium* bacteria. The demand for the crop is rising because of the added effects of its medicinal value (Directorate of Plant Production, 2011).

In Kenya, Bambara nut is mainly grown at the Coast, Eastern, Western and Nyanza regions (Wasula *et al.*, 2012; and Wasula, 2014). The crop's production has however been low because of diseases among other factors. Fusarium wilt caused by *F. oxysporum* Schlechtend f.sp. *voandzeia* is among the most important diseases of Bambara nuts in Kenya (Cook, 1978). The disease is prevalent in most Bambara nut growing regions (Begemann, 1986). *Fusarium oxysporum* attacks Bambara nut at all stages of development. The propagules gain entry into the plant through cut surfaces of seeds, damaged roots and stem tissues of young and stressed plants, infected seeds and through wounds caused by insects (Leslie *et al.*, 2006).

Generally the recommended control practices for *F. oxysporum* include use of resistant varieties, fungicidal seed treatments, adjustment of planting dates, use of biofumigants, bioagents and crop rotation techniques among others (Blanca *et al.*, 2004; Ajiloba and Babalola 2013; and Kemal *et al.*, 2015). In Kenya, farmers grow the crop during the short rainy season when the disease pressure is low. There is an increasing demand for Bambara nut arising from several alternative uses such as medicinal values, need for healthy diets and its adaptability to unpredictable climate changes. The climate change implies that farmers have to be prepared for different conditions that may affect disease incidence and severity and therefore need to plant Fusarium wilt tolerant landraces. Host plant resistance is one of the most important options for disease control but little work has been done to identify and quantify its status among Bambara nut landraces.

An experiment was carried out to determine the incidence and severity of Fusarium wilt on Bambara nut in Western Kenya with an aim of identifying the tolerant landraces.

2. Materials and methods

2.1. Site description

Bambara nut samples were collected from farmers' fields in Busia county which lies between latitudes 0°30' and 0°45' N and longitudes 33º55' and 34°25' E, at an altitude of 1200 m above sea level. The area experiences a mean annual rainfall of 900 mm-1800 mm and annual mean temperature of between 17 °C and 22 °C. The area lies within the Lower Midland 1 and 2 agro ecological zones with predominantly humic *acrisol* soils (Jaetzold *et al.*, 2006).

2.2. Sampling and fungal isolation

Bambara nut plants showing symptoms of the disease were collected from farmers' fields in Busia County and transported in press seal plastic bags (75 mm × 100 mm) in a cool box to Egerton University's Biological Sciences laboratory for pathogen isolation and identification. The Kristin and James (2000) protocol was used for fungal isolation. Root tissues were washed in running tap water, cut into 1 cm portions, surface sterilized in 1.5% NaOCl for one minute, double rinsed in sterile distilled water and blot dried between sterile paper towels.

Glass Petri dishes (Pyrex®) sterilized in the oven at 160 °C for 45 min were used to grow the isolates. The sterilized samples were aseptically plated on Czapekdox agar medium (Sodium nitrate 3 g, Potassium chloride 0.5 g, Di-potassium hydrogen phosphate 1 g, Sucrose 30 g, Magnesium sulphate 1 g, Ferrous sulphate 0.01 g, Agar 15 g and water 1 liter), a selective media for *Fusarium* growth.

The medium was autoclaved at 121 °C for 20 min allowed to cool down to touch temperatures before being amended with 5 ml/liter of streptomycin sulphate to eliminate bacteria contaminants. The sterile plates containing the samples were sealed using parafilm and incubated at 25- 26 ºC, before examination for colony formation within 2-14 days (Maina *et al.*, 2015). The Fusarium wilt fungus was purified by sub-culturing on Potato Dextrose Agar (PDA) media and identified with the help of relevant literature (Leslie and Summerell, 2006).

2.3. Pathogenicity test

The *in vivo* assay was carried out in the greenhouse at Egerton University based on the Ros *et al.* (2005) method. A pure sub culture was made from cultures isolated from infected Bambara nut roots and an inoculum prepared from these cultures. Sterilized PDA plates amended with 5 ml / liter of streptomycin sulphate were inoculated with three mycelia plugs of *Fusarium oxysporum* from actively growing regions of the mycelial growth and incubated for 7 days at 25-26 ºC. The spores from 7-day old cultures were scrapped off from the petri dishes surfaces by adding sterile distilled water so as to obtain a suspension that was filtered through one layer of miracloth and the concentration adjusted using a haemocytometer to 10^6 conidia / ml.

Seeds of the black and red landraces of Bambara nut were surface sterilized in 1% sodium hypochlorite for three minutes, rinsed in three changes of distilled sterile water and air dried in the laminar flow. Three seeds per landrace were then planted in pots (18 cm long and 19 cm wide) containing 2.5 kg of sterilized sandy loam soil (autoclaved at 120 °C for 1 h for three consecutive days) with adequate watering. At 14 days after emergence 10 ml of a 10^6 conidia / ml of spore suspension was applied over the base of the hypocotyl of the seedlings using a 10 ml syringe. Development of pathogenicity signs and symptoms was observed on a weekly basis and records taken. Re-isolation was then carried out to confirm isolates identity 12 weeks after planting.

2.4. Disease incidence and severity

The experiment was laid out as a completely randomized design replicated three times in a greenhouse. The most cultivated Bambara nut landraces in Busia county (black, red, maroon, maroon speckled, brown light eyed and brown dark eyed) were used in the study. Two seeds per landrace were germinated in pots (measuring 18 cm in length and 19 cm in diameter) containing 2.5 kg of sterilized sandy loam soil (autoclaved at 120 °C for 1 h for three consecutive days) with adequate watering. Fourteen day old seedlings were then inoculated with 10 ml of a 10^6 conidia / ml of spore suspension of *F. oxysporum* at the base of the hypocotyl using a 10 ml syringe as described by Kristin and James (2000).

2.5. Disease assessment

Wilt incidence and severity were estimated at 10-day intervals using a scale of 0-5 based on leaf yellowing rating as described by Abdou *et al.* (2001). The rating included;

0 = Healthy

1 = One leaf yellowing

2 = More than one leaf yellowing

3 = One wilted leaf

4 = More than one leaf wilted

5 = Completely dead plants

Disease Severity Index (DSI) was determined using the formula described by Liu *et al.* (1995) as;

$DSI = \Sigma d/(d\,max \times n) \times 100$ where;

d – The disease rating of each plant

d max – The maximum disease rating

n - The total number of plants / samples examined in each replicate.

Disease Incidence (DI) at 45 days after inoculation was determined using the equation proposed by Cooke (2006) for a period of 60 days.

$DI = (No.\ of\ infected\ plant\ units/total\ no.\ of\ plant\ units\ assessed) \times 100$

The area under disease progress curve (AUDPC) which is a quantitative summary of disease intensity over time for each landrace was calculated using the formula suggested by Pandy *et al.* (1989).

$AUDPC = D\,[1/2\,(Y1+Yk) + (Y2+Y3+\ldots\ldots\ldots+Yk-1)]$

where;

D = Time interval

*Y*1 = First disease severity

Yk = Last disease severity

*Y*2, *Y*3,..., *Yk* – 1 = Intermediate disease severities

3. Results

3.1. Colony characteristics

The mycelia were white cottony to pink with the aerial part becoming tinged with purple coloration (Plate 1). The reverse was non-descript pale to yellow (Plate 2). Microconidia were abundantly born on phialides with straight, curved or ellipsoidal shapes. The macroconidia were sparsely found accumulating along the hyphae or scattered. They were often 3-5 septate and pointed at both ends, more ellipsoidal than microconidia (Plate 3). Chlamydospores which developed after seven days (i.e., about 14 days of incubation) were either smooth or rough walled, abundant and formed terminally or intercalary (Plate 4).

Plate 1: Mycelial morphology – aerial view

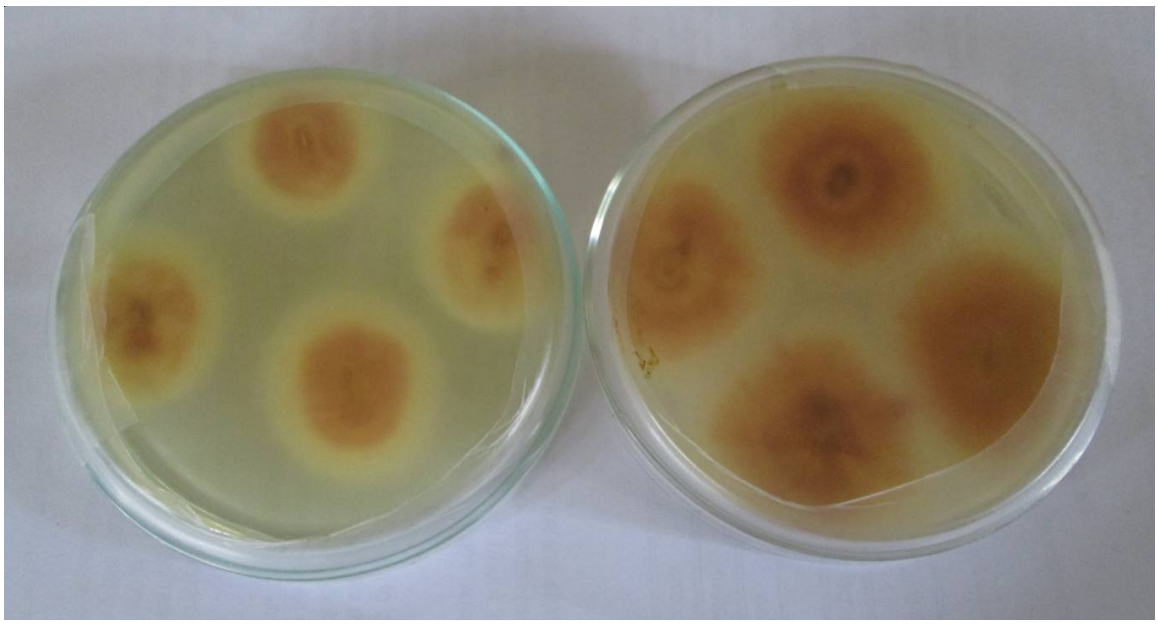

Plate 2: Mycelial morphology – reverse view

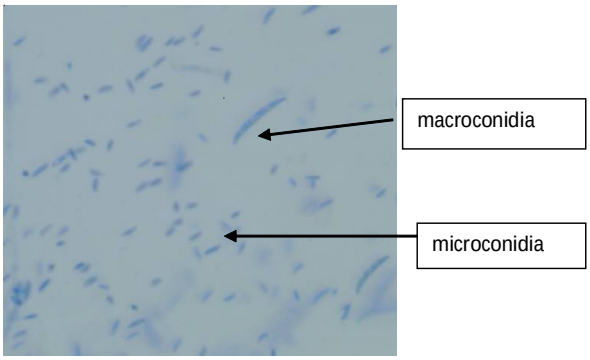

Plate 3: *Fusarium oxysporum* Macro- and Microconidia (X400 as magnification)

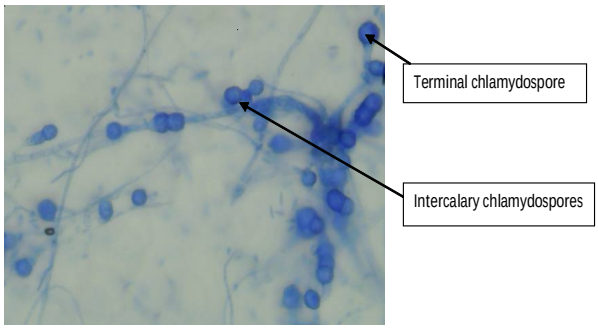

Plate 4: *Fusarium oxysporum* chlamydospores (X400 as magnification)

3.2. Pathogenicity

Fusarium wilt symptoms occurred 45 days after inoculation (Plate 5). The first indication of the disease was yellowing of leaves that intensified with time, before the entire crop eventually wilting. Destructive sampling showed brown vascular discolorations. The isolate was highly virulent with all the two landraces (black and red) being susceptible to the pathogen. *Fusarium oxysporum* identical to the parent isolate was re-isolated from the diseased root tissues.

Plate 5: Pathogenicity symptoms on black and red landraces at 8 weeks old in the glasshouse

3.3. Incidence and severity of Fusarium wilt

The landraces had high and varied disease incidence and severity (Table 1). The maroon speckled and brown dark eyed landraces had the highest disease incidences of 80.5% and 80.0%. Followed by brown light eyed, maroon and black landraces with incidences of 79.5%, 78.9% and 78.6% respectively. The red landrace had the least disease incidence of 76.2%.

Disease severity varied with landrace. The maroon and brown dark eyed landraces had the highest disease severities of 45.5% and 44.3%. Followed by the red, maroon speckled and black landraces with severities of 43.5%, 43.1% and 42.8% respectively. The brown light eyed landrace had the least disease severity of 42.6%.

3.4. Area under disease progress curves (AUDPC)

The AUDPC for severity were generated for all the landraces and at 75 days after inoculation all the landraces had similar disease severity of approximately 40% (Figure 1). Incidence and severity varied significantly with landrace as well as days after inoculation (Table 2). Interaction between landrace and days after inoculation was significant for the disease severity. The AUDPC varied with landrace (Figure 2). The maroon and brown dark eyed landraces had the highest AUDPC, followed by the red, maroon speckled and black landraces respectively. The brown light eyed landrace had the least AUDPC.

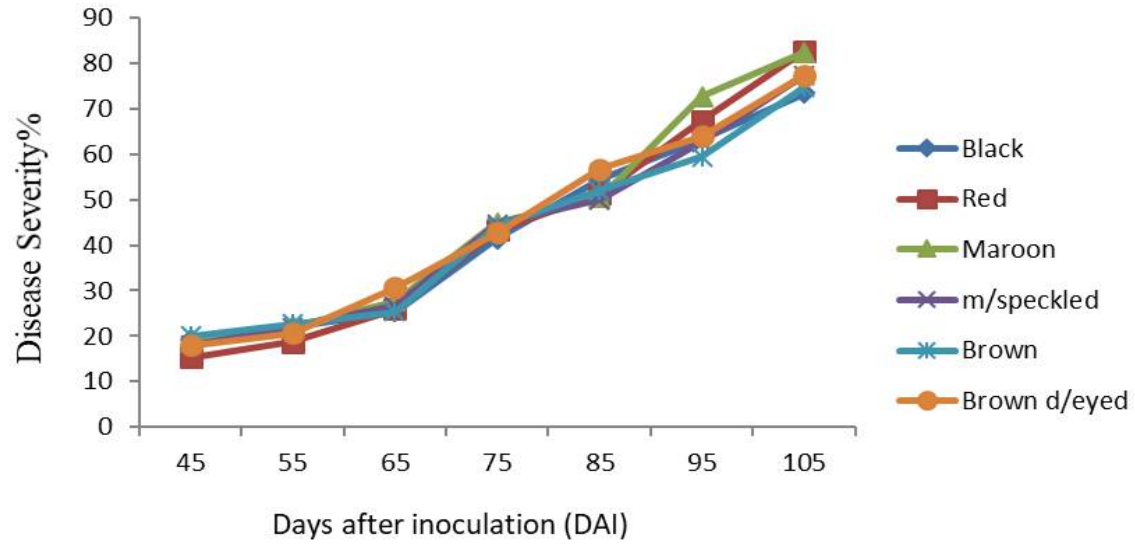

Figure 1: Disease severity progress curves of Fusarium wilt of Bambara nut for the six landraces

Table 1: Fusarium wilt incidence and severity among the local landraces of Bambara nut

Landrace	Incidence (%)	Severity (%)
Black	78.6^{ab*}	42.8^{bc}
Red	76.2^{b}	43.5^{bc}
Maroon	78.9^{ab}	45.5^{a}
M/speckled	80.5^{a}	43.1^{bc}
Brown light eyed	79.5^{ab}	42.6^{c}
Brown dark eyed	80.0^{a}	44.3^{ab}
LSD ($p \leq 0.05$)	3.62	1.67

Note: *Means followed by the same letter in the column are not significantly (pd"0.05) different from each other.

Table 2: ANOVA for Fusarium wilt incidence and severity among selected Bambara nut landraces

Source	DF	Probability Values	
		Incidence	Severity
Landrace	5	0.0368	0.0057
Days after inoculation	6	<0.0001	<0.0001
LR*DAI	30	0.7783	<0.0001

Note: LR*DAI – Interaction of landrace and days after inoculation.

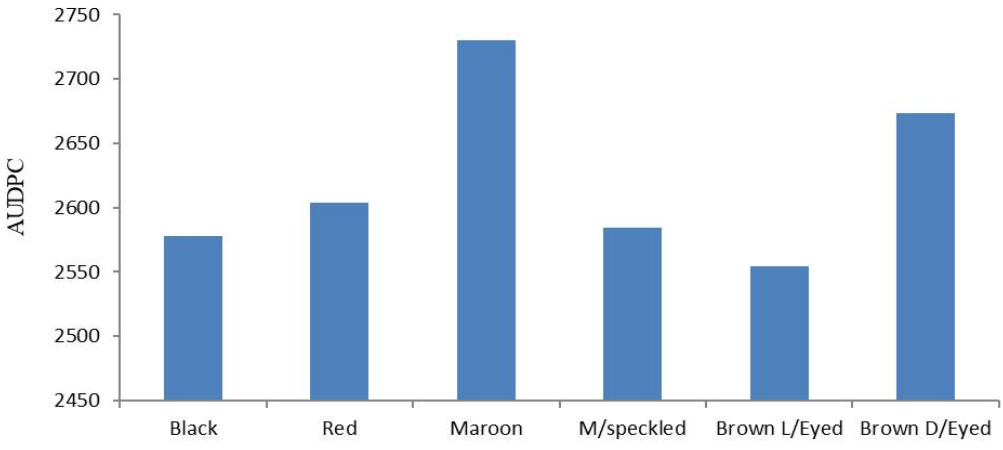

Figure 2: Area under disease progress curves of Fusarium wilt of Bambara nuts for the six landraces

4. Discussion

Fusarium wilt of Bambara nuts is among the most destructive soil-borne diseases of the crop. The disease is prevalent in most Bambara nut growing regions and it attacks Bambara nut at all stages of development. Fusarium wilt was isolated in most of the samples collected from farmers' fields in Busia. Wakelin *et al.* (2008) similarly reported abundance of *Fusarium* species in cultivated soils and mostly associated with plant roots. The colony characteristics are consistent with the descriptions by Kleczewski and Egel (2011). Pathogenicity tests proved presence of the disease since the symptoms expressed in the greenhouse were similar to those observed in farmers' fields. Ebbels and Billington (1972) in Tanzania similarly reported presence of Fusarium wilt on Bambara nut landraces. Occurrence of symptoms varied with landraces ranging from 14 days to 50 days after inoculation. The characteristic symptoms which included yellowing of leaves, wilting of the plant and brown vascular discoloration throughout the trial period were in complete compliance with those observed by Sharma (2011).

In the incidence and severity studies, the symptoms were seen starting from the vegetative phase through flowering with varying degrees among the landraces. Zemouli-Benfreha *et al.* (2013) and Landa *et al*. (2004) similarly reported presence of Fusarium wilt symptoms in all stages of chickpea development. This is probably because the pathogen is soil borne (Zhao *et al*., 2014) hence infects the plant starting from seedling phase. However at 75 days after inoculation all the landraces had similar degree of disease severity of approximately 40%. It is around this period that the crop is flowering and initiating pod set and also the inoculum levels are high due to rapid sporulation by the pathogen within the vascular system. Chaudhry *et al*. (2006) similarly observed high Fusarium wilt intensity at flowering and podding stages of chickpea development.

The high disease incidence and severity on the selected landraces is an indication of the virulence of the pathogen on the crop. Of all the tested landraces none proved to be tolerant to the disease implying that there is no resistant type. Cook (1978) also reported Fusarium wilt in Bambara nut growing areas in Kenya. There was a variation in the manner in which the landraces performed in relation to incidence and severity. Maroon speckled landrace recorded the highest disease incidence but it was not the worst hit in terms of severity. Instead the maroon landrace had the highest disease severity while the brown landrace had the least severity. This shows that genetically the brown landrace is more tolerant to the disease than the other landrace. Aslam *et al.* (2013) similarly noted influence of genetic makeup of different chickpea cultivars on the Fusarium wilt severity.

5. Conclusion

Isolation of Fusarium wilt from most of the samples collected from farmers' fields in Busia indicates presence of the disease in the fields. The high incidence and severity on the selected local landraces calls for efforts of plant breeders to work towards developing Bambara nut varieties that are resistant to the Fusarium wilt so as to boost the crop's yield performance.

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References

Abdou, El-S., Abd-Alla, H. M. and Galal, A. A. (2001). Survey of sesame root rot / wilt disease in minia and their possible control by ascorbic and salicylic acids. *Journal of Agricultural Science*. 32, 135-152.

Ajiloqba, C. F. and Babalola, O. O. (2013). Integrated management strategies for tomato *Fusarium* wilt. *Biocontrol Science*. 18, 117-127.

Aslam, M., Maqbool, M. A., Akhtar, S. and Faisal, W. (2013). Estimation of genetic variability and association among different physiological traits related to biotic stress (*Fusarium oxysporum* L.) in chickpea. *The Journal of Animal and Plant Sciences*. 23, 1679-1685.

Bamshaiye, O. M., Adegbola, J. A. and Bamishaiye, E. I. (2011). Bambara groundnut: An underutilized nut in Africa. *Advances in Agricultural Biotechnology*. 1, 60-72.

Begemann, F. (1986). Bambara groundnut (*Vigna subterranea*, (L.) Verdc.): Pests and diseases. International Institute of Tropical Agriculture (IITA), Genetic Resources Unit, Ibadan, Nigeria. Pp 18.

Chaudhry, M. A., Muhammad, F. and Afzal, M. (2006). Screening of chickpea germplasm gainst Fusarium wilt. *Journal of Agricultural Resources*. 44, 307-331.

Cook, A. A. (1978). Bambara groundnut (*Voandzeia subterrannea*). *Diseases of Tropical and Subtropical Vegetables and Other Plants,* pp 15.

Cooke, B. M. (2006). Disease assessment and yield loss. The Epidemiology of Plant Diseases (2nd Ed., pp. 43-80).

Directorate of Plant Production. (2011). Production guideline for Bambara groundnut. Directorate Agricultural Information Services, DAFF, RSA.

Ebbels, D. L. and Billington, R. V. (1972). Fusarium wilt of *Voandzeia subterranean* in Tanzania. *Transactions of the British Mycological Society.* 58, 336-338.

Jaetzold, R., Schmidt, H., Hornet, Z. B. and Shisanya, C. A. (2006). Farm management handbook of Kenya. Natural conditions and farm information (Western Province).

Kemal, S. A., Abang, M., Imtiaz, M. and Nader, A. (2015). Effect of integrated management on Fusarium wilt progression and grain yield of chickpea in Syria. *Archives of Agronomy and Soil Science*. 61, 1551-1560.

Kleczewski, N. M. and Egel, D. S. (2011). A diagnostic guide for fusarium wilt of watermelon. In Plant Health Progress, doi:10.1094 / PHP-2011-1129-01-DG

Kristin, A. S. and James, D. K. (2000). Greenhouse screening protocol for fusarium root rot in bean (*Phaseolus vulgaris*). *Journal of Horticultural Science*. 35, 1095-1098.

Landa, B. B., Juan, A., Navas-Cortes, J. A. and Jimenez-Diaz, R. M. (2004). Integrated management of fusarium wilt of chickpea with sowing date, host resistance, and biological control. *Phytopathology*. 94, 946-960.

Leslie, J. F. and Summerell, B. A. (2006). The Fusarium Laboratory Manual. Volume 2, Issue: 10 (pp. 101-117), Blackwell Publishing Ltd, Oxford- UK.

Leslie, J. F., Summerell, B. A. and Bullock, S. (2006). The Fusarium Laboratory Manual (p. 388). New York: Wiley-Blackwell. http: / / dx.doi.org / 10.1002 / 9780470278376

Liu, L., Kloepper, J. W. and Tuzun, S. (1995). Introduction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology*. 85, 695-698.

Maina, P. K., Wachira, P. M., Okoth, S. A., Kimenju, J. W., Otipa, M. and Kiarie, J. W. (2015). Effects of land-use intensification on distribution and diversity of *Fusarium* species in Machakos county, Kenya. *Journal of Agricultural Science*. 7, 48-60.

Masindeni, D. R. (2006). Evaluation of Bambara groundnut (*Vigna subterranea*) for yield stability and yield related characteristics. Master of Science Thesis, University of Free State, South Africa.

Mkandawire, C. (2007). Review of Bambara groundnut (*Vigna subterranean* (L.) Verdc. Production in sub-sahara Africa. *Agricultural Journal*. 2, 465-470.

Pandy, H. N., Menon, T. C. M. and Rao, M. V. (1989). Simple formula for calculating area under disease progress curve. *Rachis*. 8, 38-39.

Ros, M., Hernández, M. T., García, C., Bernal, A. and Pascual, J. A. (2005). Biopesticide effect of green compost against Fusarium wilt on melon plants. *Journal of Applied Microbiology*. 98, 845-854.

Sharma, P. (2011). Alarming occurrence of Fusarium wilt disease in pea (*Pisum sativum* L.) cultivations of Jabalpur district in Central India revealed by an array of pathogenicity tests. *Agriculture and Biology Journal of North America*. 2, 981-994.

Wakelin, S. A., Warren, R. A., Kongand, L. and Harvey, P. R. (2008). Management factors affecting size and structure of soil *Fusarium* communities under irrigated maize in Australia. *Applied Soil Ecology*. 39, 201-209.

Wasula, S. L. (2014). Determinants of factors influencing smallholders' adoption of Bambara (*Vigna subteerranea)* as food security crop in Kakamega County, Kenya. Unpublished PhD Thesis Masinde Muliro University of Science and Technology, Kakamega, Kenya.

Wasula, S. L., Wakhungu, J. and Palapala, V. (2012). Factors influencing adoption of Bambara nut as a food Security Crop by Smallholder Farmers' in Low Rainfall Areas of Kenya. In the Proceedings of the International Conference on Disaster Risk Reduction and Conflict Resolution for Sustainable Development Held at Masinde Muliro University of Science & Technology, Kakamega, Kenya 18th-20th July 2012.

Zemouli-Benfreha, F., Djamel-eddine, H. and Merzoug, A. (2014). *Fusarium* wilt of chickpea (*Cicer arietinum* L.) in North-West Algeria. *African Journal of Agricultural Research*. 9, 168-175.

Zhao, B., Yan, J., Zhang, S., Liu, X. and Ghao, Z. (2014). Phylogeny and pathogenicity of *Fusarium* spp. isolated from greenhouse melon soil in Liaoning Province. *Saudi Journal of Biological Sciences*. 21, 374-379.

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