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Effects of Gamma Irradiation on Post-Harvest Deterioration of Cassava (*Manihot esculenta* Crantz) Tuber by Fungi

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ABSTRACT

Three varieties of cassava (*Manihot esculenta* Crantz) (SMS 909-25, COL 2215 and COL 1734) that were gamma (γ) irradiated and non-γ irradiated were investigated for susceptibility to post-harvest fungal rot by five species of moulds (*Botryodiplodia theobromae*, *Penicillium oxalicum*, *Aspergillus niger*, *Fusarium solani* and *Macrophomina phaseolina*). These moulds were inoculated into tuber samples at wound depths of 10 mm with either single or paired cultures. The inoculated samples were stored in two environments (ambient temperature (26 ± 2°C) and polyethylene bags of 18 µm thickness). The most extensive rot development at ambient temperature was 13.50 ± 0.10 mm by *M. phaseolina* for single cultures while pair-cultures of *P. oxalicum* and *M. phaseolina* produced 19.50 ± 0.05 mm in irradiated cassava COL 2215 but 15.50 ± 0.71 mm by *M. phaseolina* for a single culture and 21.50 ± 0.71 mm for pair-culture of *P. oxalicum* and *M. phaseolina* in non-irradiated cassava. The minimum rot of 6.50 ± 0.05 mm by *P. oxalicum* and 13.00 ± 0.00 mm by *B. theobromae* and *A. niger* occurred in irradiated cassava COL 1734 but *P. oxalicum* produced 8.50 ± 0.71 mm while *P. oxalicum* and *F. solani* produced 13.75 ± 0.72 mm in non-irradiated cassava. Generally, significant (P>0.05) rot was recorded for non-irradiated than irradiated cassava varieties analyzed. In polyethylene bags storage, generally the highest zone of rot of 16.50 ± 0.05 mm by *M. phaseolina* and 21.63 ± 0.03 mm by *P. oxalicum* and *M. phaseolina* were observed in COL 2215. *M. phaseolina* showed the highest zone of rot development in γ and non-γ irradiated cassava varieties irrespective of the storage condition.

Keywords: *Aspergillus*, *Botryodiplodia*, *Fusarium*, *Macrophomina*, *Penicillium*, rot, storage

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial vegetatively propagated shrub and a nutritionally important root crop grown in tropical climates. It is the source of staple starch food for more than 600 million people, mostly in the third world countries. The total annual cassava production worldwide is over 184 million tons, out of which 50% production is in Africa, 30% in Asia and 20% in Latin American (FAO 2007). Cassava is also increasingly being used in processed food and fodder products and by the chemical, pharmaceutical, paper and textile industries (Balagopalan 1998).

The crop is faced with major constraints responsible for poor yield and that threaten food security. Among the most important are viral and bacterial diseases, weeds, and a low protein and high cyanogenic glucoside content in its storage roots and poor storability after harvest (Keresztessy *et al.* 2001; Iglesias *et al.* 2002). Rapid post-harvest deterioration normally begins 2 or 3 days after harvest followed by microbial deterioration 5-7 days later; this renders the cassava roots completely unacceptable for human consumption. This reduces their acceptability as animal feed, and lowers the quality of starch obtained from them (Diego *et al.* 2002).

The isolation of many different types of fungi from deteriorated (rotted) cassava has been reported. Some of these have proven to be pathogenic when inoculated into healthy roots, the most notable being *Botryodiplodia theobromae*, *Aspergillus flavus*, *Fusarium solani*, and *Trichoderma harzianum* (Fajola and Nwufo 1985; Efiuvwevwere and Nwachukwu 1998; Msikita *et al*. 1998). The rate of the deterioration of cassava and the type of fungi involved in the deterioration process are influenced by both wet and dry environments. Different storage techniques as preventative measures for deterioration of fresh cassava roots have been reported (Rickard and Coursey 1981).

Reducing the risks encountered during post harvest handling is almost as important a constraint as increasing output and productivity. Incentives to induce farmers to grow more cassava as an industrial raw material invariably require the genetic improvement of cassava's shelf life (Diego *et al.* 2002). Conventional genetic improvement programmes are met with reproductive and breeding barriers, such as low fertility, low hybrid seed set and poor germination rate (Nassar 2001). Such difficulties in incorporating useful and desirable traits in one genotype limit the possibility of obtaining new promising cultivars. Hence alternative ways to increase genetic variability are desirable.

Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al.* 2002). The induction and selection of mutants provides a simple, efficient, rapid and cheap method by which to alter the genetic make-up and obtain desired genotypes from otherwise well-adapted genotypes. The combined use of induced mutation techniques with *in vitro* culture methods has great potential in breeding programs. Successful long-term highly regenerable embryogenic cultures (Woodward and Puonti-Kaerlas 2001) and cyclic secondary somatic embryogenic systems (Joseph *et al.* 1999; Danso and Ford-LIoyd 2002) have been established for experimental materials for *in vitro* induction of mutations in cassava through gamma irradiation and cassava tubers from the irradiated plants were used for the inoculation.

The objective of this study was to identify the major fungal pathogens responsible for cassava rots in storage, to determine the effect of the storage methods on cassava post-harvest deterioration, and to determine the effect of gamma (γ) irradiation on cassava post-harvest deterioration by fungi and as well the pathogenic potentials of the different combinations of fungi on cassava during storage.

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MATERIALS AND METHODS

Source of materials

Three cassava varieties (SMS 909-25, COL 2215, COL 1734) irradiated with 15 Gy of γ radiation by the IAEA (International Atomic Energy Agency, Australia) were collected from the National Root Crops Research Institute (NRCRI), Umudike, Nigeria. These varieties were those available in the Research Institute and were developed by radiation treatment of 15 Gy γ dose of cassava stem cuttings. The radiated plants were maintained for 8 months.

Sterilization of cassava tubers

Whole cassava tubers of 5 to 10 cm long and 5 cm wide were surface-sterilized by dipping completely in 70% ethanol for some seconds and rinsed twice in sterile distilled water.

Source of fungal isolates

Certified fungal strains (*A. niger*, *P. oxalicum*, *F. solani*, *B. theobromae* and *M. phaseolina*) were collected from stock cultures of the International Institute of Tropical Agriculture (IITA), Ibadan. The colonial morphology and pigmentation of the isolates were recorded before sub-culturing. Each of the isolates was subcultured on potato dextrose agar (PDA) and incubated at 28°C for 3 to 5 days to ensure purity of the fungal isolates. Thereafter a portion of the fungal mycelium was teased out in a drop of lactophenol cotton blue on a grease-free microscope slide (Samson and Van Reenen-Hoekstra 1988) and examined microscopically. Cultural and morphological characteristics were observed and compared with earlier descriptions (Samson *et al*. 1984).

Inoculation of cassava samples with fungal isolates

Four similarly sized (5 to 10 cm long, and 5 cm wide), 8-months-old freshly harvested cassava roots, with peels intact, for each of the three varieties were used for the inoculation study. Surface sterilization of the tubers was done using 70% alcohol as described above. Using a 5 mm diameter cork borer, cylindrical cores (10 mm in length) were removed from the cassava samples. From the edge of 7-day old growth of the isolates, a 4 mm disc size inoculum (singly or in various combinations) were aseptically introduced into the holes made in the cassava root samples and then sealed with sterile vaseline (Weevasighe and Nagvi 1985; Nwachukwu 2006). Controls were non-γ irradiated cassava tubers inoculated with the isolates.

The experiments were carried out at two different storage environments polyethylene bags storage and ambient temperature (26 ± 2°C). Four replicate tubers of cassava were used for each test fungus and repeated twice. In storage in polyethylene bags, the inoculated cassava samples were placed individually in sterile polyethylene bags (Pentagon Plastic Ind., Nigeria) 18 µm thick. The other set of inoculated samples were kept in ambient temperature storage. Both samples were monitored and kept stored in the laboratory for four weeks. At weekly intervals the samples were examined for induction of rot. The extent of rot was determined by two measurements (i.e., vertically and horizontally) and the means recorded in mm (Nwachukwu 2006). Small portions of the lesions were aseptically transferred onto PDA to confirm that the infection was caused by the inoculants.

Statistical analysis

Data generated from evaluation of varieties and microbial deterioration was analyzed by two-way Analysis of Variance (ANOVA) and statistical significance was assessed at P=0.05. Means were separated using Least Significant Difference at P=0.05. The student's t-test was used to compare zones of rot between irradiated and non-irradiated cassava varieties used in the study.

RESULTS

Five species of moulds (*A. niger*, *F. solani*, *P. oxalicum*, *B. theobromae* and *M. phaseolina*) were used for the pathogenicity test of γ-irradiated and non-irradiated (control) cassava varieties (SMS 909-25-15GY, COL 2215-15GY and COL 1734-15GY). The pathogenicity test showed that the five fungal isolates caused decay of cassava. However, each

Table 1 Deterioration levels (mm) on irradiated cassava by fungal isolates after 4 weeks of storage at ambient temperature (26 ± 2°C).

Organism	Varieties		
	SMS 909-25	COL 2215	COL 1734
Botryodiplodia theobromae	7.50 ± 0.15 f	9.50 ± 0.05 cd	8.00 ± 0.00 ef
Penicillium oxalicum	9.50 ± 0.05 cd	13.00 ± 0.05 a	6.50 ± 0.05 g
Aspergilus niger	10.00 ± 0.00 bc	11.50 ± 0.05 ab	9.00 ± 0.10 de
Fusarium solani	10.00 ± 0.00 bc	9.50 ± 0.05 cd	8.50 ± 0.05 ef
Macrophomina phaseolina	11.50 ± 0.50 ab	13.50 ± 0.10 a	9.50 ± 0.15 cd
B + P	14.00 ± 0.00 jk	16.50 ± 0.05 ef	14.00 ± 0.00 jk
B + A	16.50 ± 0.05 ef	15.50 ± 0.05 gh	13.00 ± 0.00 m
B + F	15.00 ± 0.00 hi	16.00 ± 0.00 fg	14.00 ± 0.10 jk
B + M	17.00 ± 0.10 de	18.00 ± 0.00 bc	13.50 ± 0.05 kl
P + A	14.50 ± 0.05 ij	18.00 ± 0.10 bc	14.00 ± 0.10 jk
P + F	13.50 ± 0.05 kl	17.50 ± 0.05 cd	13.00 ± 0.00 m
P + M	15.50 ± 0.15 gh	19.50 ± 0.05 a	14.50 ± 0.20 ij
A + F	13.50 ± 0.05 kl	16.50 ± 0.05 ef	13.50 ± 0.05 kl
A + M	16.00 ± 0.00 fg	18.50 ± 0.05 b	14.00 ± 0.10 jk
F + M	15.00 ± 0.10 hi	17.50 ± 0.05 cd	13.50 ± 0.05 kl
Overall[a]			
Single	9.70 ± 0.07 b	11.40 ± 0.05 a	8.30 ± 0.07 c
Pair	15.05 ± 0.05 b	17.35 ± 0.05 a	13.65 ± 0.07 c

Each value represents the mean from two independent experiments and the overall mean ± standard error. Lesions (values) induced by each isolate or pair in the same row having different letters are significantly different at P=0.05. Pair: B+P = *B. theobromae* + *P. oxalicum*; B+A = *B. theobromae* + *A. niger*; P+A = *P. oxalicum* + *A. niger*; F+M = *F solani* + *M. phaseolina*, A+F = *A. niger* + *F. solani*, etc

[a] Each value in a column represents the overall mean of lesions/rots induced either by all the five singly or pair-inoculated organisms for 4 weeks.

Table 2 Deterioration levels (mm) on non-irradiated cassava by fungal isolates after 4 weeks of storage at ambient temperature (26 ± 2°C).

Organism	Varieties		
	SMS 909-25	COL 2215	COL 1734
Botryodiplodia theobromae	10.75 ± 1.06 d	12.75 ± 0.35 bc	10.50 ± 1.25 d
Penicillium oxalicum	11.50 ± 0.71 cd	15.50 ± 0.71 a	8.50 ± 0.71 e
Aspergilus niger	12.50 ± 0.71 bc	13.25 ± 0.35 b	10.75 ± 1.06 d
Fusarium solani	10.75 ± 0.71 d	11.50 ± 0.71 cd	10.25 ± 0.35 d
Macrophomina phaseolina	15.50 ± 0.71 a	15.50 ± 0.71 a	13.25 ± 0.35 b
B + P	15.50 ± 0.71 ij	17.50 ± 0.35 ef	14.75 ± 1.06 kl
B + A	16.75 ± 0.35 gh	17.00 ± 0.71 fg	14.50 ± 0.71 kl
B + F	16.00 ± 0.71 hi	16.50 ± 0.35 gh	14.00 ± 0.35 l
B + M	18.50 ± 0.35 de	19.75 ± 1.06 bc	15.00 ± 0.71 jk
P + A	15.50 ± 0.71 ij	19.25 ± 0.71 cd	14.50 ± 0.35 kl
P + F	14.75 ± 0.71 kl	18.50 ± 0.71 de	13.75 ± 0.71 m
P + M	17.50 ± 0.71 ef	21.50 ± 0.71 a	15.50 ± 0.35 ij
A + F	14.50 ± 0.71 kl	17.50 ± 0.71 ef	14.25 ± 0.71 l
A + M	17.00 ± 0.71 fg	20.00 ± 0.71 b	14.50 ± 0.71 kl
F + M	16.50 ± 0.35 gh	18.75 ± 0.71 de	14.00 ± 0.55 l
Overall[a]			
Single	12.20 ± 0.78 b	13.70 ± 0.57 a	10.65 ± 0.74 c
Pair	16.25 ± 0.60 b	18.63 ± 0.67 a	14.48 ± 0.55 c

Each value represents the mean from two independent experiments and the overall mean ± standard error. Lesions (values) induced by each isolate or pair in the same row having different letters are significantly different at P=0.05. Pair: B+P = *B. theobromae* + *P. oxalicum*; B+A = *B. theobromae* + *A. niger*; P+A = *P. oxalicum* + *A. niger*; F+M = *F. solani* + *M. phaseolina* , A+F = *A. niger* + *F solani*, etc.

[a] Each value in a column represents the overall mean of lesions/rots induced either by all the five singly or pair-inoculated organisms for 4 weeks.

Table 3 Influence of storage conditions on the induced lesion in irradiated cassava SMS 909-25 by fungal isolate after 4 weeks.

Inoculum	Ambient temperature (26 ± 2°C)	Polyethylene bags (18 µm)
Single		
Botryodiplodia theobromae	7.50 ± 0.05 g	11.50 ± 0.15 c
Penicillium oxalicum	9.50 ± 0.05 ef	15.00 ± 0.00 ab
Aspergillus niger	10.00 ± 0.00 de	15.00 ± 0.10 ab
Fusarium solani	10.00 ± 0.00 de	10.50 ± 0.05 cd
Macrophomina phaseolina	11.50 ± 0.15 c	15.50 ± 0.00 a
Pair		
B + P	14.00 ± 0.00 hi	16.13 ± 0.05 de
B + A	16.50 ± 0.05 cd	15.50 ± 0.04 ef
B + F	15.00 ± 0.00 fg	15.75 ± 0.05 ef
B + M	17.00 ± 0.10 bc	18.00 ± 0.03 a
P + A	14.50 ± 0.05 gh	17.50 ± 0.15 ab
P + F	13.50 ± 0.05 ij	15.88 ± 0.06 ef
P + M	15.50 ± 0.15 ef	18.25 ± 0.03 a
A + F	13.50 ± 0.05 ij	16.00 ± 0.07 de
A + M	16.00 ± 0.00 de	17.63 ± 0.10 ab
F + M	15.00 ± 0.10 fg	15.50 ± 0.08 ef

Each value represents the mean from two independent experiments and the overall mean ± standard error. Lesions (values) induced by each isolate or pair in the same row having different letters are significantly different at *P*=0.05. Pair: B+P = *B. theobromae* + *P. oxalicum*; B+A = *B. theobromae* + *A. niger*; P+A = *P. oxalicum* + *A. niger*; F+M = *F. solani* + *M. phaseolina* , A+F = *A. niger* + *F solani*, etc.

[a] Each value in a column represents the overall mean of lesions/rots induced either by all the five singly or pair-inoculated organisms for 4 weeks.

Table 4 Influence of storage conditions on the induced lesion in irradiated cassava COL 2215 by fungal isolates after 4 weeks.

Inoculum	Ambient temperature (26 ± 2°C)	Polyethylene bags (18 µm)
Single		
Botryodiplodia theobromae	9.50 ± 0.05 g	13.00 ± 0.10 de
Penicillium oxalicum	13.00 ± 0.05 de	15.50 ± 0.05 bc
Aspergillus niger	11.50 ± 0.05 f	16.00 ± 0.04 ab
Fusarium solani	9.60 ± 0.00 g	13.00 ± 0.01 de
Macrophomina phaseolina	13.50 ± 0.10 d	16.50 ± 0.05 a
Pair		
B + P	16.50 ± 0.05 hi	17.50 ± 0.05 fg
B + A	15.50 ± 0.05 jk	17.88 ± 0.06 fg
B + F	16.00 ± 0.01 ij	16.13 ± 0.05 ij
B + M	18.00 ± 0.00 ef	18.13 ± 0.04 ef
P + A	18.00 ± 0.10 ef	19.00 ± 0.04 cd
P + F	17.50 ± 0.05 fg	19.13 ± 0.07 cd
P + M	19.50 ± 0.06 bc	21.63 ± 0.03 a
A + F	16.50 ± 0.05 hi	18.38 ± 0.04 ef
A + M	18.50 ± 0.05 de	20.00 ± 0.05 b
F + M	17.00 ± 0.05 gh	17.38 ± 0.08 gh

Each value represents the mean from two independent experiments and the overall mean ± standard error. Lesions (values) induced by each isolate or pair in the same row having different letters are significantly different at *P*=0.05. Pair: B+P = *B. theobromae* + *P. oxalicum*; B+A = *B. theobromae* + *A. niger*; P+A = *P. oxalicum* + *A. niger*; F+M = *F. solani* + *M. phaseolina* , A+F = *A. niger* + *F solani*, etc.

[a] Each value in a column represents the overall mean of lesions/rots induced either by all the five singly or pair-inoculated organisms for 4 weeks.

of the fungi induced rot of different dimensions when inoculated within the cassava tissue. The extent of rot increased with storage time, irrespective of inoculum. The experiments were carried out in two different storage environments: in polyethylene bags and at ambient temperature (26 ± 2°C). The levels of rots in irradiated and non irradiated cassava roots stored in ambient temperature are shown in **Tables 1** and **2** while the levels of rots in irradiated cassava roots stored in polyethylene bags compared with those in ambient temperature are shown in **Tables 3-5**.

In irradiated SMS 909-25, the most extensive rot was observed with *M. phaseolina*, followed by *A. niger* and *F. solani* while the least level of rot development was observed with *B. theobromae* in a single isolate (**Table 1**). In paired isolates, *B. theobromae* and *M. phaseolina* showed

Table 5 Influence of storage conditions on the induced lesion in irradiated cassava COL 1734 by fungal isolates after 4 weeks.

Inoculum	Ambient temperature (26 ± 2°C)	Polyethylene bags (18 µm)
Single		
Botryodiplodia theobromae	8.00 ± 0.00 gh	10.50 ± 0.05 bc
Penicillium oxalicum	6.50 ± 0.05 i	9.00 ± 0.10 ef
Aspergillus niger	9.50 ± 0.10 de	10.00 ± 0.00 cd
Fusarium solani	8.50 ± 0.05 fg	11.00 ± 0.00 b
Macrophomina.phaseolina	9.50 ± 0.15 de	13.50 ± 0.05 a
Pair		
B + P	14.00 ± 0.00 fg	15.38 ± 0.10 de
B + A	13.00 ± 0.05 hi	14.88 ± 0.26 ef
B + F	14.00 ± 0.01 fg	15.38 ± 0.05 de
B + M	13.50 ± 0.05 gh	16.75 ± 0.13 b
P + A	14.00 ± 0.00 fg	16.50 ± 0.28 b
P + F	13.00 ± 0.00 hi	15.50 ± 0.21 cd
P + M	14.50 ± 0.20 ef	17.50 ± 0.29 a
A + F	13.50 ± 0.05 gh	14.50 ± 0.06 ef
A + M	14.00 ± 0.00 fg	14.75 ± 0.06 ef
F + M	13.50 ± 0.05 gh	16.13 ± 0.07 bc

Each value represents the mean from two independent experiments and the overall mean ± standard error. Lesions (values) induced by each isolate or pair in the same row having different letters are significantly different at *P*=0.05. Pair: B+P = *B. theobromae* + *P. oxalicum*; B+A = *B. theobromae* + *A. niger*; P+A = *P. oxalicum* + *A. niger*; F+M = *F. solani* + *M. phaseolina* , A+F = *A. niger* + *F solani*, etc.

[a] Each value in a column represents the overall mean of lesions/rots induced either by all the five singly or pair-inoculated organisms for 4 weeks.

the highest levels of rots followed by *B. theobromae* and *A. niger* while *P. oxalicum* and *F. solani* as well as *A. niger* and *F. solani* showed the least levels of rots development.

In irradiated COL 2215, higher level of rot was observed with *M. phaseolina* followed by *P. oxalicm* while *B. theobromae* and *F. solani* produced the least levels of rots (**Table 1**). But *P. oxalicum* and *M. phaseolina* produced the highest levels of rots when the moulds were combined while *B. theobromae* and *A. niger* produced the least levels of rots.

In COL 1734, *M. phaseolina* showed higher level of rot followed by *A. niger* while *P. oxalicum* induced the least rot development in single organisms. *P. oxalicum* and *M. phaseolina* induced higher levels of rots when the moulds were combined while *B. theobromae* and *F. solani* induced the least levels of rots (**Table 1**).

In non- irradiated cassava varieties (**Table 2**), the induction of more rots was observed in SMS 909-25 with *M. phaseolina*, followed by *A. niger* while the least levels of rots were observed with *B. theobromae* and *F. solani* in single organisms. Inoculated cultures of *B. theobromae* and *M. phaseolina* showed more rots, followed by *P. oxalicum* and *M. phaseolina* while *P. oxalicum* and *F. solani* showed the least level of rots development.

In the variety, COL 2215, more rots were observed with *M. phaseolina*, *P. oxalicum* followed by *A. niger* and *F. solani*. *P. oxalicum* and *M. phaseolina* produced the highest level of rot when the moulds were combined followed by *A. niger* and *M. phaseolina* while *B. theobromae* and *A. niger* produced the least level of rot (**Table 2**).

Similarly, in non-irradiated COL 1734, *M. phaseolina* induced higher level of rot followed by *Aspergillus niger* while *Penicillium oxalicum* induced the least level of rot in single organisms. *P. oxalicum* and *M. phaseolina* induced higher levels of rots when the moulds were combined followed by *P. oxalicum* and *F. solani* (**Table 2**).

In **Table 3**, *M. phaseolina* in polyethylene bags storage showed the highest level of rot (15.50 ± 0.00 mm) compared with the level of rot (11.50 ± 0.15 mm) of *M. phaseolina* in ambient temperature storage for single organisms. In the same vein, a higher level of rot was also observed in combination of *P. oxalicum* and *A. niger* compared with the level of rot in *P. oxalicum* and *A. niger* in ambient temperature storage. However, the least level of rot induction in

polyethylene bags storage was observed in *F. solani* (10.50 ± 0.05 mm) though higher than the level of rot of *F. solani* in ambient temperature.

When moulds were combined, *P. oxalicum* and *M. phaseolina* levels of rots (18.25 ± 0.03 mm) in polyethylene bags storage was higher than the levels of rots (15.50 ± 015 mm) of *P. oxalicum* and *M. phaseolina* in ambient temperature storage. The levels of rots of *B. theobromae* and *M. phaseolina* in polyethylene bags storage were higher than the *B. theobromae* and *M. phaseolina* in ambient temperature storage. The least level of rot showed by *F. solani* and *M. phaseolina* in polyethylene bags storage was higher than that of *F. solani* and *M. phaseolina* in ambient temperature storage.

In **Table 4** the level of rot induced by *M. phaseolina* (16.50 ± 0.05 mm) in irradiated cassava stored in polyethylene bags was higher than that of ambient temperature storage (13.50 ± 0.10 mm). Similar trend was also observed between the levels of rots induced by *A. niger* in irradiated cassava stored in polyethylene bags (16.50 ± 0.05 mm) and ambient temperature (11.50 ± 0.05 mm) storage for single organisms.

P. oxalicum and *M. phaseolina* produced more rots (21.63 ± 0.03 mm) in irradiated cassava tubers stored in polyethylene bags compare to that of ambient temperature storage (19.50 ± 0.06 mm) when isolates were inoculated in pairs.

In **Table 5**, *M. phaseolina* showed the highest level of rot (13.50 ± 0.05 mm) in irradiated cassava stored in polyethylene bags compared to the level of rot (9.50 ± 0.15 mm) in ambient temperature storage. In the same vein, the levels of rots produced by *F. solani* (11.00 ± 0.00 mm) in irradiated cassava tubers stored in polyethylene bags were higher than the rot (8.50 ± 0.05mm) in ambient temperature storage for single isolate while *P. oxalicum* produced rots (9.00 ± 0.10 mm) in polyethylene bags and (6.50 ± 0.05 mm) in ambient temperature storage.

For pair-isolate, *P. oxalicum* and *M. phaseolina* showed the highest levels of rots (18.25 ± 0.29 mm) in polyethylene compared with the levels of rots in ambient temperature storage (14.50 ± 0.20 mm) while the level of rot (16.75 ± 0.13 mm) was produced by *B. theobromae* and *M. phaseolina* in polyethylene bags and rots (13.50 ± 0.05 mm) in ambient temperature storage.

DISCUSSION

Moulds have been found to be the major cause of deterioration of crops (Efiuvwevwere and Nwachukwu 1998; Msikita *et al.* 1998). This is supported by the present study which showed five genera of moulds (*Botryodiplodia*, *Penicillium*, *Aspergillus*, *Fusarium* and *Macrophomina*) as the causal agents of rots of these cassava varieties; SMS 909-25, COL 2215 and COL 1734. Each of the fungi *Botryodiplodia*, *Penicillium*, *Aspergillus*, *Fusarium* and *Macrophomina* species induced rot of different dimensions when inoculated within the cassava tissue. The association of these moulds with induction of cassava rots is probably due to their widespread occurrence in the environment and their ability to produce pectinolytic enzymes (Ugwuanyi and Obeta 1997).

Generally, significant (P >0.05) deterioration was observed in the non-irradiated cassava than the irradiated (**Tables 1, 2**). Also, similar observation has been reported of irradiated Ghanaian cultivar 'Bosom nsia' with gamma radiation, mutants – VT1 and VT2 resistant to *Africa cassava mosaic virus* (Ahiabu *et al.* 1997).

This observation may be due to genetic changes in the mutants as a result of irradiations (Yu *et al.* 2000; Joseph and Yeoh 2004). According to Lee *et al.* (2002), these variations in the level of rots observed between irradiated and non-irradiated cassava might be due to genetic changes such as chromosomal aberrations or even structural mutations of some genes. Mutations dramatically altered the composition of starch (Yu *et al.* 2000), protein and dry matter, and reduction in the storage root cyanogens (Nwachukwu *et al.* 1997) content thereby causing significant changes in the physio-chemical properties. In several induced mutant plants, morphological differences in storage organs along with quantitative and qualitative differences in starch biosynthesis have been identified and characterized (Ancora and Sonnino 1987; Smith 1993; Coleman *et al.* 1995). Also, the slight or marked variations reported may be due to cassava sources including location; cultural practices during production, soil types or a combination of two or more of these factors (Jackson *et al.* 1992).

In the single inoculation the most significant rot was caused by *M. phaseolina* irrespective of the variety and the storage conditions. This result agrees with the earlier reports by Mihail and Taylor (1995) that *M. phaseolina* is one of the major pathogens of cassava probably because the fungus *M. phaseolina* is a soil pathogen distributed worldwide with a host range of more than 500 plant species. Beas-Fernandez *et al.* (2006) reported that the fungus attacks a broad spectrum of economically important crops such as beans and maize. *P. oxalicum* and/or *A. niger* also produced high rots in cassava. This similar observation was reported by Nwachukwu (2006) of the rots caused by *Penicillium* spp., *A. niger* and *Fusarium* spp. in yam. The least rot development by the fungi varied considerably within the three varieties (**Tables 1, 2**). The inoculation of single fungus in the irradiated cassava showed a similar rots trend in non-irradiated cassava.

Furthermore, more extensive rots were observed when isolates were combined especially with the combination of *P. oxalicum* and *M. phaseolina*, followed by *A. niger* and *M. phaseolina.* The induction of a larger rots when combined moulds were inoculated compared to single mould inoculation, may be due to a synergism between two moulds. The least rots development varied in the combination of moulds within the three varieties. The induction of least development of rots when combined moulds were inoculated may be as a result of antagonism and/or pathogenic activity of these moulds (Fajola and Nwufo 1985; Efiuvwevwere and Nwachukwu 1998).

The differences in the magnitude of the induction of rots by the five species of moulds in the three varieties reflect the varietal differences in their responses to these moulds. Generally more deterioration was observed in variety COL 2215 while the least deterioration in COL 1734. This observation suggests a possible existence of cassava genotypes resistant or susceptible to these moulds. The differences observed in the extents of rots induced by the fungi may also be due to the differential penetration ability of the mould into the cassava tissues.

More rots were observed in cassava stored in polyethylene bags than ambient temperature. Since injured cassava roots have a higher respiration rates, the 18 µm thick polyethylene storage bags may enhance a relatively high water vapour build-up (Hui 1992) thereby supporting fungal growth and deterioration in cassava. This may in part be responsible for the extensive rots in cassava roots stored in polyethylene bags than in ambient temperature (**Tables 3-5**). This observation agrees with the work of Efiuvwevwere and Oyelade (1991) that accumulation of condensate enhanced microbial proliferation in similar packs. However, apart from the moisture content, the type of inoculums may contribute to the extent of rots induced in the cassava varieties (Efiuvwevwere and Oyebanji 1999). Also, increase in moisture content of the stored cassava samples implies more favourable conditions for fungal growth and enzyme activities.

This study has shown that the fungal isolates (*Botryodiplodia*, *Penicillium*, *Aspergillus Fusarium* and *Macrophomina* species) induced rot of different dimensions when inoculated within the cassava tissue. The extent of rot was relatively influenced by storage condition. However, more rots were generally observed in non-irradiated than irradiated cassava varieties irrespective of the storage conditions.

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