East African Journal of Pure and Applied Science

East African Journal of Pure and Applied Science Vol. 2 (2009) 41-49

Validation of inoculation techniques of *Fusarium* wilt on pigeonpea (*Cajanus cajan* (L.) Millsp.) in the glasshouse

[1]E. K., Kiprop, A. W., Mwangombe[2], J-P., Baudoin[3] P.M. Kimani[2], and G.Mergeai[3],

[1]Department of Biological Sciences, Moi University, P.O. Box 3900, Eldoret, Kenya.

[2]Department of Plant Science and Crop Protection, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

[3]Phytotechnie des Regions Intertropicales, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage de Déportés, 2, B-5030 Gembloux, Belgium

Abstract

The effectiveness of the root-dip inoculation technique widely used for screening pigeonpea germplasm against *F. udum* was compared to colonized whole rice grain which proved to be highly successful in screening common bean against *Macrophomina phaseolina* under the glasshouse conditions. The root-dip inoculation technique was found to be very effective and reliable in inoculating pigeonpea plants with *F. udum* during pathogenicity and virulence studies under glasshouse conditions. *Fusarium* wilt symptoms appeared from 8 to 10 days and 4 weeks on plants of wilt susceptible pigeonpea varieties KAT 60/8 and NPP 670 inoculated with the isolates MR01 and TT01 using root-dip and colonized whole rice techniques, respectively. The wilt incidence on pigeonpea plants was significantly different (P = 0.05) between the two inoculation techniques, with means of 48.1% and 14.4% wilt for root-dip technique and colonized whole rice grain technique, respectively. Root-dip inoculation technique was found to be fast and reliable compared to colonized whole rice grain technique although it was tedious to inoculate and transplant seedlings. Trimming roots of the pigeonpea seedlings to about 4 cm in length from the collar region and transplanting them after inoculation does not lead to any noticeable death of plants due to transplanting shock. The root-dip inoculation technique is recommended for use in screening pigeonpea germplasm for resistance to *Fusarium* wilt in glasshouse conditions.

†Corresponding author's E-mail address: ekiprop2001@yahoo.com,
 ISSN 2070 -0903/2009

Introduction

Kenya is the world's second largest producer of Pigeonpea (*Cajanus cajan* (L.) Millsp.) after India (Omanga and Matata, 1987; Nene and Sheila, 1990). It is one of the most widely grown and eaten grain legume in the semi- arid tropics of the world. Surveys carried out by Kannaiyan *et al*. (1984) showed *Fusarium* wilt (*Fusarium udum* Butler), cercospora leaf spot (*Mycevellosiella cajani* (Henn) Rangel ex. Trotter (Syn. *Cercospora cajani = Vellosiella cajani* Rangel) and powdery mildew (*Oidiopsis taurica* (Lev.) Salmon) to be the most common diseases of pigeonpea in Eastern Africa. In Eastern Africa, wilt incidence has been found to be quite high in Kenya (15.9%), Malawi (36.3%) and Tanzania (20.4%) (Kannaiyan *et al*., 1984). In some fields, wilt incidence could be as high as 90%. Losses due to wilt vary from a negligible amount to 100% depending on the stage at which the crop is attacked (Kannaiyan and Nene, 1981). *Fusarium* wilt has been identified as one of the main causes of low yields in farmer's fields in Kenya (Onim, 1981). Surveys in Kenya showed that *Fusarium* wilt incidence and mean ranged from 5% to 60% and 12% to 14.3%, respectively (Onim, 1981; Songa *et al*., 1991; Kiprop, 2001).

Breeding *Fusarium* wilt resistant pigeonpea varieties has been conducted since the turn of the last century. In most cases it was mainly through routine methods of selection in wilt-infested fields and 'wilt-sick' soils in glasshouses (Butler, 1908; Mukherjee *et al*., 1971; Nene and Kannaiyan, 1982; Okiror, 1986; Reddy, 1991). In all cases resistant and/or tolerant cultivars/lines were reportedly identified. It is important to note that screening plant germplasm against plant pathogens should be an ongoing process as the pathogens are biological entities that tend to vary or alter their mode of action/attack. Thus, germplasm known to be resistant to the disease in one locality could succumb to the same pathogen in another locality. Some pigeonpea varieties resistant to wilt at one location have been found to be susceptible at other locations (Nene *et al*, 1979; Songa *et al*., 1995).

Fast and accurate glasshouse inoculation techniques are necessary for screening large populations of germplasm emanating from breeding programmes. Various inoculation techniques for vascular plant diseases have been developed. In this study, the effectiveness of the root-dip inoculation technique widely used for screening pigeonpea germplasm against *F. udum* (Nene *et al*., 1981; Reddy and Raju, 1993) was compared to colonized whole rice grain which proved to be highly successful in screening common bean against *Macrophomina phaseolina* (Songa, 1995) under the glasshouse conditions.

Materials and Methods

Experimental site, pigeonpea varieties and *F. udum* isolates.

The experiment was conducted in a glasshouse whose prevailing temperatures were 22 to 30°C with about 12 hours photoperiod (natural light) at the Field Station, Kabete Campus, University of Nairobi at 1820 m.a.s.l. Two wilt susceptible pigeonpea varieties namely KAT 60/8 and NPP 670, and two wilt resistant varieties namely ICP 8863 and ICP 9174 were used in this experiment. Pigeonpea varieties ICP 8863, ICP 9174 and KAT 60/8 were obtained from International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Centre (Hyderabad, India) and from ICRISAT Experimental Station (Kiboko, Kenya) while variety NPP 670 was obtained from Department of Crop Science,

University of Nairobi. Two *F. udum* isolates used in the study were MR01 and TT01. These isolates were selected at random to represent the highly pathogenic isolates.

Root-dip inoculation technique.
The inoculum was prepared from each isolate by flooding 7 day-old potato dextrose agar (PDA) cultures with 100 ml of sterile distilled water. The conidial suspension was filtered through cheesecloth, and the inoculum concentration determined using a haemocytometer and then adjusted to 1.0 x 10^6 conidia/ml. The pigeonpea seeds were surface sterilized for one minute in 1% sodium hypochlorite and washed in two series of sterile distilled water before planting in 12 cm diameter polythene bags filled with sterilized riverbed sand. The bags were kept in the glasshouse and watered at regular intervals with sterile distilled water.

Seven-day-old seedlings were uprooted carefully from the sand medium and the roots washed in running sterile distilled water. The roots were trimmed to about 4 cm from the collar region, and then dipped into 1.0 x 10^6 conidial suspension for 30 minutes before transplanting them into 20 cm diameter polythene bags containing sterilized mixture of red soil (Vertisol) and riverbed sand (3:1 v/v) (Dhingra and Sinclair, 1985; ICRISAT, 1990). For each *F. udum* isolate five seedlings were inoculated and this was replicated four times. Control seedlings were dipped in sterile distilled water. The pots were placed in the glasshouse and watered at regular intervals with sterile distilled water.

Colonized whole rice grain inoculation technique. Whole rice grains were autoclaved at 121^{o}C for 20 minutes in water in a ratio of 1:1 (w/v) in a 250 ml beaker and cooled to room temperature. Agar blocks of 1 cm^3 were cut from 7 day-old single-spore PDA cultures of *F. udum* isolates MR01 and TT01 and transferred into beakers containing the autoclaved rice seeds and incubated at 28^{o}C in a 12-hour light/dark cycle for 15 days. Three rice seeds colonized by *F. udum* were placed with one seed of pigeonpea during planting before covering with soil/sand mixture. Eight pigeonpea seeds were planted per 20 cm polythene bag containing sterilized mixture of red soil and riverbed sand (3:1 v/v) and replicated four times. Seeds of control plants were planted with un-colonized sterilized whole rice grains. The polythene bags were placed in the glasshouse and watered at regular intervals with sterile distilled water. Seven-day-old seedlings were thinned to 5 per polythene bag. The experiment was designed according to a three-factor completely randomized design in which the level of inoculation techniques was split in replicates, pigeonpea variety and *F. udum* isolates. Observations on the symptom development were carried out daily until the initial symptoms were noted. The records on per cent wilt incidence on seedlings due to *F. udum* were taken thereafter on a weekly basis from the third week after inoculation and the final records taken eight weeks later. The experiment was repeated once.

Data analysis. The data was analysed by the three-factor ANOVA procedure and means separation by LSD using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA).

Results

Incubation time to initial wilt symptoms. The initial symptoms of *Fusarium* wilt on pigeonpea plants were epinasty, interveinal yellowing of lower leaves and drooping of leaves. *Fusarium* wilt symptoms appeared from 8 to 10 days and 4 weeks on plants of wilt susceptible pigeonpea varieties KAT

60/8 and NPP 670 inoculated with the colonized whole rice techniques, respectively. Wilt resistant pigeonpea variety ICP 9174 developed symptoms after 2 weeks of incubation when inoculated with both isolates using root-dip technique while isolate MR01 induced symptoms after 5 weeks using colonized whole rice technique. No wilt symptoms developed on variety ICP 9174 eight weeks after inoculation with isolate TT01 using colonized whole rice technique. Wilt resistant pigeonpea variety ICP 8863 succumbed to infection and isolates MR01 and TT01 using root-dip and developed symptoms 2 weeks after incubation with isolate TT01 using root-dip technique with no symptoms when inoculated using colonized whole rice technique. Variety ICP 8863 did not develop wilt symptoms when inoculated with isolate MR01 using root-dip or colonized whole rice technique. Re-isolation of *F. udum* from plants with wilt symptoms was possible at 2 weeks and 5 weeks after root-dip and colonized whole rice inoculation, respectively.

***Fusarium* wilt incidence.**

Fusarium wilt incidence on pigeonpea plants was significantly different (P = 0.05) between the inoculation techniques, and among pigeonpea varieties and incubation time, but not significantly different among *F. udum* isolates (Tables 1, 2 and 3).

Table 2. Overall *Fusarium* wilt incidence on pigeonpea plants for the inoculation techniques, varieties, isolates and incubation time

Variable	Wilt incidence (%)	LSD (P = 0.05)
Inoculation technique:		
Root-dip	44.9	2.0
Colonized whole rice	7.7	
Pigeonpea variety:		
KAT 60/8	53.6	2.9
NPP 670	46.5	
ICP 9174	2.9	
ICP 8863	2.1	
F. udum isolate:		
TT01	26.8	2.0
MR01	25.8	
Incubation time:		
Week 3	20.0	3.5
Week 4	21.9	
Week 5	26.6	
Week 6	27.8	
Week 7	30.3	
Week 8	31.2	
Mean	26.3	
CV (%)	38.5	
SE	102.4	

Table 1. Wilt incidence (%) over time (weeks) of four pigeonpea varieties inoculated with two *F. udum* isolates using two inoculation techniques in a glasshouse experiment.

Incubation time (weeks)	Pigeonpea variety	*F. udum* isolate	Inoculation technique		Mean
			Root-dip	Colonized rice	
3	KAT 60/8	MR01	85	0	42.5
		TT01	80	0	40.0
	NPP 670	MR01	70	0	35.0
		TT01	75	0	37.5
	ICP 8863	MR01	0	0	0.0
		TT01	5	0	2.5
	ICP 9174	MR01	0	0	0.0
		TT01	5	0	2.5
		Mean	40.0	0.0	20.0
4	KAT 60/8	MR01	80	5	42.5
		TT01	80	5	42.5
	NPP 670	MR01	65	5	35.0
		TT01	80	10	45.0
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	0	2.5
		TT01	5	0	2.5
		Mean	40.6	3.1	21.9
5	KAT 60/8	MR01	100	15	57.5
		TT01	90	15	52.5
	NPP 670	MR01	75	20	47.5
		TT01	85	10	47.5
	ICP 8863	MR01	0	0	0.0
		TT01	5	0	2.5
	ICP 9174	MR01	5	0	2.5
		TT01	5	0	2.5
		Mean	45.6	7.5	26.6
6	KAT 60/8	MR01	100	20	60.0
		TT01	90	15	52.5
	NPP 670	MR01	75	15	45.0
		TT01	90	15	52.5
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	5	5.0
		TT01	5	0	2.5
		Mean	46.9	8.8	27.9
7	KAT 60/8	MR01	100	30	65.0
		TT01	95	25	60.0
	NPP 670	MR01	80	25	52.5
		TT01	90	15	52.5
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	5	5.0
		TT01	5	0	2.5
		Mean	48.1	12.5	30.3
8	KAT 60/8	MR01	100	30	65.0
		TT01	95	35	65.0
	NPP 670	MR01	80	25	52.5
		TT01	90	20	55.0
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	5	5.0
		TT01	5	0	2.5
		Mean	48.1	14.4	31.3
		CV (%)	95.2	158.7	
				149.6	
		SE	1828.2		
		LSD (p=0.05)	21.1	6.0	

Table 3. ANOVA for wilt incidence on pigeonpea varieties inoculated with *F. udum* using two techniques

Source	SS	DF	MS	F
Technique	132759.38	1	132759.38	1296.09**
Variety	220136.46	3	73378.82	716.38**
Isolate	84.38	1	84.38	0.82^{ns}
Time	6542.71	5	1308.54	12.77**
Technique x variety	108261.46	3	36087.15	352.31**
Technique x isolate	651.04	1	651.04	6.36**
Technique x time	384.38	5	76.88	0.75^{ns}
Variety x isolate	953.13	3	317.71	3.10*
Variety x time	5344.79	15	356.32	3.48**
Isolate x time	209.38	5	41.88	0.41^{ns}
Technique x variety x isolate	1186.46	3	395.49	3.86**
Technique x variety x time	769.79	15	51.32	0.50^{ns}
Variety x isolate x time	428.13	15	28.54	0.28^{ns}
Technique x variety x isolate x time	337.50	20	16.88	0.16^{ns}
Error	29500.00	288	102.43	
Total	507548.96	383	1325.19	

*Significant at 5% level. **Significant at 1% level. ^{ns}Not significant at 5% level.

Significant differences (P = 0.05) in wilt incidence occurred between the interactions of inoculation technique-variety, inoculation technique-isolate, variety-isolate, variety-incubation time and inoculation technique-variety-isolate, but not significant differences (P = 0.05) were observed between the inoculation technique- incubation time, isolate-incubation time, inoculation technique-variety-incubation time, variety-isolate-incubation time and inoculation technique-variety-isolate-incubation time. The wilt incidences varied from 0 to 100% with a mean of 26.3%. High wilt incidence of 44.9% was recorded when plants were inoculated using root-dip technique when compared to colonized whole rice technique which caused 7.7% wilt. Wilt incidences observed on pigeonpea varieties KAT 60/8, NPP 670, ICP 9174 and ICP 8863 were 53.6%, 46.5%, 2.9% and 2.1%, respectively. *Fusarium* wilt incidences between resistant varieties ICP 8863 and ICP 9174 were not significantly different (P = 0.05) while incidences between susceptible varieties KAT 60/8 and NPP 670 were significantly different. Wilt incidences due to *F. udum* isolates TT01 and MR01 were 26.8% and 25.8%, respectively and not significantly different (P = 0.05). Wilt incidences observed at 3, 4, 5, 6, 7 and 8 weeks after inoculation were 2.0%, 21.9%, 26.6%, 27.8%, 30.3% and 31.2%, respectively. Wilt incidences, however, on 3 and 4, 5 and 6, and 6, 7 and 8 weeks after inoculation were not significantly different (P = 0.05). The wilt incidence on the susceptible variety KAT 60/8 inoculated with isolates MR01 and TT01 using root-dip technique reached a maximum of 100% and 95%, four and seven weeks after inoculation, respectively. The plants of variety KAT 60/8 that were inoculated using colonized whole rice technique reached a maximum of 30% wilt after 7 weeks of incubation when inoculated with isolate MR01 while it was 35% wilt when inoculated with isolate TT01 after 8 weeks. The wilt incidence on the susceptible variety NPP 670 inoculated with isolates TT01 and

MR01 using root-dip technique reached a maximum of 90% wilt after 6 weeks and 80% wilt after 7 weeks, respectively. Variety NPP 670 inoculated with MR01 and TT01 isolates using colonized whole rice technique showed an incidence of 25% wilt after 7 weeks and 20% wilt after 8 weeks of incubation, respectively. The wilt incidence on the resistant variety ICP 8863 inoculated with isolate TT01 using root-dip technique reached a maximum of 10% wilt after 6 weeks with isolate MR01 inducing no wilt. Isolates MR01 and TT01 did not induce wilt on variety ICP 8863 when inoculated using colonized whole rice technique. The wilt incidence on the resistant variety ICP 9174 inoculated with isolates TT01 and MR01 using root-dip technique reached a maximum of 5% wilt after 3 weeks and 5% wilt after 4 weeks, respectively. The plants of variety ICP 9174 that were inoculated with isolate MR01 using colonized whole rice technique reached a maximum of 5% wilt after 6 weeks of incubation while plants that were inoculated with isolate TT01 did not develop wilt.

Discussion

The root-dip inoculation technique was found to be very effective and reliable for virulence studies of *F. udum* and screening pigeonpea germplasm under the glasshouse for *Fusarium* wilt resistance when compared to the colonized whole rice grain technique. Such reliable results could be obtained 3 weeks after root-dip inoculation using *Fusarium* wilt incidence as the parameter of assessment. The pathogen could be re- isolated from plants that showed wilt symptoms onto PDA medium 14 days after root-dip inoculation. Pigeonpea varieties were separated clearly into their respective susceptibility classes while *F. udum* isolates did not show any significant variability in virulence. The root-dip inoculation technique has been found to be most dependable and effective than other techniques developed when inoculating pigeonpea genotypes with *F. udum* under the glasshouse. Out of five inoculation techniques studied, Okiror (1986) found root-dip method to be more effective and reliable in inducing *Fusarium* wilt of pigeonpea than either direct sowing on sick soil, transplanting on sick soil, soaking seed in the inoculum or stem injection with inoculum. Workers at ICRISAT are currently using root-dip inoculation technique for screening germplasm for *Fusarium* wilt resistance in glasshouse experiments (ICRISAT, 1990). The findings of the present study correlated with the findings of Reddy and Raju (1993), and Changaya-Banda *et al.* (1996) who also used root-dip method with an inoculum concentration of 1×10^6 conidia/ml to study the reaction of pigeonpea genotypes to *F. udum* isolates. Wiles (1963) and Miller and Cooper (1967) found this technique to be very effective for the inoculation of cotton genotypes with *F. oxysporum* f.sp. *vasinfectum*. The disadvantages of this technique, however, are that it is tedious and time consuming, some seedlings could die after transplanting due to transplanting shock, and at very high inoculum concentrations some resistant pigeonpea genotypes show high wilt incidence. It is necessary therefore to establish the optimum inoculum concentration before starting an experiment using root-dip method, and to check for the dark/brown strip inside the stem of wilted plants and/ or plate stem pieces on PDA medium in order to confirm that the plants actually died due to *F. udum*.

Planting pigeonpea seed with colonized (with *F. udum*) whole rice grains induced low disease incidence even with the most wilt susceptible pigeonpea variety (KAT

60/8). Variety KAT 60/8 showed wilt incidence of 35% and 30% eight weeks after inoculation using colonized whole rice grain with *F. udum* isolates TT01 and MR01, respectively. The pathogen was re-isolated from wilted plants onto PDA medium six weeks after planting. This technique has been found to be very effective for screening bean germplasm for resistance against *Macrophomina phaseolina*, the causal agent of charcoal rot of common bean (Songa, 1995).

Root-dip inoculation technique was therefore a more adequate method for inoculating *F. udum* pathogen into pigeonpea plants under the glasshouse in the present study when compared to colonized whole rice grain technique. It was found to be fast and reliable although it was tedious to inoculate and transplant seedlings. Trimming roots of the pigeonpea seedlings to about 4 cm in length from the collar region and transplanting them after inoculation did not lead to any noticeable death of plants due to transplanting shock.

Acknowledgment
Financial support for this study was kindly provided by the European Union through an INCO-DC project contract number ERBIC18CT960130, which we gratefully acknowledge. We are grateful to the International Crops Research Institute for Semi-Arid Tropics (Nairobi) for the kind donation of pigeonpea varieties.

References

Butler, E. J. (1908) Selection of pigeonpea for wilt disease. *Agric. J.*, India. 3:182-183.

Changaya-Banda, A. G. A. Saka, V. W. and Msuku, W. A. B. (1996) Occurrence of pathogenic pathotypes of *Fusarium udum* (Butler): the incitant of wilt of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in Malawi. First All African Crop Science Congress, 13th-17th January 1997, Pretoria, South Africa.

Dhingra, O. D. and Sinclair, J. B. (1985) Basic Plant Pathology Methods, CRC Press, Inc. 355 pp.

ICRISAT. 1990. Annual Report 1989. ICRISAT, Patancheru, India, pp. 111-112, xxi.

Kannaiyan, J. and Nene, Y. L. (1981) Influence of wilt at different growth stages on yield loss in pigeonpea. *Trop. Pest Manage.* 27:141.

Kannaiyan, J. Nene, Y. L.; Reddy, M. V. Ryan, J. G. and Raju, T. N. (1984) Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and Americans. *Trop. Pest Manage.* 30:62-71.

Kiprop, E.K. (2001) Characterization of *Fusarium udum* Butler isolates and pigeonpea (*Cajanus cajan* (L.) Millsp.) resistance in Kenya. Ph D Thesis, University of Nairobi, 235pp.

Miller, D.A. and Copper, W.D. (1967) Glasshouse techniques for studying *Fusarium* wilt in cotton. *Crop Sci*. 7:75-76.

Mukherjee, D. De, T. K. and Parui, N. R. (1971) A note on the screening of arhor against wilt disease. *Indian Phytopathol*. 24:598-601.

Nene, Y. L. and Kannaiyan, J. (1982) Screening of pigeonpea for resistance to *Fusarium* wilt. *Plant Dis.* 66:306-307.

Nene, Y.L. and Sheila, V.K. (1990) Pigeonpea: Geography and Importance. *In*: The Pigeonpea (eds. Y.L. Nene, S.D. Hall and V.K. Sheila), C.A.B International and International Crops Research Institute for the Semi-Arid Tropics, University Press, Cambridge, UK, pp 1-14.

Nene, Y. L. Kannaiyan, J. and Reddy, M. V. (1981) Pigeonpea diseases: resistance screening techniques. *Inf. Bull*. No. 9, ICRISAT, India.

Nene, Y. L. Kannaiyan. J. Haware, M. P. and Reddy, M. V. (1979) Review of Work Done at ICRISAT on Soil-borne Diseases of Pigeonpea and Chickpea. *In*: Proceedings of the Consultants Group Discussion on the Resistance to soil-borne Diseases of Legumes. ICRISAT, Patacheru, A. P. India, 3.

Okiror, M. A. (1986) Breeding for resistance to *Fusarium* wilt of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Kenya. Ph D Thesis, University of Nairobi, 202 pp.

Omanga, P.A. and Matata, J.B.W. (1987) Grain legume production in Kenya. *In*: Research on Grain Legumes in Eastern and Central Africa. Summary Proceedings of the Consultative Group Meeting for Eastern and Central African Regional Research on Grain Legumes (Groundnut, Chickpea, and Pigeonpea), 8-10 December 1986, International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia. Patancheru, A.P.; India: ICRISAT, pp. 51-56.

Onim, J. F. M. (1981) Pigeonpea Improvement Research in Kenya. *In*: Proceedings of the International Workshop on Pigeonpeas, 15-19 Dec. 1980, ICRISAT, India. Vol. I.

Reddy, M. V. (1991) Disease Problems of Pigeonpea in Eastern Africa-Progress and Future Research Needs. *In*: Proceedings of the First Eastern & Southern Africa Regional Legumes (Pigeonpea) Workshop, 25-27 June 1990, Kenya. EARCAL, ICRISAT. pp 60-64.

Reddy, M.V. and Raju, T.N. (1993) Pathogenic variability in pigeonpea wilt-pathogen *Fusarium udum*. *In*: K. Muralidharan and C.S. Reddy (eds), Plant Disease Problems in Central India, Proceedings of the Symposium of Central Zone, Indian Phytopathological Society, Directorate of Rice Research, Hyderabad, pp 32-34.

Songa, W.A. (1995) Variation and survival of *Macrophomina phaseolina* in relation to Screeining common bean (*Phaseolus vulgaris* L.) for resistance. PhD Thesis, University of Reading, UK, pp 276.

Songa, W. A. King, S. B. and Omanga, P. A. (1995) Pigeonpea Pathology Research in Kenya. *In*: Improvement of Pigeonpea in Eastern and Southern Africa, Annual Research Planning Meeting, 21-23 Sep.; 1994, Nairobi, Kenya. (S. N. Silim, S. B. King and S. Tuwaje, eds). ICRISAT, Patanchru 502 324, Adhra Pradesh, India. pp. 30-37.

Songa, W.A.; Omanga, P. and Reddy, M.V.(1991) Survey of pigeonpea wilt and other diseases in Machakos and Kitui districts of Kenya. *Int. Pigeonpea News*. 14:25-26.

Wiles, A.B. (1963) Comparative reaction of certain cottons to *Fusarium* and *Verticillium* wilts. *Phytopathol*. 53:586-588.