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Effects of Radiation and Chemical Mutagenesis on Expression of Aflatoxigenic Traits in *Aspergillus parasiticus* SMS08-C

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ABSTRACT

The study presented the effects of some chemical and radiation mutagens on aflatoxigenic traits in *Aspergillus parasiticus* SMS08-C. This was an attempt to enhance its bio-reagent standard production potential and repress aflatoxins production as a control measure. The fungal spores were suspended into fixed concentrations of sodium azide (NA), Ethyl Methane Sulphonate (EMS), nitrous oxide (HNO), hydroxylamine (NH_2OH) and some exposed to ultra violet rays (UV) over a period of 90 min. Mutants were recovered at every 15 min of the treatment. The mutants were then screened for aflatoxins in yeast extract sucrose medium (pH 5.5) at 30°C for 10 days in the dark. Aflatoxin analysis was carried out using ELISA-based techniques. The effects of the mutagens were significantly different on expression of aflatoxigenic traits ($p<0.05$). Substantial reduction in aflatoxin production was showed by mutants listed with the corresponding percentage, TM_{EMS}-90 (75.75%), TM_{HNO}-90 (73.13%), TM_{NA}-60 (57.07%) and TM_{NH_2OH}-60 (73.55%); while TM_{UV}-75 showed the least reduction (30.19%). Ultra violet radiation resulted into hyper-production of aflatoxins in TM_{UV}-15 (97.55%). Out of the mutant strains of sodium azide, ethyl methane sulphonate, nitrous oxide and hydroxylamine, high level of biosynthesis was exhibited by TM_{EMS}-45 (62.14%), TM_{HNO}-15 (37.79%), TM_{NA}-30 (49.03%) and TM_{NH_2OH}-15 (17.95%), respectively. The relevance of different mutagens as tools in improving biotechnological potentials of *Aspergillus parasiticus* either to repress or promote aflatoxins biosynthesis has been revealed.

Key words: *Aspergillus parasiticus*, aflatoxigenic traits, ELISA, mutagenesis, mutagens, aflatoxins, bio-reagent standard

INTRODUCTION

The role of *Aspergillus parasiticus* in enzyme biotechnologies and bio-reagents production cannot be over emphasized. *Aspergillus parasiticus* has been identified as a source of invertase (Matrai *et al*., 2000), efficient producer of α-galactosidase (Shivam *et al*., 2009), extracellular β-D-fructofuranosidase (Lucca *et al*., 2013), other enzymes and bio-solvents. But, *Aspergillus parasiticus* are well known majorly, because of its economic importance in agriculture and storage. *A. parasiticus* are aflatoxigenic and potential aflatoxins producers (Salaman *et al*., 2015) that mainly infect maize, peanuts and tree nuts (Youssef *et al*., 2008). Foods get contaminated by aflatoxigenic fungi, especially in tropical countries during pre-harvesting, processing, transportation and storage (Fente *et al*., 2001). The risks of *A. parasiticus* come from its ability to produce Aflatoxins spatially (Salaman *et al*., 2015).

Despite the facts that the biotechnological advantages of *Aspergillus parasiticus* are two-sided, little or no attention as being devoted to enhancing its biotechnological potential and utilization in bio-reagents production. Strains producing aflatoxins of more types as been described by Labuda *et al.* (2009) could be potentially used in production of reference substances either for further research or in the control of food quality and safety. Thus, *A. parasiticus* which are both copious and broad spectral producers of Aflatoxins (AFs) B1, B2, G1, G2 (AFB1, AFB2, AFG1 and AFG2) unlike *Aspergillus flavus* that only have the capability to produce aflatoxins B1, B2 (AFB1 and AFB2). *Aspergillus parasiticus* in turn, could be employed in large scale production of aflatoxins as bio-reagent standards. Furthermore, aflatoxins produced by *A. parasiticus* could find suitable application as antigens in the production of specific monoclonal and polyclonal antibodies. These are needed in enzyme-linked immunosorbent assay for detection of any species of aflatoxins in foods and other biosafety procedures. To harness this two-sided potential of *A. parasiticus*, there is a need to suppress undesirable traits and simultaneously enhance the targetted ones. This will depend on the direction we would prefer to channel its biotechnogical potentials. One of the ways in achieving such goal is mutagenesis directed toward exerting up-regulation or down-regulation of aflatoxin biosynthesis. The present investigation studies the ability of chemical and radiation mutagens to promote controls of aflatoxin biosynthesis in aflatoxigenic *A. parasiticus* SMS08-C.

MATERIALS AND METHODS

Aflatoxigenic *Aspergillus parasiticus* SMS08-C: *Aspergillus parasiticus* SMS08-C previously isolated from sawdust, identified according to Barnett and Hunter (1972) and confirmed to be active producer of cellulase and aflatoxins in the Microbiology Research Laboratory, Federal University of Technology, Akure, Nigeria, was used in this study.

Preparation of conidial suspension of *Aspergillus parasiticus* SMS08-C: A spore suspension of the isolate was prepared by serial dilution method from 5 days old culture according to Elliaiah *et al.* (2002). Spore suspension of the fungal strain was prepared by adding sterilized phosphate buffer (pH 7.0) into the 5 days old slant. The spores were dislodged with the aid of a sterile inoculating loop from the mycelia and mixed thoroughly. Inoculum size was measured with a haemocytometer as described by Sharma (1989). A 10 µL of homogenous spore solution was pipette into one of the chambers of the haemocytometer. Then, the spores were enumerated.

Chemical mutagenesis

Ethyl methane sulfonate treatment of *Aspergillus parasiticus* SMS08-C: Ethyl methane sulphonate mutant strains of *A. parasiticus* SMS08-C were generated according to Nadeem *et al.* (2010). A 6.0 mg stock solution of EMS was prepared. Volumes of 2.0 mL of spore suspension (10^8) was taken and incubated with equivalent amount of EMS stock solution for 15, 30, 45, 60, 75 and 90 min at 28±2°C. After the period of treatment, spores were centrifuged and washed with sterilized phosphate buffer (pH 7.0) twice at 6000 rpm for 15 min at 4°C. A volume of 10 µL of EMS-treated fungal suspension was plated onto potato dextrose agar at 28±2°C for 5 days. Pure mutant colonies were thereafter selected for further studies.

Nitrous acid treatment of *Aspergillus parasiticus* SMS08-C: Nitrous acid-mutant strains were generated according to the modified method of Iftikhar *et al.* (2010). Spore culture of *A. parasiticus* SMS08-C prepared in Vogel's medium was subjected to nitrous acid (0.1 M sodium nitrite in phosphate buffer, pH 5.0) treatment for 15, 30, 45, 60, 75 and 90 min, with incubation at 28±2°C. The treated spores were washed thrice at 6,000 rpm for 15 min to remove traces of mutagen. After suspending the spores in saline, 10 μL was plated onto potato dextrose agar at 28±2°C for 5 days. Pure mutant colonies were thereafter selected for further studies.

Sodium azide treatment of *Aspergillus parasiticus* SMS08-C: The sodium azide mutagenesis of *A. parasiticus* SMS08-C was conducted according to Rajeshkumar and Ilyas (2011). To 9.0 mL of the spore suspension, 1.0 mL sterile solution of sodium azide (400 μg mL^{-1} in phosphate buffer) was added. The reaction was allowed to proceed at room temperature. The control tube was also kept without any chemical mutagen. Sample of 1.0 mL was withdrawn from the reaction mixture at 15, 30, 45, 60, 75, 90 min and centrifuged at 6000 rpm for 15 min. The spores were washed three times with sterile distilled water and again re-suspended in 10.0 mL sterile buffer. A 10 μL of the treated spore suspension was plated onto potato dextrose agar at 28±2°C for 5 days. Pure mutant colonies were thereafter selected for further studies.

Hydroxylamine treatment of *Aspergillus parasiticus* SMS08-C: Hydroxylamine-mutant strains of *A. parasiticus* SMS08-C were developed according to the modified procedure of Damisa *et al.* (2013). One molar stock solution of hydroxylamine was prepared by reacting 1 M hydroxylammonium chloride with 1 M NaOH solution. A 100 mM solution of hydroxylamine was therefrom prepared using phosphate buffer (pH 7.5).

To 9.0 mL of the spore suspension in phosphate buffer (pH 7.5), 1.0 mL sterile solution of hydroxylamine (100 mM) was added. The reaction was allowed to proceed at 28±2°C. The control tube was also kept without hydroxylamine. One milliliter of the sample was withdrawn from the reaction mixture at 15, 30, 45, 60, 75 and 90 min. After the period of treatment, spores were centrifuged and washed with sterilized phosphate buffer (pH 7.5) twice for 15 min at 6000 rpm (Rajeshkumar and Ilyas, 2011). A 10 μL of the treated spore suspension was thereafter plated onto potato dextrose agar at 28±2°C for 5 days. Pure mutant colonies were selected for further studies.

Physical mutagenesis

Ultra Violet (UV) radiation treatment of *Aspergillus parasiticus* SMS08-C: Ultra violet-mutant strains were created according to the method of Irfan *et al.* (2011). A 2.0 mL quantities of spore suspension was aseptically pipetted into sterile petri dishes and exposed to UV radiation (366 nm) at a distance of 13.0 cm away from the centre of a germicidal lamp (Model UVL-21, San Gabriel, Calif. USA) for various time intervals (15, 25, 35, 45, 55, 65, 75 and 85 min). Constant agitation of suspension was achieved by gently rotating the plate in-between treatment intervals Elliaiah *et al.* (2002). The UV exposed spore suspension was stored over night in dark to avoid photo-reactivation and for stabilization of Thymine-Thymine (T-T) dimmers (Irfan *et al.*, 2011). After over night incubation, irradiated spore suspension was serially diluted in a phosphate buffer (pH 7.0). The diluted spore suspension (10 μL) was plated onto potato dextrose agar at 28±2°C for 5 days. Pure mutant colonies were further selected for studies.

Screening of *Aspergillus parasiticus* SMS08-C mutant strains for aflatoxins production: Aflatoxin production by the mutants was assessed according to the method of Zaini *et al.* (2009). Ten mycelial plugs of 10 mm diameter of the 5 days old culture of the mutant was harvested and inoculated into a 250 mL Erlenmeyer flask. The flask contained 50 mL Yeast Extract-Sucrose

(YES) broth medium (2% yeast extract, 20% sucrose) with initial pH 5.5. The flask was incubated at 30°C for 10 days in the dark. Afterwards, the culture was filtered and centrifuged at 10,000 rpm for 5 min.

Determination of aflatoxins produced by *Aspergillus parasiticus* SMS08-C mutant strains: Total aflatoxin production was evaluated by using Ridascreen Aflatoxin Total Kit (R-Biopharm AG, Darmstadt, Germany). The reagents and materials used for the analysis include microtiter plate (12×8 strips) coated with captured antibodies, calibrators/standards (6): 0, 50, 150, 450, 13500 and 4050 ρg mL^{-1} (ppt) of aflatoxin B (1 mL) each, methanol/water and aflatoxin conjugate (6 mL) (peroxidase conjugated aflatoxin B). Others include anti-aflatoxin antibodies (6 mL) (monoclonal), substrate/chromogen solution (10 mL) (stained red because of tetramethylbenzidin), stop solution (14 mL) that contained 1 M sulfuric acid and washing buffer salt (0.05% Tween20), for preparation of 10 mM phosphate buffer (pH 7.4). Reagents and materials were stored at 4-8°C.

The assay test was carried out according to the procedure provided with the kit (R-Biopharm AG, Darmstadt, Germany). One milliliter (1 mL) of the sample supernatant prepared from the culture filtrate was pipetted into a centrifuge tube. This was additionally spun at 14,000 rpm for 5 min using MiniSpin centrifuge (Eppendorf AG, model 2231, Hamburg Germany). One hundred microlitres (100 μL) of the standard solution or sample (as the case may be) was pipetted into pre-labelled wells of the test strips using a multi-channel pipette. The standard solution and sample positions were recorded accordingly. One hundred microlitres (100 μL) of the conjugate enzyme and one hundred microlitres (100 μL) of the antibody solution was thereafter added into the entire wells successively with the multi-channel pipette. The plate was gently mixed by manual shaking and incubated at 23±2°C for 30 min in dark. Afterwards, contents of the wells were poured out. The microwell holder was tapped upside down vigorously (three times in a row) against a stack of absorbent paper towels to ensured complete removal of liquid from the wells. Each well was subsequently washed three times with 250 μL diluted washing buffer solution in every washing step. The remaining buffer in wells was removed by patting the plate dry on a stack of paper towels. One hundred microlitres (100 μL) of substrate (color) solution was added to all the wells and gently mixed by manual shaking. The strip was finally incubated at 23±2°C for 15 min in the dark. The reaction was afterwards terminated by the addition of 100 μL stop solution to each well in the same sequence the substrate was added. The strip was gently mixed as soon as the substrate was added. The absorbance was read at 450 nm with a microplate ELISA photometer (Model ELx800, BioTek, USA) within 30 min after the addition of stopping solution (R-Biopharm AG, Darmstadt, Germany). Aflatoxin production was compared between the wild strain and the mutants as Relative Aflatoxin Production (RAP) and as Difference in Aflatoxin Production (DAP) calculated as:

$$\text{RAP (\%)} = \frac{\text{AFm}}{\text{AFw}} \times \frac{100}{1}$$

where, AF_m is aflatoxin produced by the mutant, AF_w is aflatoxin produced by the wild strain.

$$\text{DAP (\%)} = \text{RAPm-RAPw}$$

where, RAP_m is Relative aflatoxin production of the mutant, RAP_w is Relative aflatoxin production of the wild strain.

Statistical evaluation: Data obtained were subjected to Analysis of Variance (ANOVA) of Statistical Package for Social Sciences (SPSS) software version 17 (Microsoft Corporation, USA) at 95% confidence interval. Data presented on the average of three replicates (Mean±SE) were obtained from duplicate determinations. Means were separated using Duncan's New Multiple Range Test and differences were considered significant at $p \leq 0.05$.

RESULTS

Production of aflatoxins by chemical mutant strains of *Aspergillus parasiticus* SMS08-C: The effects of different chemical mutagens on aflatoxin production by the resultant mutants of *A. parasiticus* SMS08-C are represented in Table 1-4. Aflatoxin synthesis was statistically significant among mutants generated through the same chemical treatment at varied contact time ($p<0.05$). Reduction in aflatoxin biosynthesis was showed by 83.33% of the EMS mutants while only 16.67%, revealed more yield than the wild strain (Table 1). The TM_{EMS}-75 (241.73±0.006 ppt) and TM_{EMS}-90 (244.59±0.42 ppt) attained 76.03 and 75.75% reduction in aflatoxin synthesis, respectively. High value of aflatoxin increase (62.14%) was produced by TM_{EMS}-45 (1635.15±1.15 ppt).

Table 1: Aflatoxins production by EMS-mutants of *Aspergillus parasiticus* SMS08-C

Mutants	Concentration (ppt)	RAP (%)	DAP (%)
SMS08-C	1008.50±3.50^{e}	100.00	0.00
TM_{EMS}-15	797.60±0.10^{b}	79.09	20.91*
TM_{EMS}-30	846.15±1.15^{d}	83.90	16.10*
TM_{EMS}-45	1635.17±0.17^{f}	162.14	62.14**
TM_{EMS}-60	806.00±1.00^{c}	79.92	20.08*
TM_{EMS}-75	241.73±0.06^{a}	23.97	76.03*
TM_{EMS}-90	244.59±0.42^{a}	24.25	75.75*

Values are presented as Mean±SE (n = 2). Means with the same superscript letter(s) along the same column are not significantly different ($p<0.05$). SMS08-C: Wild type, 15, 30…75 and 90 treatment duration (min), RAP: Relative aflatoxin production, DAP: Difference in aflatoxin production of mutant and the wild, **Increase and *Decrease

Table 2: Aflatoxins production by nitrous acid-mutants of *Aspergillus parasiticus* SMS08-C

Mutants	Concentration (ppt)	RAP (%)	DAP (%)
SMS08-C	1075.00±3.50^{d}	100.00	0.00
TM_{HNO}-15	1481.25±0.10^{f}	137.79	37.79**
TM_{HNO}-30	1121.25±0.06^{e}	104.30	4.30**
TM_{HNO}-60	360.00±0.11^{c}	33.49	66.51*
TM_{HNO}-75	359.00±0.10^{b}	33.40	66.60*
TM_{HNO}-90	288.90±0.15^{a}	26.87	73.13*

Values are presented as Mean±SE (n = 2). Means with the same superscript letter(s) along the same column are not significantly different ($p<0.05$). SMS08-C: Wild type, 15, 30…75 and 90 treatment duration (min), RAP: Relative aflatoxin production, DAP: Difference in aflatoxin production of mutant and the wild, **Increase and *Decrease

Table 3: Aflatoxins production by sodium azide-mutants of *Aspergillus parasiticus* SMS08-C

Mutants	Concentration (ppt)	RAP (%)	DAP (%)
SMS08-C	1008.50±3.50^{e}	100.00	0.00
TM_{NA}-15	1003.50±3.50^{e}	99.55	0.45*
TM_{NA}-30	1503.00±3.00^{f}	149.03	49.03**
TM_{NA}-45	538.79±0.22^{b}	53.43	46.58*
TM_{NA}-60	432.96±0.10^{a}	42.93	57.07*
TM_{NA}-75	903.58±0.58^{d}	89.60	10.40*
TM_{NA}-90	645.50±0.51^{c}	64.01	35.99*

Values are presented as Mean±SE (n = 2). Means with the same superscript letter(s) along the same column are not significantly different ($p<0.05$). SMS08-C: Wild type, 15, 30…75 and 90 treatment duration (min), RAP: Relative aflatoxin production, DAP: Difference in aflatoxin production of mutant and the wild; **Increase and *Decrease

Table 4: Aflatoxins production by hydroxylamine-mutants of *Aspergillus parasiticus* SMS08-C

Mutants	Concentration (ppt)	RAP (%)	DAP (%)
SMS08-C	1008.50 ± 3.50^{d}	100.00	0
TM_{NH_2OH}-15	1189.50 ± 0.30^{g}	117.95	17.95**
TM_{NH_2OH}-30	1131.17 ± 0.74^{e}	112.16	12.16**
TM_{NH_2OH}-45	1151.58 ± 0.53^{f}	114.19	14.19**
TM_{NH_2OH}-60	281.95 ± 0.35^{b}	27.96	72.04*
TM_{NH_2OH}-75	341.95 ± 0.65^{c}	33.91	66.09*
TM_{NH_2OH}-90	266.72 ± 0.27^{a}	26.45	73.55*

Values are presented as Mean±SE (n = 2). Means with the same superscript letter(s) along the same column are not significantly different ($p<0.05$). SMS08-C: Wild type, 15, 30...75 and 90 treatment duration (min), RAP: Relative aflatoxin production, DAP: Difference in aflatoxin production of mutant and the wild, **Increase and *Decrease

Table 5: Aflatoxins production by UV-mutants of *Aspergillus parasiticus* SMS08-C in YES medium

Mutants	Concentration (ppt)	RAP (%)	DAP (%)
SMS08-C	1005.22 ± 0.22^{e}	100.00	0.00
TM_{UV}-15	1985.76 ± 0.77^{i}	197.55	97.55**
TM_{UV}-25	1748.00 ± 3.00^{g}	173.89	73.89**
TM_{UV}-35	865.83 ± 0.83^{c}	86.13	13.87*
TM_{UV}-45	875.23 ± 0.23^{d}	87.07	12.93*
TM_{UV}-55	1504.51 ± 4.51^{f}	149.67	49.67**
TM_{UV}-65	810.37 ± 0.37^{b}	80.62	19.38*
TM_{UV}-75	701.78 ± 1.78^{a}	69.81	30.19*
TM_{UV}-85	1757.28 ± 2.28^{h}	174.82	74.82**

Values are presented as Mean±SE (n = 2). Means with the same superscript letter(s) along the same column are not significantly different ($p<0.05$). SMS08-C: Wild type, 15, 30...75 and 90 Treatment duration (min), RAP: Relative aflatoxin production, DAP: Difference in aflatoxin production of mutant and the wild, **Increase and *Decrease

Out of the five nitrous acid mutants screened for aflatoxin biosynthesis, 60% showed reduced level of aflatoxin yield when compared to the parent strain (Table 2). Highest reduction in aflatoxin production was achieved by TM_{HNO}-90 which was a 73.13% reduction in the synthesis. Conversely, maximum level of aflatoxin yield was recorded by TM_{HNO}-15 (37.79%, 1481.25 ppt).

Although, 83.33% of sodium azide mutant strains showed reduction in aflatoxin level, the values range was very small, with the highest reduction (49.03%) recorded by TM_{NA}-30 (1503.00±3.00 ppt) (Table 3). Forty-nine-point-zero-three percent (49.03%) highest increase in aflatoxin level was obtained in TM_{NA}-30 (1503.00±3.00 ppt) among sodium azide mutant strains.

For the hydroxylamine mutants, 50% showed appreciable reduction in aflatoxin biosynthesis but others gave considerable increase (Table 4). The TM_{NH_2OH}-90 (266.72±0.027 ppt) and TM_{NH_2OH}-15 (1189.50±0.300 ppt) yielded 73.55% reduction and 17.95% increase in aflatoxin level, respectively compared with the wild strain's.

Production of aflatoxins by physical mutant strains of aflatoxigenic *Aspergillus parasiticus* SMS08-C: Radiation mutagenesis trial conducted to effect control on aflatoxin synthesis ability of *A. parasiticus* SMS08-C is portrayed in Table 5. Aflatoxin biosynthesis was significantly different among the mutants ($p<0.05$). Out of the eight UV mutants developed from *A. parasiticus* SMS08-C, 50% showed increase level of aflatoxin synthesis. Highest yield and reduction of aflatoxin level was displayed by TM_{UV}-15 (1985.76±0.77 ppt: 97.55%) and TM_{UV}-75 (701.78±1.78 ppt: 30.19%), respectively among the UV mutants.

DISCUSSION

Radiation and chemical mutagenesis was conducted to exert control on elaboration of aflatoxigenic traits in *A. parasiticus* isolated from sawmill soil in a two-ways driven goal. Firstly, to get rid of aflatoxin production, to enhanced high titre cellulase production from the strain (result not shown) and secondly, to improve the strain for hyper-production of aflatoxin as bio-reagent standard. Schmidt-Heydt *et al.* (2010) have earlier reported that greater than 90% of *A. parasiticus* isolated from soils are able to synthesize high concentrations of aflatoxins.

All the mutagens employed in this study exercised influence on aflatoxins production in the strain. This could be attributed to the unique mechanism by which different mutagenic agent causes mutation. The efficiency of any mutant in aflatoxin production could depend on simultaneous and/or multi-platform effects of pH, temperature, culture conditions, concentration of mutagen used and the treatment duration. El-Mokadem and Mostafa (2014), reported the effects of similar conditions in sodium azide mutagenesis of *Browallia speciosa*. The ethyl methane sulphonate, nitrous acid and hydroxylamine mutagenesis resulted into a substantial reduction of aflatoxin production. This could be due to any mutation that led to recession of some genes that are involved in aflatoxin pathway-specific regulatory genes. Also, it could be adduced to any chromosomal disruption of DNA that directly affected genes that are involved in aflatoxins biosynthesis. Chang *et al.* (2002), as earlier reported some aflatoxin pathway-specific regulatory genes.

Mutations in the genes that encoded some important enzymes in aflatoxin synthesis, could in addition, led to differences in the level of aflatoxin production. Enzymes such as polyketide synthase (Crawford *et al.*, 2008), β-ketoacyl synthase, acyltransferase, acyl carrier protein and thioesterase (Chang *et al.*, 1995), have been documented to be involved in conversion of some intermediate precursors in aflatoxin synthesis. These mutagens in addition, could cause incomplete expression of some genes required in aflatoxin biosynthesis as a result of altered genomes in the mutants. Fractional expression of some genes in aflatoxin biosynthesis was reported by Yu (2012), to lead to generation of norsolorinic-acid-accumulating mutants in aflatoxigenic *A. flavus*. Mutations could also invoked impairment in DNA. This may affect gene products required in catalysis of any important intermediate precursors in aflatoxin production. This is similar to the report of Ehrlich (2009) in oxidation of norsolorinic acid anthrone to averantin. Lack of aflS transcript in aflS-knockout mutants of *A. parasiticus* was also revealed by Yu (2012), to be associated with 5-20 fold reduction of expression of some aflatoxin pathway specific genes, such as aflC (pksA), aflD (nor-1), aflM (ver-1) and aflP (omtA).

Differential capability of mutants in gene reconstitution following mutagenic treatment could signify variation in toxin production. Yu (2012) has reported the relevance of aflS (aflJ), AflS) and aflR in genetic control of aflatoxin biosynthetic pathway. Any form of alteration in genes that are associated with genetic control of secondary metabolism, fungal development and aflatoxin formation, could exert significant influence on aflatoxin production as noticed in the mutants. This is in line with Yu (2012).

High level of aflatoxin production seen in some mutants generated with ultra violet radiation could be due to duplicating effect of ultra violet radiation on some aflatoxigenic traits. *Aspergillus parasiticus* was reported by Atalla *et al.* (2004) to became a highly toxin producer especially deoxynivalenol, nivalenol and T-2 toxin, when exposed to UV light of short wavelength. Our observations coincided with Atalla *et al.* (2004) on exposure of *A. parasiticus* to UV long wave which resulted into production of large quantities of aflatoxin G2, ochratoxin A and nivalenol.

Similar results obtained in the chemical mutagenic treatments of the strain, might predict activation of certain genes as an outcome of mutations in the mutants. Flaherty and Payne (1997) reported that mutation led to over-expression of aflR in *A. flavus*, up-regulated aflatoxin pathway gene transcription and aflatoxin accumulation. In addition, transformation that involved *A. parasiticus* aflR gene might be ascribed to the increased production of aflatoxin by some chemical mutants of *A. parasiticus*. Chang *et al*. (1995) has earlier documented that, *A. parasiticus* aflR mutants showed significantly enhanced production of aflatoxin pathway intermediates. Likewise, transmutation activities of the agents that resulted into a point deletion of any gene associated with aflatoxin production in *A. parasiticus* strains, could signalled either an up-regulation or down regulation of aflatoxin-producing ability of the mutants.

CONCLUSION

Conclusively, the study has revealed the effects of mutagens on aflatoxins production and the possible direction towards, which, these potentials could be harnessed either in the control of toxigenic fungi or production of reference substances (standard reagents) from their strains. Radiation mutagens could be useful in producing strains for commercial bio-reagent production whereas, chemical agents would be suitable in the control of toxin-producing *A. parasiticus*.

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