A Research Article in AJRTC (2010) Vol. 8 No. 1 : Pages 1–5

Cassava yield response to sources and rates of potassium in the forest–savanna transition zone of Ghana

S. Agyenim Boateng and S. Boadi

Soil Research Institute, Academy Post Office, Kwadaso-Kumasi, Ghana

Corresponding author: S. Agyenim Boateng
Email: sagyenimboateng@yahoo.com

Manuscript revised received: 27/09/2007; accepted: 30/06/2008

Abstract

The response of cassava yield to two sources of potassium (K) fertilizer–muriate of potash (KCl) and sulfate of potash (K_2SO_4) was studied on a savanna Ochrosol in the forest-savanna transition zone of Ghana. The crop received basal applications of 60 kg of nitrogen (N)/ha and 60 kg of phosphorus (P)/ha in a randomized complete block design with four replications. Levels of K were 0, 30, 60, 90 kg K/ha for the two sources. Results indicated that K fertilizer was necessary for achieving higher yields in cassava. The higher response of cassava to K was irrespective of the source. Although sulfate of potash generally produced higher figures than muriate, there were no significant differences between the two K sources. Mean responses to KCl treatments relative to control were 124% in stover and 84% in tuber weights. Responses to K_2SO_4 treatments were 128% in stover and 79% in tuber weights. Significant differences existed between the K treatments and the controls (including the NP treatment). Cassava did not show a significant response to NP treatments, likely to be due to the absence of K, confirming the importance of K in cassava nutrition. A minimum of 30 kg K/ha in the presence of N and P (i.e., 60–60–30 kg NPK/ha) was found to be enough to produce a significant response of cassava at the site with very low inherent soil fertility.

Key words: cassava, muriate of potash, sulfate of potash, forest-savanna transition, yield, Ghana.

Introduction

Cassava (*Manihot esculenta*) is an important starchy crop in Ghana. It is cultivated in all the agroecological zones and in all the ten regions of the country. It is the most widely cultivated in Ghana (Doku 1993), and consumed in various forms by a large percentage of the populace. Doku (1969) has estimated that over 90 cultivars are grown in Ghana.

Cassava production has increased considerably in recent years, partly as a direct result of the importance of the crop as an industrial crop in natural starch manufacture with special reference to the Presidential Special Initiative (PSI) on cassava and also as an export commodity. It is also partly due to its significance as a staple food crop that is eaten in various forms such as *fufu*, *ampesi, abetee, gari* and tapioca. It is used in varied combinations in the preparation of many other foods in Ghana and elsewhere.

The crop can grow on soils that are too impoverished to support other staple crops. This is because cassava has an extensive root system and is able to utilize plant nutrients less accessible to other crops. It can produce a modest fresh tuber yield of 5–6 t/ha on low fertility soils that would not support other crops (IITA 1990). However, to produce high yields, the crop does require large supplies of nutrients and this requirement can be met through the use of fertilizers. The crop has been reported to respond to good soil fertility and adequate fertilizer (Gomez et al 1980; Howeler 1996).

Fertilizer use is generally very low in sub-Saharan Africa and, in particular, on root and tuber crops such as cassava. Farmers do not fertilize cassava, partly because they think that cassava does not require it and partly because they are contented with the minimal yields obtained from using limited inputs or even from their infertile soils (Agbaje and Akinlosotu 2004). Cassava is usually the last crop in a rotational system.

Schultess et al 1997 noted that, 4–7 months after the application of soil treatments, differences in N,

P, and K in the first 50 cm of the soil had mostly disappeared, indicating that the soil nutrients had been taken up by the cassava plant. Fertilizing with NPK maintained high annual cassava crop yields per ha of 6–10 t cassava in sole cropping; without NPK, final crop yields seemed to stabilize at about 4 t/ha (Akonde et al 1996). An experimental second year of cassava cropping without any fertilizer caused a decline in the yield of roots and tubers to 60% less than the comparable value for the first year of cassava (Mailly et al 1997).

Other soil amendments also made improvements in cassava yields. Escalada and Ratilla (1998) reported that an application of 7.23 t/ha *Leucaena* biomass promoted vigorous growth in cassava resulting in a high yield and better values of yield components. Alley cropping with *Cajanus cajan* in a fertilized sole-cropped cassava induced significant crop yield increases of about 50% (Akonde et al 1996).

As a root crop producing a large amount of starch, cassava draws heavily on soil nutrients, and has an extraordinarily high reliance on K for high tuber yields. High yields export large amounts of nutrients. Mailly et al (1997) intimated that cassava cultivation reduced the content of exchangeable K in the mineral soil during the second year of cropping. Sanchez (1976) estimated that nutrient removal in 30 t/ha of fresh cassava (15–20% dry matter) can be as high as 120 kg N, 40 kg P, and 187 kg K/ha.

Takyi (1972) reported that on an undisturbed silt loam forest-savanna Ochrosol cropped intermittently for several years by local farmers without fertilization, an application of K had little effect on cassava yields, producing a mean response of only 1.4%. On a plowed (disturbed) soil, a sandy loam forest Ochrosol cropped almost continuously for over 15 years, the mean response to K was negative (-2.7%). Stephens (1960b) had earlier reported decreases in cassava yields with continuous cropping which he thought indicated a developing deficiency of K.

Two common sources of K fertilizers are currently being used by farmers in the country. These are muriate of potash and sulfate of potash. The objective of this study was to determine the response of cassava yield to the two K fertilizer sources and their respective rates at a site in the forest-savanna transition zone of Ghana.

Materials and Methods

The study was conducted at Wenchi outstation of the Soil Research Institute on a soil that appeared to have been in fallow for 2–3 years. The place is within the forest-savanna transition zone of Ghana. This zone, covering an area of 8 300 km^2, has a bimodal rainfall regime with annual precipitation between 1200 and 1400 mm. The soil at the experimental site was sampled and analyzed for pH, organic carbon, total nitrogen (N), available phosphorus (P), and exchangeable cations according to the methods described by Anderson and Ingram (1998) by bulked samples of treatments at the start of the experiment.

A randomized complete block design with four replications was employed with each plot measuring 50 m^2. The plots received a basal N application as urea at a level of 60 kg N/ha, and P application as triple superphosphate at a level of 60 kg P/ha. Fertilizers were applied by hill placement 4 weeks after planting. Two treatments served as an absolute control and a relative control: the absolute control did not receive any fertilizer while the relative control received only the basal N and P fertilizers. The K levels were 0, 30, 60, 90 kg K/ha for the two sources, muriate and sulfate of potash.

Cassava cuttings were planted slanted, at an angle on small mounds. The cultivar was a local *Ankra*. This was chosen because it is preferred by farmers and popularly consumed by virtue of its good characteristics, such as flavor, texture, taste, and cooking and pounding properties. It also has moderate resistance to cassava mosaic disease. The spacing was 1 m × 1 m giving 50 plants to a plot. The fields were maintained by regular manual weeding and inspection of any disease or pest attack. Mature crops were harvested manually after 12 months of growth. Yield data were taken on fresh tuber weight and stover weight. Stover was the above-ground parts, made up of cassava stems cut at the soil surface, leaves, and branches. Harvest Index (HI) was also determined by the relationship below and expressed as a percentage:

$$HI = Economic\ Yield \times (Biological\ Yield)^{-1}$$

where,
Economic yield refers to tuber yield and biological yield refers to total biomass yield.

Statistical analyses were done using a two-way analysis of variance (ANOVA) with MSTATC statistical package.

Results and Discussion

Soil Analysis. Initial analysis of the soil (Table 1) indicated that the soil was low in inherent fertility, having very low levels of N, organic matter, P and exchangeable cations. The pH was acidic. The soil was a sandy loam belonging to Damongo series, a savanna Ochrosol (Chromic Lixisol). The texture was ideal for cassava cultivation (Daisy 1987). It was a brownish–grey, well–drained soil. These features are characteristics of the soils found in the area. With these low fertility levels, it was expected that the cassava crop would respond to fertilizer application. According to Kang and Okeke (1991), soils with low N (< 0.10% total N) and K (< 0.15 meq/100g) will require additional fertilizer for optimum tuber yield. There was no evidence of the soil having been disturbed, as is common with mound making in the area for yam cultivation.

Stover Yield. Table 2 shows the stover yield response of the crop to K fertilization. The relative control (60–60–0 NPK kg/ha) did not produce a significant increase in stover yield over the absolute control, although it recorded an increase of 18%. This inability of the crop to show significant response to as much as 60 kg N/ha and 60 kg P/ha in the absence of K may be attributed to imbalanced nutrition. With the addition of 30 kg K/ha to N and P, a significant increase in yield (of 71% for the chloride and 66% for the sulfate) was obtained. Significant yield increases were observed following further addition of K to 60 kg/ha. However, additional increases to 90 kg K/ha did not result in a significant increase of stover yield. Schultess et al 1997 found that K made the highest contribution to cassava growth rate in Bénin.

Tuber Yield. Results obtained indicated that K fertilizer was necessary for achieving higher tuber yields in cassava (Table 2). This has also been noted by many investigators in various places at various times (e.g. Kang and Okeke 1984; Sanchez 1976; Takyi 1972 and Stephens 1960a). A similar trend to the stover yield was also observed with the tubers. Addition of 30 kg K/ha made a significant contribution to fresh tuber yields, increasing above 80% over absolute control and 40% over the N × P treatment (relative control) in the case of the muriate, and 63% over absolute control and 28% over the N × P treatment in the case of the sulfate. Akonde et al (1985) observed that cassava fresh tuber yields were increased 74% by K fertilizer application at a site in southern Bénin.

Significant differences ($p > 0.05$) existed between all the K treatments and the controls (including the relative control) in both stover and tuber weights. Cassava did not show any good response to the N × P treatments probably due to imbalanced nutrition although N × P gave 18% increase over the absolute control in stover weight and 30% in tuber weight. This was likely due to the absence of K, confirming the importance of K in cassava nutrition.

Thus, responses to only N and K were minimal and non-significant, which might have arisen from unbalanced fertilization. Starch formation and translocation of sugars to all parts of the plant require K. It is useful for root formation and therefore tuber crop production and also helps the uptake of nitrates from the soil (Akinsanmi 1999; Brady and Weil 1999). Therefore, its absence can badly affect the tuber yield of cassava. A minimum of 30 kg K/ha in the presence of N and P (i.e., 60–60–30 kg NPK/ha) was found to be enough to produce a significant response of cassava at this site with low inherent soil fertility level.

The partial factor productivity (PFP) of the crop due to the applied K fertilizer is presented (Table 4). The PFP is a relative measure of the actual output of production (in this case, tuber yield) compared to the actual input of resources (in this case, fertilizer), measured across time or against common entities. It is, thus, an indication of the tuber yield response of the crop as affected by the fertilizer applied. It shows that 30 kg K/ha produced the highest response from both K sources, confirming that 30 kg K/ha was adequate to produce a significant response in the tuber yield of cassava.

The higher response of cassava tubers to K was irrespective of the source. There were no significant differences between the two K sources. Mean responses to KCl (30, 60, 90 kg K/ha) relative to the absolute control were respectively 124% of stover weight and 84%, of tuber weight while responses to K_2SO_4 (30, 60, 90 kg K/ha) were 128% of stover weight and 78.5% of tuber weight (Table 3). This observation, that there were no differences between muriate and sulfate of potash, had been reported by some workers (SRI 1977; 1988).

Harvest Index. The HI (Table 2) were generally high, exceeding 41% in all cases, indicating a fairly effective redistribution of photosynthate and conversion of assimilates from leaves and stems into the tubers. However, the HI was reduced by fertilizing

with K probably due to excessive stover production. At 60–60–60 kg NPK/ha, HI was reduced to 41%. Leihner et al 1996 found that the HI in sole-cropped cassava was reduced by fertilizing from 40–55% to 30–40%, due to excessive leaf growth at 60–190 days after planting. It is also reported that on very rich soils the plant may produce stems and leaves at the expense of roots (FAO 2007). There was no difference in HI between the two K sources, although it was expected that the sulfate would be a better converter of assimilates since cassava, like most of the other tropical root crops, is sensitive to the chloride ion (Onwueme and Sinha 1991).

Table 1. Some chemical and physical properties of the experimental site (0–20 cm) at the start of the experiment.

Parameter	Value
pH	5.50
Organic matter (%)	0.07
Total N (%)	0.05
Available P_2O_5 (mg/kg)	1.20
Exchangeable cations (me/100g):	
Ca	1.88
Mg	0.47
Mn	0.19
K	0.15
N	0.18
CEC	2.87
Soil texture	sandy loam

Table 2. Cassava yields at final harvest.

Treatments	Stover weight (t/ha)	Tuber weight (t/ha)	HI (%)
0–0–0	8.4	9.3	52.5
60–60–0	9.9	12.1	55.0
KCl			
60–60–30	16.9	16.9	50.0
60–60–60	21.0	14.9	41.5
60–60–90	19.0	18.5	49.5
K_2SO_4			
60–60–30	15.8	15.2	49.0
60–60–60	20.3	14.7	41.4
60–60–90	21.3	19.9	48.3
Lsd (5%)	4.2	3.3	-

Conclusion

To achieve higher stover and tuber yields in cassava K fertilizer was necessary. Although sulfate of potash generally produced higher figures than muriate of potash, there were no significant differences between the two K sources. Mean response to muriate was 126% of stover yield and 84% of tuber yield; response to sulfate was 128% of stover yield and 79% of tuber yield. Significant differences existed between the K treatments and the two controls. Cassava did not show a significant response to N × P treatments, probably due to imbalanced nutrition arising from the absence of K. A minimum of 30 kg K/ha, 60 kg N/ha, and 60 kg P/ha (i.e., 60–60–30 kg NPK/ha) was enough to produce a significant response of cassava at the site which had very low inherent fertility.

Table 3. Percentage increases of various treatments over absolute control.

Treatments	Stover weight	Tuber weight
60–60–0	17.8	30.1
KCl		
60–60–30	101.2	81.7
60–60–60	150.2	72.0
60–60–90	126.2	98.9
Mean	125.8	84.2
K_2SO_4		
60–60–30	88.1	63.4
60–60–60	141.7	58.1
60–60–90	153.6	114.0
Mean	127.8	78.5

Table 4. Partial factor productivity (PFP) of cassava.

K rate (kg/ha)	Tuber weight (kg/ha)	PFP
0	9,300	-
0	12,100	-
KCl		
30	16,900	563
60	14,900	248
90	18,500	205
K_2SO_4		
30	15,200	506
60	14,700	245
90	19,900	221

References.

Agbaje G.O. and Akinlosotu T.A. 2004. Influence of NPK fertilizer on tuber yield of early and late-planted cassava in a forest alfisol of south-western Nigeria. African Journal of Biotechnology 3 (10): 547–551.

Akinsanmi O. 1999. Senior Secondary Certificate Agricultural Science. Longman Group Ltd. UK 245 pp.

Akonde T.P., Leihner D.E., and Steinmuller N. 1996. Alley cropping on an Ultisol in subhumid Bénin. Part 1: long-term effect on maize, cassava and tree productivity. Agroforestry Systems 34 (1):1–12.

Anderson J.M. and Ingram J.S.I. 1998. Tropical Soil Biology and Fertility – A Handbook of Methods, Second Edition. CABI Publishing, Wallingford, UK. 221 pp.

Brady N.C. and Weil R.R. 1999. The Nature and Properties of Soils. 12th Edition. Prentice Hall Inc., Upper Saddle River, NewJersy, USA, 881pp.

Daisy E.K. 1987. Root Crops – Tropical Development and Research Institute, London. Crop and Product Digest No. 2, Second Edition, 380 pp.

Doku E.V. 1993. The challenges of achieving sustainable production of starchy staples in Ghana. Proceedings of the Second National Workshop on Root and Tuber Crops and Plantain, 21–22 October 1993, Kumasi, Ghana.

Doku E.V. 1969. Cassava in Ghana. GU Press, Accra, Ghana.

Escalada R.G. and Ratilla B.C. 1998. Effects of *Leucaena* biomass application in conjunction with fertilizers on cassava and taro yields in the Philippines. Agroforestry Systems 41(3): 251–266.

FAO. 2007. Cassava processing. FAO Plant Production and Protection Series No. 3. www.fao.org/docrep/X5032E/x5032E01.htm. 19/11/2007.

Gomez J.C., Howeler R.H., and Webber E.J. 1980. Cassava production in low fertility soils. *In*: Cassava cultural practices, edited by M.J.C. Toro and M. Graham. Bowker Publishing. Co Ltd, Epping, UK.

Howeler R.H. 1996. Diagnosis of Nutritutional Disorders and Soil Fertility Maintainance of Cassava. *In*: Kurup G.T. et al eds. Tropical Tuber Crops:problems, prospects and future strategies, 181–93. Oxford and IBA Publishing Co. prt.LTD. New Delhi.

IITA. 1990. Cassava in Tropical Africa: A reference manual. International Institute of Tropical Agriculture, Ibadan. Nigeria. 176 pp.

Kang B.T. and Okeke J.E. 1984. Nitrogen and potassium responses of two cassava varieties grown on an alfisol in southern Nigeria. *In*: Proceedings, 6th symposium of the International Society of Tropical Root Crops, 1984, Lima, Peru. 231–234.

Leihner D.E., Ernst Schaeben R., Akonde T.P. and Steinmuller N. 1996. Alley cropping on an Ultisol in subhumid Benin. Part 2: Changes in crop physiology and tree crop competition. Agroforestry Systems 34 (1): 13–25.

Mailly D., Christanty L. and Kimmins J.P. 1997. "Without bamboo, the land dies": nutrient cycling and biogeochemistry of a Javanese bamboo talun-kebun system. Forest Ecology and Management 91 (2/3): 155–173.

Onwueme I.C. and Sinha T.D. 1991. Field Crop Production in Tropical Africa. CTA , Ede, The Netherlands, 480 pp.

Sanchez P.A. 1976. Properties and Management of Soils in the Tropics. John Wiley and Sons Inc., NewYork, USA. 618 pp.

Schultess F., Neuenschwander P. and Gounou S. 1997. Multi-trophic interactions in cassava, *Manihot esculenta*, cropping systems in the subhumid tropics of West Africa. Agriculture, Ecosystems and Environment 66 (3): 211–222.

SRI. 1977 (Soil Research Institute). Annual Report, Kwadaso- Kumasi, Ghana.

SRI. 1988. Annual Report of the Soil Research Institute, Kwadaso- Kumasi, Ghana.

Stephens D. 1960a. Fertilizer trials on peasant farms in Ghana. Empire Journal of Experimental Agriculture 28: 1–15.

Stephens D. 1960b. Three rotation experiments with grass fallows and fertilizers, Empire Journal of Experimental Agriculture 28: 165–178.

Takyi S.K. 1972. Effects of potassium, lime, and spacing on yields of cassava (*Manihot esculenta* Crantz). Ghana Journal of Agricultural Science 5: 39–42.

A Research Article in AJRTC (2010) Vol. 8 No. 1: Pages 6–12

Setting priorities: case study of IITA's root and tuber crops systems program

V. M. Manyong[1], R.U. Okechukwu[1], P. Ojiambo[2], and R. Asiedu[1]

[1]International Institute of Tropical Agriculture, PMB 5320 Oyo Road, Ibadan, Nigeria.
[2]Department of plant pathology, North Carolina State University, Raleigh, USA.

Corresponding Author: V.M. Manyong
Email: v.manyong@cgiar.org

Manuscript received: 30/04/2008; accepted: 30/06/2008

Abstract

This report presents results from a priority setting exercise conducted on the strategic objectives and activities of the Root and Tuber Systems Program as a part of a process to develop strategic plans to guide the activities of IITA for the following 10 years. The scoring method was applied to rank activities and statistical tests were used to validate the ranking from the scoring method. Thirty-five Project members participated in data generation.The results indicate the proportion of each strategic objective between 18 and 22%. Within each strategic objective, the score of each activity relative to the maximum achievable score resulted in the ranking of activities. Statistical tests showed some activities to be significantly not different in ranking from others. There is a clear importance attached to activities of strategic objective 1, compared to those of the others. Activity 4 of strategic objective 1 emerged as a top activity among all the activities. The disciplines of scientists did not affect the scores significantly or the perceived importance of activities. It is advocated that priority setting is an iterative process that needs to be implemented continuously in various steps to improve the efficiency of the project operations.

Key words: priority setting, Root and Tuber Systems Project, IITA

Introduction to priority setting

Priority setting is relevant for decision-making and resource allocation at the institute level, i.e., for investments across agroecological zones or across projects. Priority setting is important to achieve a better understanding of the factors leading to changes in the use of technology at the farm, regional, or ecoregional level. Priority setting can also take place at the Project level, i.e., for the choice of research activities within the Project. Priority setting is important because it results in greater efficiency of operations through a better choice of interventions among alternative options, and it leads to an optimal allocation of scarce resources to operations. During the process of priority setting there is a greater participation of stakeholders leading to increased ownership of the Project.

IITA has a long tradition of setting priorities for its research agenda. The Institute has been using a bottom to top approach to define its research priorities across programs and ecoregions. For example, the strategic plans and priorities developed by the three regional agricultural research organizations provided a solid basis for the new research agenda in the IITA Strategic Plan 2001–2010. The process of setting priorities at the Institute level takes place through several mechanisms. During the development of the rolling three-year Medium Term Plan (MTP) program, priorities are discussed. Yearly, scientists meet during the Strategic Planning Week organized to agree on the future directions of the Institute. Occasionally, members of an MTP Project also meet to define priorities within their Project.

There are many approaches that can be used in priority setting. Manyong et al 2001 identified 11 approaches. Each has its strengths and weaknesses and priority setting may involve a combination of two or more. One of these is the scoring method that was deemed appropriate for setting priorities within the MTP

for the Roots and Tuber Crops System Project. It can be conducted in a relatively short period of time and no advanced analytical skills are required. It is possible to include all disciplines in the priority setting exercise and both qualitative and quantitative information can be used. It is based on a multitude of criteria that reflect a set of research objectives. Relative weights are attached to the objectives and finally priority setting takes place. Some of the disadvantages are from the overlap on objectives, duplicate criteria, or subjectivity in assigning weights. A more complete description of the scoring method can be found in Alston et al 1995.

Process for setting priorities of the MTP R & T crops. To advance IITA's seven MTP Projects from reporting units into planning and operational ones , the Institute's Management asked all Projects to develop strategic plans that would guide their activities for the next 10 years. In line with this directive, the members of the Root and Tuber Systems Project met in November 2006 and developed a draft strategic plan. This draft was presented early in 2007 to IITA's Research-for-Development Directorate, IITA's Board of Trustees, and the panel for the Institute's sixth External Program and Management Review. A major comment from these reviews was the need to reduce and prioritize the number of proposed activities (tactical objectives) for the Project. A workshop was therefore organized in Maputo, 13–15 October 2007, to review the draft, with emphasis on prioritization. Several Project members and representatives of partner institutions attended this workshop. The participants at the workshop actually increased the total number of activities from 57 to 59 following the revision of proposed activities in the November 2006 meeting (Table 1). These 59 activities were subjected to the first round of priority setting using the methodology described below Twenty-six Project members present in Maputo contributed to this first round. Based on an extensive discussion of the results, some activities were eliminated or merged with others, leading to a total of 28 activities that were subjected to a second round of prioritization.

Materials and Methods for setting priorities

Source of data and data input. The results from the first round of priority resulted in five strategic objectives and 28 activities (Table 1), which were submitted to the second round of priority setting. Data were from 35 respondents who are members of the MTP Root and Tuber Systems Project. The Project members were from 11 major disciplines, agronomy (2), biotechnology (5), crop protection (2), economics (4), entomology (1), food technology (2), GIS (1), pathology (4), plant breeding (8), soil science (3), and virology (1).

Each respondent was asked to assess each activity under each strategic objective, based on five outcome criteria that more and less describe the IITA mission: contribution to food security (Cr1), contribution to income generation (Cr2), contribution to protection of the environment (Cr3), contribution to building institutions involved in research for the development of root and tuber crops in Africa (Cr4), and contribution to quality of science (Cr5). During the 2007 meeting, Project members assigned relative weights such that most important criteria had a heavier weight (see details on weights in annex 1).

Using a structured questionnaire (annex 1), respondents were asked to score each activity between 1 and 10 for each criterion. The score of 1 indicates

Table 1. Project strategic objectives and corresponding numbers of proposed activities in 2006 and 2007.

	Project Strategic Objective	No. of Activities		
		Nov 2006	Oct 2007, first round of priority setting	Oct 2007, second round of priority setting
1	Increase productivity of root and tuber crops	6	8	7
2	Reduce production and consumer risks	13	15	7
3	Add value and expand markets	7	8	5
4	Analyze policies and advocate those supportive of the root and tuber sector	15	12	6
5	Strengthen NARS capacity for root and tuber crop research	16	16	3
	Total	57	59	28

a low contribution of an activity to an outcome criterion and 10 indicates a high contribution. Data management used the aid of an Excel spreadsheet. All completed forms were received from respondents through emails. Each received form was cross-checked to confirm that it was filled according to the guidelines, and whether each data cell had been completed. Where there was non-compliance, attempts were made to get the respondent to resubmit the completed form or to clarify it.

Analytical models. Two analyses were conducted: first to define priorities and then to validate results from the priority setting.

Defining priorities. The definition of priorities is based on the weighted mean score of positive responses; that is the product of mean score and frequency of responses with scores more than 0 over the total number of responses. This method combines the relative importance scientists attach to a criterion, as shown by the mean score, and the popularity of that criterion, as shown by the proportion of scientists who chose it.

score showed the relative importance of the activity for a strategic objective (SO). A plot of the proportion of each grand mean score of each SO to total maximum achievable mean score for SOs indicated the importance of an SO relative to all the others.

Validating the ranking of priorities. The results from priority setting were further subjected to general linear model test using SAS GLM procedure (SAS 2003) to evaluate if weighted means scores for activities within each SO were statistically different ($p< 0.05$). Means were separated for each SO by means of Duncan multiple range test (DMRT) (Gomez and Gomez 1984) when the analysis of variance showed a significant difference in activities.

Because of the non-uniform distribution of the disciplines of respondents, the effect of discipline on scores provided was also tested. To reduce this variance a reclassification of disciplines was done, based on expert advice. Group 1 was constituted with technical scientists (plant breeders, agronomists, biotechnologists, and soil scientists). Group 2 were Integrated Pest Management scientists (pathologists, entomologists, virologists, nematologists, and others in crop protection). Group 3 were "human" scientists (economists, food technologists, and GIS specialists). The distribution of respondents under this grouping is shown (Fig. 1). Using this new classification, Chi-square analysis was performed to test unweighted scores obtained for activities by the discipline doing

Let us assume

N = total number of responses
n_j = number of respondents with a weight > 0 to criterion j
s_{ij} = score given by Respondent i to criterion j (I = 1…35)
i = Respondent i (i = 1…35)
j = Criterion j (j = 1…5)

$\bar{X}_j$ = *Mean score for criterion j*

$W\bar{X}_j$ = *Mean score weighted by frequency for criterion j*

Then

$$\bar{X}_j = \frac{\sum_{i=1}^{N} s_{ij}}{N} \quad \text{and}$$

$$W\bar{X}_j = \frac{n_j}{N} * \bar{X}_j = \frac{n_j}{N} * \frac{\sum s_{ij}}{N} \qquad \text{---------(1)}$$

Equation (1) describes the set of formulas that were embedded in the Excel spreadsheet to derive the weighted mean score used in ranking of activities or strategic objectives.

A plot of the proportion of each weighted score for each activity to the maximum achievable weighted

the assessment for each criterion using SAS FREQ procedure. For confirmation, SAS GLM was also used to assess the three groups of disciplines for each activity and criteria based on the unweighted scores provided by respondents.

The weighted mean scores of each SO were subjected to SAS GLM procedure and means separation, where significant, was done using DMRT ($p< 0.05$).

Results and Discussion

Ranking among SOs. For the SOs in descending order of ranking, results showed SO1 (22.2%), SO3 (20.4%), SO2 (20.3%), SO4 (18.8%), and SO5 (18.4%). While these percentages are used to show the relative ranking of each SO, they can serve as well as ways to share resources among SOs within the Project.

Ranking within SOs. Within SO1, in descending order, the activities were ranked as follows: Act. 4 (80.6%), Act. 6 (76.6%), Act. 2 (74.7%), Act. 5 (70.0%), Act. 1 (68.8%), Act. 3 (65.4%), and Act. 7 (58.4%). The analysis of variance (ANOVA) test showed a significant difference ($p< 0.05$) among activities. Three major groups emerged: Act. 4 alone, Acts 6, 2, 5, 3 in a cluster, and Act. 7 alone. In terms of the "disciplinary bias" test, both the Chi square and GLM analyses showed no significant difference ($p< 0.05$).

Within SO2, in descending order, the activities ranked as follows: Act. 3 (68.4%), Act. 1 (68.2%), Act. 5 (67.7%), Act. 2 (67.0%), Act. 6 (61.7%), Act. 4 (60.7%), and Act. 7 (58.7%). The ANOVA showed no significant ($p> 0.05$) difference between activities, and between assessments made by disciplines.

Within SO3, in descending order, the activities ranked as follows: Act. 4 (68.9%), Act. 3 (68.8%), Act. 2 (66.7%), Act. 1 (60.5%), and Act. 5 (59.4%). The ANOVA showed no significant ($p> 0.05$) difference between activities, or between assessments made by disciplines.

Within SO4, in descending order, the activities ranked as follows: Act. 4 (71.4%), Act. 6 (65.1%), Act. 1 (61.2%), Act. 2 (55.0%), Act. 3 (53.2%), and Act. 5 (53.1%). The ANOVA test showed a significant difference ($p\leq0.05$) among activities. Two major groups were evident. Group 1 was made of Acts 4, 6, and 1 that are not statistically different from one another. Group 2 was Acts 2, 3, and 5. In terms of disciplinary bias, both the Chi square and GLM analyses showed no significant difference ($p < 0.05$).

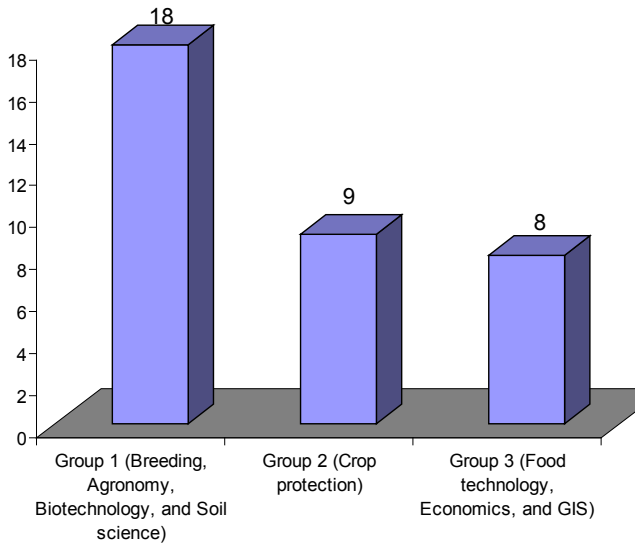

Figure 1. Classification of number of respondents in three discipline groups for the priority setting of the IITA Root and Tuber Crops Systems Project.

Within SO5, in descending order, the activities ranked as: Act. 1 (60.7%), Act. 3 (58.1%), and Act. 2 (56.7%). The ANOVA showed no significant ($p > 0.05$) difference between activities, and between assessments made by disciplines.

Comparing activities across SOs. The main objective of this exercise was to define priorities within and among SOs. However, results from the analyses give an additional opportunity to compare activities across SOs. This is facilitated by the use of common criteria, the same analytical tools in data analysis, and scoring by the same respondents (=same perception in ranking). In descending order, the ten first activities are as follows: SO1Act. 4 (80.6%), SO1Act. 6 (76.6%), SO1Act. 2 (74.7%), SO4Act. 4 (71.4%), SO1Act. 5 (70.0%), SO3Act. 4 (68.9%), SO3Act. 3 (68.8%), SO1Act. 1 (68.8%), SO2Act. 3 (68.4%), and SO2Act. 1 (68.2%). Five activities belong to SO1, with three activities taking the lead out of 10. Across SOs and on the whole, respondents perceived five out the seven activities of SO1 as being very important. SO3 has two activities in ranks six and seven. SO2 also has two activities but in the last two ranks out of ten. The only activity for SO4 was ranked fourth. None of the activities of SO5 appear among the top 10, probably because respondents perceive the activities of capacity building of partners as an integrated component of technical activities in the other objectives. This raises the issue of mutually exclusive criteria required for a proper priority setting. Where criteria overlap, some

of the activities under priority setting cannot appear in the final ranking exercise, not because they are un important but because criteria applied to rank them are not mutually exclusive.

Identifying strengths and weaknesses within SOs. Another interesting outcome from a priority setting exercise is the identification of weaknesses and strengths. This would assist managers and Project members in developing strategies to overcome weaknesses and to reinforce strengths, therefore improving overall efficiency.

An example can be given (Fig. 2) for the activity that ranked first within its SO and across all SOs. That is Activity 4 of SO 1 (SO1Act4). This activity scored 80.7% out of the maximum achievable 100%.

Results in Figure 2 compare the score of Act. 4 to the average of scores for all activities of SO1 per criterion. Although Act. 4 realizes a score above 100% compared to the average in all criteria except one (which was expected), its highest perceived benefit is in its contribution to Cr 4 about building of institutions and the lowest contribution is in Cr 1 about food security. Building on the above results, Project members should challenge themselves on answering the question why an SO that is all about increasing productivity of root and tuber crops is perceived by its members as having lowest contribution to food security out of the five criteria used in the analysis. Answering such questions could lead to the design of new strategies to correct for possible weaknesses identified in the Project operations.

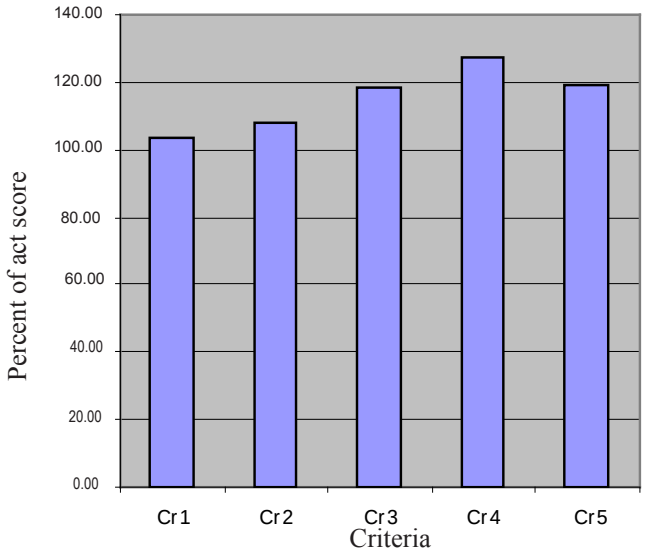

Figure 2. Comparing the score of first ranked Act. 4 of SO 1 to the average score of all activities of same SO.

Conclusion

The results from priority setting provide a scientific means of deciding where a project should emphasize the focus on its operations, thus contributing to a better planning of operations and allocation of resources. While the ranking among SOs was done in this study, the same resulting proportion calculated for each SO could serve as a basis for allocating resources to the five SOs of this Project.

The ranking of activities indicated priorities for each SO. Across SOs, the assessment revealed the perceived high rank attached to the activities relative to SO 1 about increased productivity. Project members also identified at least one activity from each of the other SOs among the top 10 activities ranked for this Project (the exception was SO 5 that did not show any ranked activity among the top 10).

The hypothvesis about "discipline bias" in priority setting was not validated by the statistical tests. Project members made a fair assessment of the activities, regardless of their discipline of origin.

Priority setting is also a useful tool for the management of a project as the analysis can lead to the design of new strategies to improve efficiency. This report shows how the process of priority setting began in November 2006 and was followed by two levels of priority setting in 2007. More insights can be gained through a further analysis of input data. Therefore , a one-time priority setting is not an end in itself. It can be the beginning of an iterative process whereby results from one step become a valuable input for the next step. Project members can fully benefit from priority setting only when they consider and value it as an important and permanent element in the planning and implementation of their activities. Priority setting must be embedded in our culture of everyday business for impact.

The participation of many is a key to a good priority setting exercise. It is important to mobilize all Project members to take part in the priority setting exercise for their Project. To facilitate the involvement of all, it is important to adopt approaches, methods, and techniques that allow all the disciplines to participate effectively.

Annex 1: Priority Assessment Scoring template

Date:
Name of assessor (optional):

Discipline of assessor (write the name):
Code for discipline of assessor (see below):

Activities	(1)	(2)	(3)	(4)	(5)
	Criteria				
	Food Security	Income Generation	Protection of Environment	Building Institutions	Quality of Science
Weight for Criteria:	21	22	19	19	19

SO1: Increasing Productivity of Root and Tuber Crops

1. Design sustainable nutrient management options for root and tuber systems
2. Breed more nutrient-responsive, water and nutrient-use-efficient, and pest resistant varieties
3. Improve seed systems for root and tuber crops
4. Develop sustainable integrated soil, water, crop and pest management systems
5. Adapt and promote appropriate methods of farm mechanization and other labor-saving technologies
6. Develop and apply biotechnology tools to introduce tolerance / resistance to biotic and abiotic stresses into improved germplasm
7. Expand production and use of root and tuber crops into non-traditional areas

SO2: Reduction of production and consumer risks

1. Generate knowledge on emerging biological risks, consumer risks for root and tuber products, and develop strategies to mitigate them
2. Develop and promote diagnostics to manage biological risks
3. Identify and promote strategies to minimize postharvest risks and biological risks to seed systems
4. Conduct risk analysis of transgenics
5. Determine the impact of environmental risks on root and tuber crop systems and develop strategies to reduce them
6. Develop strategies to minimize the impact of root and tuber crop systems on the environment
7. Develop and promote standard guidelines for compliance with trade and quarantine obligations

SO3: Adding value and expanding markets

1. Analyze market preferences for traditional and novel products from root and tuber crops and study the relevant functional properties
2. Develop and apply biotechnology tools to improve germplasm for specialty traits
3. Breed and select germplasm for market and nutritional traits
4. Develop safe and competitive products from roots and tubers, and determine safety thresholds in food and feed products
5. Design, adapt and promote appropriate machines to add value and expand markets

	(1)	(2)	(3)	(4)	(5)
	Criteria				
Activities	Food Security	Income Generation	Protection of Environment	Building Institutions	Quality of Science
Weight for Criteria:	21	22	19	19	19
SO4: Advocacy and policy					
1. Model competitiveness of root and tuber crops under different fertilizer policy regimes and land intensification					
2. Monitor and evaluate return to investment of research and training on root and tuber crops and assess their economic and social impacts					
3. Make an inventory of policies and other economic factors that influence development of root and tuber crops					
4. Advocate policies that support and promote development and utilization of root and tuber crops					
5. Provide relevant information on genetically modified organisms to African national governments and assist them in the development of biosafety guidelines					
6. Promote and assist the development of national policies and protocols for release of new varieties, and development and regional harmonization of seed regulations.					
SO5: Strengthening partners					
1. Identify and respond to appropriate training needs of partners					
2. Promote and assist efforts to strengthen infrastructural capacity of partners for research and development					
3. Promote public-private partnerships					

Scales for scores: 1-10 (1 = Very bad and 10= Very good)

Code for discipline of assessor: 1 = Plant breeding, 2 = Food technology, 3 = Weed science, 4 = Economics (Agric. Economics), 5 = Biotechnology (including Tissue culture), 6 = Statistics, 7 = Pathology, 8 = Agronomy, 9 = Crop protection, 10 = Soil science, 11 = Agroenterprise, 12 = Entomology, 13 = Virology, 14 = Agric. Extension, 15 = Animal science, 16 = GIS

Acknowledgement

The authors acknowledge the contributions of all of the members of the Root and Tuber Crops System Project in this priority setting.

References

Alston J., Norton W. and Pardey G. 1995. Science under scarcity. Principles and practices for agricultural research evaluation and priority setting. CAB International, Wallingford, Oxford, UK.

Gomez K.A. and Gomez A.A.1984. Statistical PRocedures for agricultural research. Second Edition, John Wiley & Sons Inc. NY, USA.

Manyong V.M., Kormawa P., Diels J., Dixon A. and Hughes J. 2001. Priority setting at IITA: Report by the task force on priority setting. IITA. Mimeo. Ibadan, Nigeria.

SAS 2003. SAS/STAT software, version 9.1. for Windows. Cary, NC, USA.

A Research Article in AJRTC (2010) Vol. 8 No.1: Pages 13–16

Evaluation of some botanicals as an alternative to chemical fungicide in the rapid multiplication of cassava

O.N. Eke-Okoro, F. Ogbe and J.K.U. Emehute

National Root Crop Research Institute Umudike, P.M.B 7006, Umuahia, Abia State, Nigeria.

Corresponding Author: O.N. Eke-Okoro
Email: ekeokorono@yahoo.com

Manuscript revised received: 27/09/2007; accepted: 30/06/2008

Abstract

The use of plant extracts (botanicals) such as standard neem, pepper fruit, *uziza* and ash as local substitutes or alternatives to chemical fungicides (Tecto-60E) in the rapid multiplication of cassava was studied for two years (2004 and 2005) in Nigeria. Percentage sprouting, sprouting vigour and percentage fungus soot were dependent on the treatments and were optimized in 2–node cassava stakes treated with standard neem extract. Standard neem extract and Tecto 60 (chemical fungicide) had similar, significant (p = 0.05) control over fungus soot. The significant growth differences among the treatments with the optimum at standard neem extract indicate the need to adopt neem extract as the best local plant extract as a substitute for or alternative to chemical fungicides using 2–node cassava technique in the rapid multiplication of cassava planting material.

Keyword: cassava, neem, botanicals, fungicides,Tecto–60

Introduction

A major constraint to increased cassava production in Nigeria is an insufficiency of improved planting materials. However, the use of 2–node stakes for rapid multiplication of cassava stakes (IITA, 1990, Eke-Okoro et al 2005) has reduced this problem considerably. Farmers now multiply their planting materials with this technique, using materials such as transparent polyethylene-bags and chemical fungicides. The fungicides are used to prevent fungus attack on the cut surfaces of 2–node stakes. Some of the chemical fungicides usually applied are Tecto 60 (Thiabendazole), Benlate –T (Benomyl + Thiram), Apron plus (Metalaxyl + Carboxin + Furathiocarb), Mancozeb (Dithane M45), (Nnodu and Okwuowulu 1990; Eke-Okoro et al 2005).

Fungus infection is a serious problem in pre-germination or pre-sprouting nursery operations. In most farms, fungus soot reduces sprout energy, vigour, growth and in severe cases damage or delay the emergence of new buds (Wheeler 1978). The control of fungus infection in most cases has been primarily through the use of chemical fungicides. However, some plant extracts (botanicals), e.g., neem, have been found to be effective fungicides. The neem extract has been reported to have insecticidal, fungicidal, and nematicidal properties which are useful in controlling the pests, diseases, and micro-organisms that spoil agricultural produce (Chelfant et al 1990; Prakash and Rao 1997, Jansson and Raman 1991; Hawang 1994). Neem leaves extract has been found to be effective against nursery ants, termites, and garden pests (Stoll 1998, IITA 1999). Nwuzor et al (2005) controlled *Cylas puncticollis* in sweetpotato by applying neem leaves powder in Nigeria. Ginger, cashew, dry chilli pepper, onion scale leaves, and neem seed kernel have been used as surface protectants in the control of cowpea beetle in storage (Ofuya 1986; Sowummi and Akinwusi 1983). Stoll (1998) and Offor (2004) reported that pepper fruit and neem have properties that inhibit the growth of micro–organisms. The non-toxic nature some of plant extracts (neem) to most parasitoids and predators of rice pests have also been reported (Thiam and Dujommun 1993).

At the rural farm level in Nigeria, the use of chemical disinfectants (fungicides) in the pre-sprouting of stems has been difficult to adopt in the rapid multiplication of cassava. This is because the chemical fungicides or disinfectants are scarce, costly when available, and unfriendly to man and the environment. These reasons render the adoption of the rapid multiplication technique by farmers very slow. In most cases, farmers reluctantly use this technique in multiplying cassava stems. To overcome this problem, this study explored the use of locally available, less expensive, and safe plant extracts, such as neem (*Azadirachta indica*) leaves, pepper fruit (*Dennettia tripetala*),

and wood ash as sprout protectants or fungicides against fungus attack on the cut surfaces of cassava stakes as alternatives to chemical fungicides.

Materials and Methods

Three standard plant extracts: standard neem, pepper fruit and *Uziza* seed were evaluated against three controls: ordinary ash, fresh water, and Tecto 60 (chemical fungicide) using 2-node cassava stakes. Standard plant extracts were prepared based on the combined procedures of Karim et al (1992) and Stoll (2000). The chemical fungicide was applied at the rate of 10 g per 20 liters of fresh water. Standard 1 kg of dried neem leaves powder was soaked in 5 liters of fresh water for 24 hours and filtered with muslin cloth before application. The ash extract was the National Root Crop Research Institute's standard ash and was applied by dissolving one sachet of 1 kg in 5 liters of fresh water. Twenty-five 2-node cassava stakes were soaked in the prepared plant extracts and the fungicide solution in a plastic container and left to stand for 10 min. After which, 25 stakes per treatment were packed into perforated transparent bags to a level sufficient to allow for the mouth to be tied with a rope or string, leaving enough empty space inside the polyethlene bags for air circulation. The bags were laid out in a randomized complete block design with five replications under tree shade. Estimation of number of sprouts were done by cumulative counting of the new sprouts on the cassava stakes from 3 to 6 weeks after placement in the bags and were converted to percentages. Sprouting vigour was estimated by measuring the length of the new sprouts using a transparent metric ruler. Fungus soot (black powder or substance on the surface of the cut stake) was estimated by recording the incidence (%) on cassava stakes during the experimental period of 6 weeks.

Data collected were analysed according to the procedure for a randomized complete block design using a Genstat computer program. The significance of treatment effect was done by Duncan's New Multiple Range Test (DNMRT) at 5% level of probability.

Results and Discussion

The sprouts, sprouting vigour, and the stakes infected by fungus soot were monitored during the experimental period in 2004 and 2005. The number of sprouts, vigour of sprouts and fungus soot were significantly ($p< 0.05$) influenced by the treatments. The maximum percentage sprouting during the two seasons ranged from 88% in 2004 to 97% in 2005 and was obtained by 2-node cassava stakes treated with standard neem extract. The minimum percentage sprouting during the two seasons ranged from 71.2% in 2004 to 68.7% in 2005 and was obtained by 2-node cassava stakes treated with standard ash extract. The vigour of sprouting was significantly enhanced by standard neem extract with a sprout length of 3.7 cm in 2004 and 4.3 cm in 2005. The vigour of sprouts was, however, lower across all other treatments in 2005 but fresh water sustained the lowest vigour (2.0 cm). The maximum number of stakes infected by fungus soot (black exudate or powder) during the two seasons ranged from 44% in 2004 to 52.1% in 2005 and was observed in the non-treated 2-node cassava stakes (fresh water). The minimum number of stakes infected by fungus soot during the two seasons ranged from 12.% in 2004 to 17.3% in 2005 and was observed in 2-node cassava stakes treated with Tecto -60 (chemical fungicide)

Sprout determinants, such as number of sprouts, vigour of sprouts, and fungus soot, were dependent on the treatments. Number of sprouts, and sprouting vigour were optimized by the application of standard neem extracts; the lowest fungus attack (soot) was obtained by 2-node cassava stakes treated with Texto 60 (chemical fungicide) and standard neem extract in the two seasons. This suggests that treatment of stakes with either standard neem extracts or chemical fungicide is necessary for minimizng fungus attack and sprout failure in the rapid multiplication of cassava. Higher percentage sprouting, sprouting vigour, and comparative low levels of fungus soot were obtained in stakes treated with standard neem extract in more than any other treatment, indicating that 2-node cassava stakes treated with standard neem extract resulted in a higher stake sprout performance, less fungus attack and sprout failure relative to chemical fungicide.

The ability of plant extract (standard neem) and (chemical fungicide) Tecto 60 to reduce fungus attack on the cut surface of 2-node cassava stakes was significantly similar. This suggests that the (standard neem extract) plant extract is the best local substitute for or alternative to chemical fungicide (Tecto 60) in the rapid multiplication of cassava. Neem is locally available and environmentally friendly. Similarly, Prakash and Rao 1997, Nwuzor et al (2005), Stoll (1998), and Offor (2004) reported the ability of plant extract (neem) in inhiting the growth of micro–

organisms in agriculture. In addition, Chelfant et al (1990) and IITA (1990) noted that neem is an effective fungicide, insecticide, and nematicide in controlling pests, diseases and micro-organisms that spoil agricultural produce.

In conclusion, the use of standard neem extract is recommended to farmers as the best local substitute for or alternative to chemical fungicide in the rapid multiplication of cassava.

Table 1: Number of sprouts, fungus soot, and vigour of cassava sprouts as affected by botanical extracts in 2004.

Botanical/ Chemical Fungicide	Percentage Sprouting	Sprouting Vigour (cm)	Fungus Soot (%)
Standard Neem Extract	88.0 a	3.7 a	15.2 b
Standard Pepper Fruit Extract	85.6 ab	2.5 b	25.6 b
Standard *Uziza* Extract	79.2 bc	2.3 b	12.8 b
Standard Ash Extract	71.2 d	2.3 b	13.6 b
Tecto 60-chemical fungicide (control I)	81.6 abc	2.8 b	12.0 b
Fresh water (Control II)	75.2 cd	2.9 b	44.0

Mean separation in columns by DMRT at 5% level

Table 2: Number of sprouts, sprouting vigour, and fungus soot as affected by botanical extracts in 2005.

Botanical / Chemical fungicide	Percentage Sprout	Sprouting Vigour (cm)	Fungus Soot (%)
Standard Neem Extract	97.0a	4.3a	16.7b
Standard Pepper Fruit Extract	90.4ab	3.1b	22.4b
Standard *Uziza* Extract	72.6b	3.0b	18.3b
Standard Ash Extract	68.7c	2.9b	20.4b
Tecto 60- Chemical Fungicide (Control 1)	87.4ab	3.4b	17.3b
Fresh water (Control 11)	79.7d	2.0c	52.1a

Mean separation in columns by DMRT at 5% level

References

Chelfant R.B., Joansson P.R., Scal D.R. and Schalk J.M. 1990. Ecology and management of sweet potato insects. Annual Review of Entomology 35: 157–180.

Das G.P and Karim A.A. 1986. Effectivenes of neem seed kernel oil as surface protectant against the pulse beetle. (*Callosobruchus, chinensis*). Grain and Legume Bulletin 33: 30–33.

Echendu T.N.C. 1991. Ginger, cashew and neem as surface protectants of cowpea against infestation and damage by *Callosobruchus maculatus*. Tropical Science 31: 209–211.

Eke-Okoro O.N., Ekwe K.C. and Nwosu K.I. 2005. Cassava stem and root production:
A practical manual. Atlas Press Umuahia, Nigeria. 52 pp.

Hawang J.S. 1994. Sweetpotato insect pest management in Taiwan. *In* Proceedings of the symposium on upland crop production. Council on Agriculture plant protection series No. 2 Taina District Agric. Improvement Station, Taiwan, Taiwan. ROC: 191–200.

IITA 1990. Cassava in Tropical Africa. A reference manual: 415 pp. IITA, Ibadan, Nigeria.
IITA 1999. Plant Health Management Division. Annual Report. 4–5. IITA, Ibadan, Nigeria.

Jansson R.K. and Raman K.V. 1991. Sweetpotato plant management. A global perspective Westview Press Inc., Boulder, Colorado, USA. 439 pp.

Karim A.N.M.R., Chowdhurg M.N.A. and Mozadd M.N.A. 1992. Current research on neem in Bangladesh. *In* Botanical pest control project phase 2. Proceedings of the final workshop 18–31, July 1992. IRRI. Los Banos, Laguna, The Philippines 30–32.

Nnodu E.C. and Okwuowulu P.A. 1990. Evaluation of fungicides for the control of leaf spot of ginger. Nigeria Journal of Plant Protection 13: 19.

Nwuzor E.C., Afuonye S.O., Okoriocha D.S. and Ezulike T.O. 2005. Studies on the use of neem leaf preparation to control *Cylas puncticollis* damage of sweetpotato root tubers. National Root Crops Research Institute, Annual Report, 2005. Umudike, Nigeria. 92–94.

Ofuya T.I. 1986. Use of wood ash, dry Chilli pepper fruits and onion scale leaves for reducing *Callosobruchus maculatus* damage in cowpea during storage. Journal of Agricultural Science (Cambridge) 107: 467–468.

Offor P.C. 2004. Pesticidal potentials of neem. National Root Crops Research Institute, NRCRI, Umudike Nigeria. Seminar series 2: 1–10.

Prakash A. and Rao Jagadiswari 1997. Botanical Pesticides in Agriculture. CRC Press Inc.

Florida,USA. 40 pp.

Sowummi E.O. and Akinwusi A.O. 1983. Studies on the use of neem kernel in the control of stored cowpea beetles. *Callosobruchus maculatus*. Tropical Grain and Legume Bulletin 27: 28–30.

Stoll G. 1998. Natural crop protection in the tropicsa and Sub-Tropics. AGRECOL, Switzerland 188pp.

Stoll G. 2000. Natural Crop Protection in the Tropics: Letting Information come to life. 2nd edition; Magraf Verlag, Germany. pp:101–139

Thiam S. and Dujommun. G. 1993. Protection Naturelle des vègètaux en Afrique. *In* Producing food without pesticides. CTA Postbus 300, 67 pp. A.J. Wageningen, The Netherlands.

Wheeler B.E.J. 1978. An introduction to plant disease. The English Language Book Society and John Wiley and Sons Ltd. Chichester. UK.

A Research Article in AJRTC (2010) Vol. 8 No. 1: Pages 17–23

Exploring storage protocols for yam (Dioscorea spp.) pollen genebanking

I.O. Daniel

Department of Plant Breeding and Seed Technology, University of Agriculture, PMB 2240, Abeokuta, Nigeria.

Email: drdayodaniel@yahoo.com

Manuscript revised received: 27/06/2007; accepted: 30/06/2008

Abstract

Implementation of pollen genebanks allows the conservation of plant genetic resources at the haploid level, pollen genetic manipulations, scheduling of hybrid seed production, and improvement of breeding efficiency. To establish pollen storage protocols for various genotypes of West African yams, laboratory and field experiments were conducted on fresh pollen and pollen stored under various conditions at the Genetic Resources Unit International Institute of Tropical Agriculture Ibadan, Nigeria (IITA). The storage treatments examined were air-dried storage, freeze-drying followed by storage in liquid nitrogen, and hermetic cold storage without previous drying ("wet-cold" storage). Pollen maintained under dry conditions (dry-air and freeze-dried) maintained aceto-carmine stainability up to 400 days but drastically lost germination capacity, most notably under dry-air storage. But pollen samples maintained at "wet-cold" conditions under -80°C retained germination capacity after 2 years. Hand pollination with pollen of *D. rotundata* that was "wet-frozen" under -80°C for 365 days gave 69.5% fruit set and 50% fruit set after 730 days in storage. The results provide evidence to suggest recalcitrance of yam pollen grains in view of poor pollen survival with drying. Therefore, the "wet-cold" storage procedure appears to be the most promising method for the sustainable implementation of yam pollen genebanks. Pollen storage protocols for active and base collections were proposed, based on these findings.

Key words: Yams, pollen genebank, preservation protocols

Introduction

The improvement of yam through conventional breeding is constrained by complicated flowering biology characterized by dioecy, unsynchronized male and female flowering, and poor and irregular flower production which altogether limit wide hybridization (Akoroda 1981, Asiedu et al 1992). A way to overcome these barriers is by combining flowering induction with pollen storage. There are reports of the successful induction of flowering in different types of yam through agronomic manipulation, for example, staggered planting (Bai and Jos 1986) and siting crossing blocks at locations conducive for flowering (Abraham and Nair 1990, Asiedu et al 1992). Pollen storage has not been effectively implemented.

Pollen storage is important for germplasm conservation, exchanges and handling, and it enhance improved breeding efficiency (Hanna 1994). The application of pollen storage as an integrated method for the long-term conservation of yam genetic resources and for use in yam improvement programs holds great promise. First, it will allow pollen to be available throughout the breeding period, thus enhancing wide and controlled hybridization. Secondly, it will remove the constraint to wide hybridization from fluctuations in flowering in genotypes that flower irregularly from season to season. It will also circumvent the limitations to yam hybridization of unsynchronized male and female flowering by making pollen available whenever the stigma is receptive. Moreover, it will permit crosses across geographical locations. Furthermore, pollen storage will enhance conservation of the haploid level gene pool as a valuable resource base for biotechnological manipulations in yam improvement.

Storage of pollen under low temperatures as a means of plant genetic resources conservation has been widely discussed by many authors for various species (Harrington 1970, Bajaj 1987, Rajasekharan et al 1994, Kozlowski and Pallardy 2002). For yam, Akoroda (1981, 1983) reported about 5% viability in pollen lots of Guinea yam after 14 months of storage under dry-cold conditions (5 °C over-concentrated sulphuric acid). But Daniel (1997) and Daniel et al (2002) concluded that the maintenance of pollen

grains of yam would be better at high moisture levels, arguing that the potential fertility of pollen grains is highest at shedding and, at this stage, yam pollen grains are sticky (Ngu 1991), indicating a high moisture content. This paper examines potential protocols for sustainable pollen storage based on results from various experiments conducted on the pollen grains of different West African yam genotypes.

Materials and Methods

Freshly opened flowers containing mature but unshed pollen grains were collected during yam flowering seasons of 1994 through to 1996 from the germplasm maintenance and nature conservation sites of International Institute of Tropical Agriculture Ibadan, Nigeria (7 °30N', 3 °54¹E).

Flowers were collected between 9.00am and 11.00am and brought to the Seed Laboratory of Genetic Resources Unit, IITA. Collection was done by severing spikes with mature flower buds from mother plants with scissors. Anthers were scooped out from recently opened flowers (mature flower buds) with a microscope mounting needle and placed on slides smeared with a drop of aceto-carmine stain. Pollen grains were teased out of the anthers with blunt mounting needles and pollen morphological observations were done with a Labourlux™ compound light microscope.

Pollen physiology studies. Laboratory pollen viability and fertility were evaluated by aceto-carmine stainability and pollen germination capacity *in vitro*. Anthers with clusters of sticky pollen grains were placed on slightly modified Brewbaker and Kwack (BK) (1963) culture medium and incubated under room conditions for 3 hr. The modified medium consisted of 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulphate, 100 ppm potassium nitrate, and 7% bacto-agar in deionized water. Percentage pollen germination was estimated and tube length was measured in micrometers (µm) using the microscopic scale. An estimate of pollen fertility was calculated as a product of percentage pollen germination and the tube length. These values were log transformed to derive a pollen fertility index (PFI).

All data were collected from three different microscopic fields, each field observation representing a replicate. Mean values of percentage viability, tube length, and the estimated PFI were calculated.

Pollen storage trials

Air-dried storage. In this trial, freshly excised anthers in male flower buds were collected in vials. Each vial contained at least 15 anthers. The vials containing the anthers were left uncapped and placed in a cold room at 5 °C with a relative humidity of about 35% for 2 days. The anthers containing the pollen were then packed and sealed in an aluminium envelope containing 1g of silica gel. The sealed envelopes were stored at -80 °C, -20 °C, 5 °C, and 15 °C. Pollen samples were taken out from each storage treatment for assessment of viability and stainability at 10day intervals for the first 100 days and at 100 day intervals thereafter.

Freeze-drying and storage in liquid nitrogen. Freshly sampled male flower buds of *D. rotundata* accessions TDr 3577, TDr 3370, and TDr 1766, were freeze-dried in a Ohaus™ lyophilizer set at −60 °C and 50mm Hg vacuum for 24 hr. The viability of the pollen grains was evaluated immediately after freeze-drying. The freeze-dried male flower buds were placed in cryo-vials and plunged into liquid nitrogen in Dewar™ flasks for 1 hr. Viability of the pollen was assessed after the vials were allowed to warm and thaw at room temperature.

"Wet-cold" storage. Male flower buds containing mature anthers were placed either in cellophane packs and heat sealed or in glass vials and sealed with film tapes without drying. The sealed packs and vials were immediately stored under four cold storage conditions, 15 ± 2 °C, 5 ± 2 °C (active collection cold stores), –20 °C (base collection cold store) and −80 °C (freezing in ultra-low temperature freezer). The stored pollen of *D. rotundata* (TDr 1424) and *D. praehensilis* was examined for laboratory viability and fertility after 100 and 700 days. Fruit setting capacity during pollination with fresh and stored pollen was also investigated using pollen of *D. rotundata* accessions TDr 199, TDr 3303, TDr 3577, and TDr 3605 after 365 and 730 days of storage.

Pollen storage trials were initiated during the 1994 and 1995 cropping seasons. All the materials that were air-dried or freeze-dried were from the 1994 pollen collections.

Hand pollination trials. Unripe female flower buds were covered with thrips-proof cloth bags, tightened at the base of the spike to avoid insect pollination. After 5 days when the pistil matured, the most recently opened flowers (receptive female flowers) on the spikes were pollinated by placing test pollen grains (from fresh or stored anthers) on the stigma inside the flowers. Flowers not pollinated on a spike were removed and the spikes were re-bagged. The pollinated flowers were observed for fruit setting after 8 days. Swelling of fruit indicated successful fertilization and percentage fruit set was estimated as the number of swollen fruits per total number of pollinated flowers.

Hand pollination trials were conducted at the yam breeding plots of IITA, in 1995 (365 days of storage) and in 1996 (730 days of storage) using only test pollen of pollen lots maintained under the "wet-cold" storage method to compare results with freshly harvested pollen. The trials involved only *D. rotundata* accessions which represent the bulk of the yam improvement programme at IITA. In 1995, pollen of TDr 3577 frozen under –80 °C was used to pollinate female line 90/00/308. Fresh pollen samples from accession TDr 199 were used to pollinate as control. In 1996, the same pollen lot (TDr 3577 frozen under –80 °C) was used to cross female line 93-1 and compared with fresh pollen samples of TDr 3577, TDr 3303, and TDr 3605 used on female line 3099.

Statistical procedure. Data collected on pollen physiological characteristics were subjected to One-way ANOVA on the effects of genotype on the pollen fertility variables. Means of pollen viability and fertility variables were estimated for pollen lots. Pollen lots with the highest mean values of the various genotypes were used in the analysis and mean comparison among species was done by estimates of Least Significant Differences (LSD) at p=0.05 and the Duncan Multiple Range Tests (DMRT).

The analysis of pollen viability and fertility data collected during the pollen storage trials were subjected to factorial ANOVA on the effects of genotype and storage treatments on arc-sine equivalents of percentage *in vitro* germination. Means of pollen germination and fertility under the different storage treatments were compared with control (fresh pollen) using the DMRT letters generated by the ANOVA. Percentage germination data were transformed with the arc-sine transformation table in Gomez and Gomez (1984). The ANOVA procedure was performed on Statview™ statistical package.

Results and Discussion

Yam pollen sizes ranged from 0.25 µm diameter in *D. bulbifera* and *D. dumetorum* to 0.70 µm diameter in *D. preussii* (Table 1). Yam pollen grains were mostly round or oval in shape.

Laboratory viability and fertility assays of fresh yam pollen revealed wide variations in the *in vitro* germination capacity among the various genotypes but the PFI estimates varied narrowly. *D. praehensilis* consistently had the highest percentage pollen viability in the 1994 trial and the highest pollen fertility in the 1996 trial (Table 2). All genotypes examined through the staining test stained aceto-carmine red, implying biochemical viability. Functional viability shown by the percentage germination capacity was over 30% for all the species and PFI estimates ranged between 4 and 6, except for the exotic *D. bulbifera* accessions that appeared to be sterile.

The results showing high pollen germination capacity from the various viability tests conducted on the pollen of various West African yam confirmed that yam pollen grains are naturally viable, (Akoroda 1983). The viability of freshly harvested pollen as the *in vitro* germination and aceto-carmine stainability tests is an indication of the high potential viability necessary for the successful implementation of pollen banks, since genebanking is about maintaining the materials in living and functional condition. The result suggest that the poor seed setting commonly reported in West African yam (Doku 1978, Hanson 1986, Okoli 1991) is not as a result of poor pollen viability, but is probably evidence of the poor efficiency of natural pollinators, i.e., thrips, or of non-synchronized male-female flowering. This further intensifies the potential benefits for establishing and maintaining yam pollen genebanks.

Results from the dry air storage trial are shown (Table 3). Dry air storage under -80, 5, and 15 °C resulted in a total loss of germination capacity and acetocarmine stainability in all yam pollen. But after air-dry storage of yam anthers inside -20 °C cold stores, some pollen lots maintained over 40% stainability in aceto-carmine after 30 and 400 days of storage but had totally lost *in vitro* germination capacity at both periods (Table 3).

The pollen grains of all the genotypes of yam subjected to the freeze-drying procedure maintained very high percentages of pollen stainability, but percentage germination capacity was lost in all genotypes except *D. alata* that still maintained 15% germination capacity (Table 4). The procedure for freeze-drying in the experiments appeared to have detrimental effects on most of the yam pollen grains.

Both dry storage procedures resulted in high pollen stainability but very poor *in vitro* germination capacity in most of the species, indicating the activity of certain enzymes. Impairment of germination, might be due to the low moisture availability for the process. Previous reports on pollen storage of various yam showed the same trend of low germination capacities in response to dry-storage treatments (Akoroda 1983, Ng and Daniel 2000). Hong et al 1999 reported that the longevity of *Typha latifolia* pollen improved with dry and low temperature storage, indicating "orthodox" storage behaviour. These results suggest that yam pollen grains exhibit some degree of recalcitrance, that is, yam pollen, though viable, require certain level of moisture content to germinate. Connor and Towill (1993) proposed the need to determine optimum

moisture levels at which to maintain viability at low temperatures for different pollen species instead of a general routine desiccation. There is, therefore, a research gap especially in yam pollen drying procedures to investigate the threshold moisture levels of dried flower buds that permit pollen germination, before implementation for practical pollen storage.

Table 5 shows pollen viability of two yam species after 100 and 730 days of "wet-cold" storage. At 100 days of storage, germination capacity was maintained in pollen lots stored under 5, -20, -80°C, but viability was totally lost in pollen of both species refrigerated at 15°C. Pollen frozen under -80 and -20°C germinated comparably well with fresh pollen at 100 and 730 days. A comparison of pollen *in vitro* germination data at 100 days with pollen *in vitro* germination at 730 days of "wet-cold" storage showed an insignificant loss of viability in pollen frozen under -80 and -20°C between the two periods. Pollen of *D. rotundata* wet-frozen under -80°C for 365 days gave a high laboratory pollen germination and a fertility index of 2.4, corroborated by 69.5% fruit set on the field which was insignificantly different from the fruit set when fresh pollen was used (Table 6). After 730 days (2 years) of wet-freezing, the fruit set when frozen pollen was used was not quite as close to the fruit set when pollination was done with fresh pollen, even though the laboratory PFI estimates were lower in frozen pollen than in all the fresh pollen lots (Table 6).

When compared with results from dry-cold storage trials (Akoroda 1981, 1983; Ng and Daniel 2000), "wet-cold" storage appeared to be the most effective method for the preservation of pollen viability and fertility in yam. Though desiccation was recommended for the freeze preservation of plant cells and tissues (Bajaj 1987, Hughes and Lee 1991), preserving moisture in fresh yam pollen appears more beneficial than desiccation for maintaining their fertility. The pollen grains did not exhibit the expected injuries that were apparent in the surrounding tissues of the anthers after freezing and thawing. These results demonstrated the possibility of successfully preserving the viability and fertility of yam pollen grains by ultra-low temperature storage of fresh anthers or flower buds ("wet-cold" storage).

Table1. Pollen sizes in different yam genotypes

Species	Pollen diameter	Pollen shape
D. alata	0.60a	Round
D. bulbifera	0.25b	Round
D. dumetorum	0.25b	Oval
D. praehensilis	0.30b	Round
D. preussii	0.70a	Oval
D. rotundata	0.30b	Round
D. togoensis	0.30b	Round
LSD (p=0.05)	0.163	

Means in a column followed by the same letters are not significantly different at P=0.05 by the DMRT

Protocols

The aim of this paper is to suggest protocols for preserving the haploid level genetic diversity of yam in the form of pollen form under genebank conditions to supplement classic clonal preservation methods. In genebanks, seed germplasm is maintained as active and base collections in medium and long-term conditions. In active collection genebanks, materials are usually retrieved for use within a short period of time. The results of this study showed that active collections of yam pollen grains can be maintained within a breeding season for 1-3 months when stored "wet-cold" under 5 °C as shown in protocol 1 (Table 7). This is most beneficial when the problem of non-synchronization of male and female flowering is limiting breeding efficiency.

Base collections are materials to be maintained on a long-term basis under conditions of storage that induce minimal losses in viability over time. The maintenance of pollen viability under frozen conditions for 2 years in this study implies that pollen can be maintained as base collections when wet-frozen. Protocol 2 (Table 7) shows the procedure used for wet-freezing of yam pollen in ultra-low freezers. A cheaper freezing protocol that could still be investigated for yam pollen grains is the liquid nitrogen (LN) storage in flasks. Though the results of this study did not suggest that this approach effectively preserved pollen grains, several aspects of the protocol still needs to be investigated and fine-tuned. These include pollen drying methods for LN storage, application of cryostats for LN storage, and retrieval procedures that will result in improved germination capacity of the pollen of various yam genotypes.

From this study,, the "wet-cold" preservation procedure was the most promising approach to implement the establishment of pollen banks from this study. Protocols for the implementation of this method were presented in five stages of operations including collection, sorting, packaging, storage, and retrieval. When these protocols are made available to genebank managers the objectives of pollen storage for yam breeding and improvement would be achieved.

Acknowledgement

The author acknowledges the Graduate Research Fellowship granted by the International Institute of Tropical Agriculture, IITA to conduct the research.

Table 2. Viability and fertility of fresh yam pollen grains (1994 and 1996 data).

Species	1994			1996		
	% Germ.	% Stain	PFI	% Germ.	% Stain	PFI
D. alata	64.3a	22.1b	-	53.1bc	61.9b	5.7a
D. bulbifera (exotic)	0b	0c	-	0	3.8d	0
D. bulbifera (wild)	-	-	-	71.3	-	5.1a
D. dumetorum	79.66a	83.30a	-	49.1bc	79.9a	4.9ab
D. praehensilis	100a	100a	-	87.9	-	5.9a
D. preussii	-	-	-	65.7b	-	5.3ab
D. rotundata	69.89a	73.36a	-	33.1c	63.8a	4.1bc
LSD (p=0.05)	38.528	39.236		25.429	32.507	1.811

Means in a column followed by the same letters are not significantly different at P=0.05 by the DMRT

Table 3. Percentage pollen viability (germination in vitro and aceto-carmine stainability) of yam genotypes after air-drying and storage at −20°C.

Genotype Accession	Storage period (days)					
	0		30		400	
	% Germ.	% Stain	% Germ.	% Stain	% Germ.	% Stain
D. alata 0247	34.6b	93.8a	0	65.6a	0	51.3a
D. dumetorum 2788	86.7a	62.5ab	0	66.7a	0	44.7bz
D. rotundata 3577	59.0ab	50.9b	0	50.3a	0	48.9ab
LSD (p=0.05)	35.360	30.108		12.385		3.031

Means in a column followed by the same letters are not significantly different at P=0.05 by the DMRT

Table 4. Percentage pollen germination in vitro, percentage aceto-carmine stainability and fertility index of yam genotypes in response to 24 hours of freeze-drying and 1-hour storage at vapor phase liquid nitrogen

Genotype Accession	% Germ.	% Stain	PFI
D. alata 0487	15.4a	100.0a	1.8a
D. dumetorum 2788	0b	100.0a	0b
D. praehensilis (wild)	0b	100.0a	0b
D. rotundata 0276	0b	88.8b	0b
LSD (p=0.05)	9.048	6.58	1.058

Means in a column followed by same letters are not significantly different at P=0.05 by the DMRT

Table 5. Percentage in vitro germination of yam pollen after 100 days and 2 years under hermetic "wet-cold" at various temperature regimes. Data for fresh and stored pollen were analysed separately for each temperature regime.

Genotype Accession		−80°C	−20°C	5°C	15°C
D. rotundata 1424	fresh	70a	70a	70a	70a
	100 days	74a	78a	37b	18b
	730 days	56b	--*	0c	0c
D. praehensilis (wild)	fresh	100a	100a	100a	100a
	100 days	81a	62b	47b	0c
	730 days	78a	73a	0c	0c
LSD (p=0.05)		13.865	32.407	37.718	41.454

Means in a column followed by the same letters are not significantly different at P=0.05 by the DMRT

*No data due to exhaustion of pollen samples in this store

Table 6. In vitro germination, laboratory fertility, and fruit setting capacity of fresh and wet-frozen yam pollen at –80°C. (Female parent was line 90/00/308 on IITA's breeding plot in the first year trial; female parents 93-1 and 3099 were used in the second year trial and data for each year were analysed separately).

Pollen genotype 1 year	% Germination	Pollen tube length (μm)	Fertility index (log)	% fruit setting
Fresh pollen				
D. rotundata 0199	56.0a	2.2a	2.1a	80.0a
Frozen pollen (−80 °C)				
D. rotundata 3577	75.8a	3.3a	2.4a	69.5a
LSD (p=0.05)	23.265	1.293	0.352	12.338
2 years				
Fresh pollen				
D. rotundata 3577	73.0a	2.75a	2.3a	68.0a
D. rotundata 3303	60.0a	2.75a	2.2a	85.0a
D. rotundata 3605	68.0a	2.15a	2.2a	50.0b
Frozen pollen (−80 °C)				
D. rotundata 3577	40.0b	1.50b	1.8b	50.0b
LSD (p=0.05)	17.061	0.703	0.261	19.740

Means in a column followed by the same letters are not significantly different at P=0.05 by the DMRT

Table 7. Pollen storage protocols for implementation in genebanks.

Operation	Protocol 1 Active collection	Protocol 2 Base collection
Collection	i) Cut freshly opened flowers between 9.00 and 11.00 am	i) Cut freshly opened flowers between 9.00 and 11.00 am
Sorting and Packaging	i) Conduct laboratory pollen viability assessments (in-vitro) germination and aceto-carmine staining tests) on excised anthers from randomly sampled flowers ii) Discard flowers and anthers with poor pollen viability iii) Excise anthers from flower buds iv) Place in glass vials v) Seal with parafilm and label	i) Package fresh flowers in polythene pouches ii) Heat seal pouches and label
Storage	Place sealed glass vials under 5 °C cold store or refrigerator	Place packed polythene pouches in ultra-low freezers (-80°C or -20°C)
Retrieval	Draw from store and pollinate directly on dry ice throughout transit.	i) Draw pouches from store ii) Thaw on dry ice for at least 2 hours in lab when pollination* plot is nearby. For distant pollination, place pouches iii) Thaw at field condition for at least 20 min before pollination iv) Excise anthers from flower buds to pollinate thereafter.

*Pollinate only open flower buds in the morning hours

References

Abraham K. and Nair P.G. 1990. Floral biology and artificial pollination of *D. alata*. Euphytica 48: 45–51.

Akoroda M.O. 1981. Studies on the genetics and floral biology of yams (*D. rotundata* and *D. cayenensis*). PhD thesis, Department of Agronomy, University of Ibadan, Ibadan, Nigeria.

Akoroda M.O. 1983. Long-term storage of yam pollen. Scientia Horticulturae 20: 225–230.

Asiedu R., Bai K.V., Terauchi R., Dixon A.G.O. and Hahn S.K. 1992. Status of wide crosses in cassava and yam. Pages 63-68. *In*: Biotechnology: Enhancing Research on Tropical Crops in Africa, edited by G. Thottappilly, L.M. Monti, D.R. Mohan Raj, A.W. Moore,, CTA/IITA co-publication, IITA, Ibadan, Nigeria.

Bai K.V. and Jos J.S. 1986. Female fertility and seed set in *Dioscorea alata* L. Tropical Agriculture (Trinidad*)* 63:7–10.

Bajaj Y.P.S. 1987. Cryopreservation of pollen and pollen embryos, and the establishment of pollen banks. Page 397–420. *In*: International Review of Cytology Vol. 107, Pollen: Cytology and Development, edited by K.L. Giles and J. Prakash. Academic Press, Orlando, Florida. USA.

Brewbaker J.L. and Kwack B.H. 1963. The essential role of calcium ion in pollen germination and tube growth. American Journal of Botany 50: 859–865.

Connor K.F. and Towill L.E. 1993. Pollen handling protocol and hydration/dehydration characteristics of pollen for application to long-term storage. Euphytica 68: 77–84.

Daniel I.O. 1997. Conservation of West African yam (*Dioscorea* spp.) germplasm: Physiology of seed and pollen storage, PhD. thesis, University of Ibadan, Nigeria. 148 p.

Daniel I.O., Ng N.Q., Tayo T.O. and Togun A.O. 2002. Wet-cold preservation of West African yam (*Dioscorea* spp.) pollen. Journal of Agricultural Sciences, Cambridge 138: 57–62.

Doku E.V. 1978. Sterility in female white yams, *D. rotundata*. Pages 61-67. *In* Proceedings of 1st International Seminar on Yams.

Gomez K.A. and Gomez A.A. 1984. Statistical procedures for agricultural research. Wiley-Interscience publication, John Wiley and Sons, Inc. New York, Chichester, Brisbane, Toronto and Singapore.

Hanna W.W. 1994. Pollen storage in frostless and conventional frost-forming freezers. Crop Science 34: 1681–1682.

Hanson J. 1986. Methods of storing tropical roots crops germplasm with special reference to yam. IBPGR Newsletter 64: 24–32.

Harrington J.F. 1970. Seed and pollen storage for conservation of plant genes. Pages 501-521. *In*: Genetic Resources in Plants – their Exploration and Conservation, (edited by O.H. Frankel and E. Bennett). Blackwell, Oxford, UK.

Hong T.D., Ellis R.H., Buitink J., Walters C., Hoekstra F.A. and Cranes J. 1999. A model of the effect of temperature and moisture on pollen longevity in air-dry storage environment. Annals of Botany. 83: 167–173.

Hughes H.G. and Lee C.W. 1991. Low temperature preservation of *Clianthus formosus* pollen. Scientia Horticulturae 26: 1411–1412.

Kozlowski T.T. and Pallardy S.G. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Botanical Review 68(2): 270–334.

Ng N.Q. and Daniel I.O. 2000. Storage of pollens for long-term conservation of yam genetic resources. Pages 136-139 *In*: Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application.edited by F. Englemann and H. Tagaki). JIRCAS/IPGRI co-publication, JIRCAS, Tsukuba, Japan.

Ngu M.A. 1991. In vitro Culture of Yam Embryo. M.Sc dissertation, Department of Agricultural Biology, University of Ibadan, Nigeria.

Okoli O.O. 1991. Yam germplasm diversity, uses and prospects for crop improvement in Africa. Page 109–117. edited by Q. Ng, F. Perrino, Attere, H. Zedan, *In*: Crop genetic resources in Africa, Vol. 2 IITA, Ibadan, Nigeria.

Rajasekharan P.E., Rao T.M., Janakiram T. and Ganeshan S. 1994. Freeze preservation of gladiolus pollen. Euphytica 80: 105–109.

A Research Article in AJRTC (2010) Vol. 8 No. 1: Pages 24–30

Effects of ten plant extracts on mycelial growth and conidial production of four fungi associated with yam tuber rot

T.E. Sangoyomi[1], R. Asiedu[2] and E.J.A. Ekpo[3]

[1]Department of Crop Production, Soil and Environmental Management, Bowen University, Iwo, Osun State, Nigeria
[2]International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
[3]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria

Corresponding Author: T.E. Sangoyomi
Email: t.sangoyomi@yahoo.com

Manuscript recieved:27/09/2007; accepted: 30/06/2008

Abstract

The effects of extracts from ten plant species on the mycelial growth and conidial production of four major fungi associated with yam tuber rot were investigated. Extracts of *Allium sativum, Ocimum gratissimum, Cassia alata, Azadiracta indica,* and *Hibiscus rosa-sinensis* were found to be effective in reducing mycelial growth as well as conidial production at varying degrees *in vitro.* Their incorporation into plant protection programs will be a good complement in integrated crop disease management.

Key words: Plant extracts, mycelial growth, fungi and yam rot

Introduction

Yam (*Dioscorea* spp.) constitute a major food crop of West and Central Africa, with West Africa as the most important yam-producing region of the world (Coursey 1967). The West African yam belt comprises Nigeria, the Republic of Bénin, Togo, Ghana, Cameroon, and Côte d'lvoire. This, sub-region produces about 90% of the world's yam, estimated at 43 million t and Nigeria alone produced 31.1 million in 2007 (FAO 2007).

There are about 600 species of *Dioscorea*, however *D. rotundata* is the most important yam species in West Africa. It is the most widely cultivated in the yam belt of Nigeria because of its economic value and uses (Degras 1993). The tuber is the only economically important part of the crop and it is consumed roasted, boiled, pounded, or as dough prepared in hot water from flour. Cultivated yams are a source of carbohydrate, protein, amino acids, and minerals (Degras 1993). In addition to the nutritional value, yam has considerable social and cultural significance, especially among the people of south-east Nigeria (Nweke and Winch 1980; Okorji and Obiechina 1985).

Pests and diseases affect the crop both in the field and in storage. A conservative estimate indicates that about 15 % of yam produced annually never reach the market, because of post harvest losses. Coursey (1967) reported that over one million t of yam tubers are lost annually in storage in West Africa. Okoh 1997 also reported an economic loss of 10.45% of the expected revenue during storage. The Food and Agriculture Organization reported that 7.9 of the 26.4 t of yams produced in Nigeria in 1999 were declared as wasted (FAO 2000). The invasion of yam tubers by microbial pathogens, especially fungi, is considered the critical factor in yam decay (Degras 1993). Early work on fungi associated with the post harvest rot of yam in Nigeria include those of Okafor (1966), Adeniji (1970), Ogundana et al (1970), Noon (1978) and Ikotun (1983). The colour, magnitude, and texture of the symptoms vary with the organisms responsible for the decay (Efiuvwevwere and Nwachukwu 1998). Three fungal genera, *Botryodiplodia, Fusarium,* and *Penicillium,* have been reported to cause extensive rotting of tubers. These genera are highly pathogenic and are extremely widespread but the level of damage varies with the region, yam variety, and season (Degras 1993). *Botryodiplodia theobromae* causes dark brown rot which may be pink at the beginning on *D. trifida* L.*,* or dirty grey on *D. rotundata* (Ricci and Arnolin 1973; Ogundana et al 1970). *Fusarium oxysporum* causes pale-pinkish dry rot which may be darker around the edges (Degras 1993; Efiuvwevwere and Nwachukwu 1998). The *Fusarium* rots are often considered to be secondary to nematode attacks

because they are superficial (Bridge 1972). *Penicillium* species cause a hard but dry brown rot which turns wet and soft when invaded by bacteria (Adeniji 1970). *Penicillium oxalicum* is one of the most widely studied of the *Penicillium* species. Lesions on infected tubers are usually covered with green sporulation (Degras 1993). Ikotun (1983) reported *Aspergillus niger* to be among the fungi responsible for severe decay of yam tubers in Nigeria. These fungal pathogens may cause infections either singly or in combination with several others. Other fungi that have been reported to cause storage rot of yam include *Rhizopus nodosus* (Ikotun, 1983), *Sclerotium rolfsii* (Ejechi and Ilondu 1998), and *Rhizoctonia solani* (Sangoyomi 1995).

Preservation of yam using chemicals (Ogundana 1972) and gamma irradiation (Adesuyi 1978) are either too sophisticated or too costly for peasant farmers who are responsible for nearly all yam produced in Nigeria. The use of plant products and extracts in disease control has been reported for some crops such as maize and cowpea, (Awuah 1989, 1994; Ekpo 1991, 1999; Owolade et al 1999) but has been sparsely used in the control of yam diseases.

Materials and Methods

Effects of 10 plant extracts on mycelial growth inhibition. The experiment was conducted in the Yam Pathology Laboratory of IITA using a completely randomized design with four replicates. Fully expanded leaves of *Acalypha wilkersiana, Azadirachta indica, Cassia alata, Chromolaena odorata, Cymbopogon citratus, Ocimum gratissimum,* flowers of *Hibiscus rosa-sinensis,* bulbs of *Allium sativum*, stems of *Enantia chlorantha* and rhizomes of *Zingiber officinale* were used in the preparation of extracts. Five water extract concentrations were prepared by blending 1 g, 5 g, 10 g, 50 g, and 100 g of the plant part in sterile distilled water that was made up to 100 ml to produce 1%, 5 %, 10 %, 50 %, and 100 % extract concentrations. The extracts were sieved through four layers of cheese-cloth and their effects were studied on the growth of four fungal pathogens (*Botryodiplodia theobromae, Sclerotium rolfsii, Fusarium oxysporum* and *Penicillium oxalicum*) using the food poisoning technique (Nene and Thapliyal 1979). Extracts were used soon after preparation. One millilitre of each extract was dispensed per Petri dish and 9 ml of molten potato dextrose agar (PDA) was added to prepare a PDA-extract mixture giving corresponding 0.1%, 0.5%, 1%, 5% and 10% extract concentrations. These concentrations were used to test their effects on mycelial growth and the production of the conidia, pycnidia, and sclerotia of appropriate fungi.

The plates were gently rotated to ensure even dispersion of the extracts. The agar-extract mixture was allowed to solidify and then inoculated at the centre with a 4 mm-diameter mycelial disc obtained from the colony edge of a 7-day old culture of each of the test fungi. Four plates per replicate and three replicates per fungus per extract were inoculated. The control set-up consisted of blank agar plates (no extracts) inoculated with the test fungi as described above. All plates were incubated at 27 ºC and radial growth was measured daily for four days. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. Percentage inhibition of mycelial growth was calculated according to the method described by Whipps 1987:

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_1} x \frac{100}{1}$$

where R_1 is the furthest radial distance of pathogen in control plates
and R_2 is the furthest radial distance of pathogen in extract-incorporated agar plates

The inhibition percentage was determined as a guide in selecting the minimum inhibitory concentrations that will be effective in controlling rot-causing fungi. Extracts were rated for their inhibitory effects using the scale:

≤ 0% inhibition (not effective);
>0-20% inhibition (slightly effective);
>20-50% inhibition (moderately effective);
>50-<100% inhibition (effective);
100% inhibition (highly effective).

Effects of 10 extracts on the production of conidia, pycnidia, and sclerotia. Plant extracts at a concentration of 1% were prepared and used as described above. Inoculated plates were incubated at 27 °C for 10 days after which the production of conidia, pycnidia, and sclerotia was investigated using completely randomized design with four replicates. For conidial production, extract-impregnated plates were inoculated with 4-day old mycelial discs of test fungi. The control set-up consisted of blank agar plates with no extracts incorporated but inoculated with the mycelial discs of the test fungi. Conidial suspensions were obtained from the 10-day old cultures of *Fusarium oxysporum* and *Penicillium oxalicum* using the method of Fajola and Nwufo 1985. Ten ml of sterile distilled water containing 0.01%

Tween 80 was added to the surface of each culture and the surface was gently rubbed using a fine brush to dislodge conidia into suspension and then filtered through four layers of cheese cloth. Each replicate plate was washed into a conical flask and the number of conidia was counted using a hemacytometer (Fisher scientific CIAT 0267110). The pycnidia produced by *Botryodiplodia theobromae* and sclerotia produced by *Sclerotium rolfsii* were directly counted on plates using a binocular stereo miscroscope. Two perpendicular lines were drawn on the reverse sides of the plates and then pycnidia and sclerotia were counted in each of the four sectors in four replicate plates of *Botryodiplodia theobromae* and *Sclerotium rolfsii*. Data obtained were subjected to the general linear model (GLM) of Statistical Analysis System (SAS 1999). Means separation was carried out using standard error of difference.

Results

Effects of ten plant extracts on mycelial growth of *Botryodiplodia theobromae.* The effects of plant extracts on the mycelial growth inhibition of *Botryodiplodia theobromae* are shown in Table 1. At 0.1% extract concentration, extracts of *A. wilkersiana* , *C. odorata*, *E. chlorantha*, *H. rosa-sinensis* and *O. gratissimum* had no inhibitory effect on mycelial growth. At 0.5% extract concentration, mycelial growth inhibitions ranged from 0.8 to 88.5% in *O. gratissimum* and *A. sativum*. At 1% extract concentration, extract of *A. sativum* caused 100% inhibition of mycelial growth. Extract of *E. chlorantha* and *H. rosa-sinensis* had no inhibitory effects on mycelial growth of *B. theobromae* while extracts of *A. indica, C. alata,* and *C. citratus* caused 51-71% inhibition of mycelial growth. The inhibitory effects of plant extracts at 5% concentration were similar to those at 1% concentration and were only slightly lower than those of the corresponding 10% concentration.

Effects of ten plant extracts on mycelial growth of *Penicillium oxalicum.* The effects of plant extracts on mycelial growth of *Penicillium oxalicum* are shown in Table 2. There was a general increase in mycelial growth inhibition of *P. oxalicum* with an increase in extract concentration. At 0.1% extract concentration, inhibition ranged from 0.7 to 31.0%. Extracts of *A. wilkersiana*, *C. citratus,* and *E. chlorantha* had no inhibitory effect on mycelial growth while others were either slightly effective (<20% inhibition) or moderately effective (21–50% inhibition). At 0.5% extract concentration, all extracts inhibited mycelial growth to varying degrees (2.4–75.1%). Extracts of *C. odorata*, *A. indica,* and *O. gratissimum* were moderately effective (21–50% inhibition) and *A. sativum* had 75.1% inhibition. At 1% extract concentration, *A. sativum* extract caused 100% inhibition of mycelial growth. In general, inhibition percentages (14.7–57.5%) at 1% extract concentration in the remaining nine extracts were only slightly lower than those of the corresponding 5 and 10% extract concentrations.

Effects of ten plant extracts on mycelial growth of *Fusarium oxysporum.* All plant extracts, except that from *E. chlorantha* at 0.1 and 0.5% concentrations, caused mycelial growth inhibition of *F. oxysporum* (Table 3). There was a general increase in percentage inhibition with an increase in extract concentration. At 0.1% extract concentration, inhibitions ranged from 1.4 to 38.8% and extracts of *A. sativum* (38.8%) and *Z. officinale* (23.7%) were moderately effective. At 1% extract concentration, five of the tested extracts (*A. indica, A. sativum, C. citratus, H. rosa-sinensis* and *Z. officinale* were effective (>50 <100% inhibition) in mycelial growth inhibition. The least inhibition was observed using the extract from *A. wilkersiana* (7.8%). At 5% extract concentration, extract of *A. sativum* caused 100% inhibition of mycelial growth and the inhibition percentages (8.6–89.9%) at 10% extract concentration in the remaining nine extracts were slightly higher than those of the corresponding 1% concentration.

Effects of ten plant extracts on mycelial growth of *Sclerotium rolfsii.* The effects of plant extracts on mycelial growth of *Sclerotium rolfsii* are shown in Table 4. At 0.1, 0.5, and 1% extract concentrations, extracts of *A. wilkersiana*, *C. odorata* and *H. rosa-sinensis* had no inhibitory effect on the mycelial growth of *Sclerotium rolfsii*. However, the extract of *E. chlorantha* had a stimulatory effect on the mycelial growth of the pathogen at 0.1% concentration. At 0.5% extract concentration, mycelial growth inhibition ranged from 1.8 to 38.4% and *A. sativum* extract caused 100% inhibition. At 1% concentration, extracts of *A. indica, C. alata,* and *C. citratus* were effective (>50<100% inhibition) in reducing the mycelial growth of *S. rolfsii* while others were slightly effective (<20% inhibition). Radial growth inhibition percentages at 5% extract concentration ranged from 0.8 to 87.0%. At 10% extract concentration, inhibition percentages were slightly higher than those of the corresponding 5% extract concentration.

Effects of ten percent concentration of plant extracts on production of pycnidia, sclerotia, and conidia. There were significant differences in the number of pycnidia produced on different PDA/ extract combinations (Table 5). Crude extracts of *A. sativum, C. citratus, and H. rosa-sinensis* completely inhibited pycnidium formation while significantly ($p \leq 0.05$) fewer pycnidia (2 or 3) were recorded on plates containing extracts of *C. alata* and *Z. officinale* compared to a count of 16 pycnidia in the control. Significantly ($p \leq 0.05$) more sclerotia (31) were recorded on medium containing *E. chlorantha* extract than on the control. The remaining extracts had no significant effects on the production of pycnidia.

Crude extracts of *A. sativum* and *Z. officinale* completely inhibited formation of sclerotia. Fewer ($p \leq 0.05$) sclerotia (7–160 per plate) were produced on PDA containing the other extracts than on the control (225 per plate). There were significant differences in the number of *P. oxalicum* conidia produced in different PDA/extract combinations. *Allium sativum* extract completely inhibited the production of conidia. There were no significant differences in the number of conidia of *S. rostrata* produced in various PDA/extract combinations. However, significantly ($p \leq 0.05$) higher conidia counts were recorded on media containing *C. odorata* extract than on the control.

Discussion

Crude extracts from ten plants were used in this study to develop cheap and simpler methods of yam rot control for use by farmers. The investigation showed that the most fungitoxic extracts obtained were from *Allium sativum, Ocimum gratissimum, Cassia alata, Azadiracta indica,* and *Hibiscus rosa-sinensis*. These extracts were able to inhibit growth of mycelia and reduce production of conidia in the four major fungi associated with yam rot during storage. This agrees with the reports of Okigbo and Nmeka (2005); and Okigbo and Ogbonnaya (2006). This study identifies the potential of these plant extracts and therefore recommends their use as natural fungicides. *Allium sativum* extracts, even when used at low concentrations, were found to be effective against the test fungi in this study. Allicin (the main biologically-active compound of garlic) has been reported to be soluble in water as well as a natural antimicrobial botanical that can disable an unusually wide variety of infectious organisms (Shashiskanth et al 1986) although it is highly unstable (Freeman and Kodera (1995). Further studies into how to stabilize the active ingredients are essential. *Ocimum gratissimum* was also effective *in vitro* in the control of rot-causing fungi in agreement with the reports of Awuah (1994), where it was reported to reduce radial growth of *Rhizopus* spp. and *Ustilaginoidea virens.* Crude steam distillate sprayed onto infection courts also inhibited the pathogen and lesion development (Awuah 1989).

The preparation of crude extracts from plants for disease control is relatively cheap and can easily be accepted by peasant farmers. From the point of view of environmental impact assessment, it is also a safer alternative to the hazardous and expensive conventional fungicides. The use of plant extracts in the control of fungal rot of yam in storage presents no potential toxicity to man. The extracts investigated in this study are from plants that are commonly used for medicinal purposes and have been widely studied.

Table1. Effects of plant extracts on the mycelial growth inhibition of *Botryodiplodia theobromae* incubated at 27 ºC for 4 days.

	Mycelial growth inhibition (%) at specified extract concentration				
Botanical	0.1%	0.5%	1%	5%	10%
Acalypha wilkersiana	0.0	0.0	9.3	9.5	9.8
Allium sativum	28.3	88.5	100.0	100.0	100.0
Azadirachta indica	8.0	26.5	70.6	76.4	80.2
Cassia alata	2.4	8.3	53.2	59.1	66.0
Chromolaena odorata	0.0	0.0	1.9	1.9	2.1
Cymbopogon citratus	10.0	19.9	60.2	65.6	65.9
Enantia chlorantha	0.0	0.0	0.0	0.0	0.0
Hibiscus rosa-sinensis	0.0	0.0	0.0	0.5	0.7
Ocimum gratissimum	0.0	0.8	2.7	2.9	3.6
Zingiber officinale	0.4	8.2	3.9	39.6	39.7
Mean	4.9	15.2	30.2	35.6	36.8
S E D	1.4	2.4	2.8	2.8	2.8

Table 2. Effects of plant extracts on the mycelial growth inhibition of *Penicillium oxalicum* incubated at 27 ºC for 4 days

	Mycelial growth inhibition (%) at specified extract concentration				
Botanical	0.1%	0.5%	1%	5%	10%
Acalypha wilkersiana	0.0	18.4	34.8	35.0	35.2
Allium sativum	20.0	75.1	100.0	100.0	100.0
Azadirachta indica	2.0	24.4	57.5	59.2	61.1
Cassia alata	4.8	10.6	49.2	49.8	52.0
Chromolaena odorata	0.0	20.3	38.0	41.0	42.0
Cymbopogon citratus	0.0	20.0	46.9	48.0	48.0
Enantia chlorantha	0.0	2.4	31.4	35.8	38.1
Hibiscus rosasinensis	2.3	12.3	14.7	15.0	16.1
Ocimum gratissimum	24.8	31.0	50.8	61.3	71.5
Zingiber officinale	0.7	6.0	40.5	42.8	46.6
Mean	5.5	22.1	46.3	48.8	51.1
S E D	1.5	2.0	21.0	2.1	2.2

Table 3. Effects of plant extracts on the mycelial growth inhibition of *Fusarium oxysporum* incubated at 27 ºC for 4 days.

	Mycelial growth inhibition (%) at specified extract concentration				
Botanical	0.1%	0.5%	1%	5%	10%
Acalypha wilkersiana	1.4	6.4	7.8	8.2	8.6
Allium sativum	38.8	50.1	63.8	100.0	100.0
Azadirachta indica	21.4	32.0	62.8	71.0	76.5
Cassia alata	8.0	20.1	43.0	60.4	64.4
Chromolaena odorata	3.9	25.0	30.5	31.2	36.4
Cymbopogon citratus	15.1	25.0	61.5	64.5	68.0
Enantia chlorantha	-2.3*	-2.2	9.4	12.0	12.9
Hibiscus rosa-sinensis	13.0	33.1	81.0	86.8	89.9
Ocimum gratissimum	4.3	14.2	43.0	46.0	49.1
Zingiber officinale	23.7	50.2	62.0	66.0	71.0
Mean	12.7	25.4	46.5	48.1	57.7
S E D	1.6	1.9	2.2	2.5	2.5

* Negative sign indicates stimulatory effects of extracts

Table 4. Effects of ten plant extracts on the mycelial growth inhibition of *Sclerotium rolfsii* incubated at 27 ºC for 4 days.

	Extract concentrations				
Botanical	0.1 %	0.5 %	1 %	5 %	10 %
Acalypha wilkersiana	0.0	0.0	0.0	5.0	7.0
Allium sativum	9.6	100.0	100.0	100.0	100.0
Azadirachta indica	32.1	38.4	71.6	78.5	82.6
Cassia alata	9.6	23.8	86.4	87.0	87.9
Chromolaena odorata	0.0	1.8	7.3	8.7	12.2
Cymbopogon citratus	9.9	23.2	59.0	61.0	63.5
Enantia chlorantha	-1.2*	0.0	0.0	0.8	1.0
Hibiscus	0.0	0.0	0.0	9.5	9.6
Ocimum gratissimum	4.7	6.3	9.0	9.6	9.9
Zingiber officinale	2.8	11.9	16.0	22.7	29.1
Mean	5.7	17.5	34.9	38.3	40.3
S E D	1.4	2.5	2.8	2.7	2.7

* Negative sign indicates stimulatory effects of extracts

Table 5. Effects of 10% concentration of plant extracts on the production of pycnidia by *Botryodiplodia sclerotia* by *Sclerotium rolfsii* and conidia by *Penicillium oxalicum* after 10 days of incubation at 27 ºC

Extracts	Number per plate		Sporulation intensity		
	B. theobromae pycnidia	*S. rolfsii* sclerotia	*P. oxalicum* ($x10^5$)	*F. oxysporum* Micro ($x10^5$)	*F. oxysporum* Macro ($x10^5$)
Acalypha wilkersiana	21.5	159.80	18.2	8.0	5.1
Allium sativum	0.00	0.00	0.0	4.0	1.1
Azadirachta indica	19.00	10.80	6.0	1.6	0.1
Cassia alata	2.50	7.30	1.7	7.0	0.5
Chromolaena odorata	11.80	122.50	15.8	3.4	0.1
Cymbopogon citratus	0.00	0.30	5.2	1.1	0.1
Enantia chlorantha	31.30	85.50	13.9	37.3	2.9
Hibiscus rosa-sinensis	0.00	149.00	5.1	28.1	2.4
Ocimum gratissimum	12.30	6.80	11.3	0.0	0.0
Zingiber officinale	2.30	0.00	0.5	1.5	0.4
Control	15.80	224.50	20.0	10.8	0.6
Mean	12.50	69.70	8.9	9.3	1.0
S.E.D.	4.37	18.38	0.8	0.2	0.3

References

Adeniji M.O. 1970. Fungi associated with storage decay of yam in Nigeria. Phytopathology 60:590–592.

Adesuyi S.A. 1978. The use of gamma radiation for control of sprouting in yams (*Dioscorea rotundata*) during storage. Nigeria Journal of Plant Protection 2: 34–39.

Awuah R.T. 1989. Fungitoxic effects of extracts from some West Africa plants. Anuals of Applied Biology 115: 451–453.

Awuah R.T. 1994. *In vitro* use of extracts from *Ocimum gratissimum* and *Cymbopogon citratus* against *Phythophthora palmivora* causing blackpod disease of cocoa. Annals of Applied Biology.124:173–178.

Bridge J. 1972. Nematode problems with yams (*Discorea* spp.) in Nigeria. PANS 18: 89–91.
Degras L. 1993. The yam (A Tropical Root Crop). Macmillan Press, London. 408 pp

Coursey D.G. 1967. Yams. Longman Green London: 230 pp.

Efiuvwevwere B.J.O. and Nwachukwu E. 1998. Incidence of yam (*Dioscorea rotundata* Poir.) rots, inoculation-induced quality changes, and control by chemical fungicides and modified atmosphere. Postharvest Biology and Technology 14:235–243.

Ejechi B.O. and Ilondu M.E. 1998. Control of yam tuber (*Dioscorea rotundata*) rot agent *Sclerotium rolfsii* with camwood (*Baphia nitida* Lodd) sawdust extract. African Journal of Root and Tuber Crops 3: 13–15

Ekpo E.J.A. 1991. Antifungal activity of leaf powder and extract of *Vernonia amygdalina* Del. on maize seed isolates of *Curvularia lunata* Wakker and *Fusarium semitectum* Berk and Rau. Zimbabwean Journal of Agricultural Research 29: 129–132.

Ekpo E.J.A. 1999. Control of seed-borne fungi of cowpea (*Vigna unguiculata* (L.) Walp.) with neem (*Azadirachta indica* A. Juss.) extracts. Nigerian Journal of Science 33: 39–47.

Fajola A.O and Nwufo M. 1985. Control of corm rot of Cocoyam (*Colocasia esculenta*) caused by *Botryodiplodia theobromae* and *Sclerotium rolfsii*. Fitopathologia Brasileira. 10:43–50.

FAO 2007. Food and Agriculture Organisation Food Balance Sheet. http:/apps.fao.org/lim500/wrap.pl?FoodBalanceSheet&Domain=FoodBalanceSheet&] Accessed 19 August 2007.

Freeman F. and Kodera Y. 1995. Garlic chemistry: stability or S- (2-Propenyl) -2-propene -1-Sulfinnothionate (allicin) in blood, solvents and stimulated physiological fluids. Journal of Agriculture and Food chemistry. 43:2332–2338

Ikotun T. 1983. Post-harvest microbial rot of yams in Nigeria. Fitopathologia Brasileira. 8: 1–7.

Nene Y.C and Thapliyal P.N. 1979. Fungicides in plant disease control. New Delhi, Bombay, Calcutta, Oxford and IBH Publishing Company. Pp 406–425

Noon R.A. and Colhoun J. 1979. Market and storage diseases of yams imported the United Kingdom. Phytopath. Zeitscrift. 94: 94:289–302.

Ogundana S.K., Naqvi S.H. and Ekundayo J.A. 1970. Fungi associated with soft rot of yams (*Dioscorea* spp*)* in Nigeria. Transactions of the British Mycological Society 54: 445–451.

Ogundana S.K. 1972. The control of soft rot of yams in Nigeria. International. Biodeterioration Bulletin 3: 75–78.

Okafor N. 1966. Microbial rotting of stored yam (*Dioscorea* spp) in Nigeria. Experimental Agriculture. 2: 179–182.

Okigbo R.N and Nmeka I.A. 2005. Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale.* African Journal of Biotechnology. 4 (8): 804–807.

Okigbo R.N. and Ogbonnaya U.O. 2006. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframmomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot. African Journal of Biotechnology. 5 (9): 727–731.

Okoh R.N. 1997. Economic evaluation of losses in yams stored in traditional barns. Tropical. Science 38 :125–127.

Okorji E.C. and Obiechina C.O.B. 1985. Bases for farm resource allocation in the traditional farming systems: A comparative study of productivity of farm resources in Abakaliki area of Anambra State. Nigeria. Agricultural Systems 17: 197–210.

Owolade O.F., Osikanlu Y.O.K. and Amusa N.A. 1999. Efficacy of certain indigenous plant extracts against seed-borne infection of *Fusarium moniliforme* on maize (*Zea mays* L.) in South western Nigeria. Crop Research 19 : 462–465

Ricci P. and Arnolin R. 1973. Fungicidal treatment of cush yam tubers useful against storage decay. Proc. 3rd Inter. Symp. Trop. Root Crops, IITA. Ibadan, Nigeria.

Sangoyomi T.E. 1995. Studies to evaluate the importance of *Rhizoctonia solani* as a pathogen of yam (*Dioscorea* spp.) in Nigeria. MSc. Dissertation, University of Ibadan. 69 pp.

SAS Institute 1999. SAS/STAT User's Guide, release 8.0. SAS Institute, Inc., Cary, NC, USA.

Shashiskanth K.N., Basapp S.C. and Muurthy V.S. 1986. Effect of feeding raw and boiled garlic (*Allium sativvm)* on the growth, ceacal microfloral and serum proteins of Albino rats. Nutrition Reports Int. 33: 313–319.

Whipps J.M. 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. New Pathologist 107: 127–142.