Mansoura Journal of Biology, Vol. 35 (2), December, 2008

BIOCHEMICAL AND MOLECULAR EVALUATION OF GENETIC EFFECTS OF *RHAZYA STRICTA* (DECNE) LEAF EXTRACT ON *ASPERGILLUS TERREUS*

N. A. Baeshin, S. H. Qari*, J. S. M. Sabir and A. M. Alhejin**

Department of Biological Science, Faculty of Science, King Abdulaziz University, P.O. Box 9028, Jeddah - 21413, Saudi Arabia
*Department of Biology, Teachers College, Umm Al-Qura University, P.O. Box 2203, Makkah, Saudi Arabia (e-mail: qarinet@gmail.com)
**Medical Biology Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

(Received: 18 / 9 / 2007)

ABSTRACT

Dense conidial suspensions of *Aspergillus terreus* were treated with elevated concentrations of aqueous extracts of the leaves of the wild *Rhazya stricta* plant. Samples were taken at regular intervals in each treatment and assayed for survival percentage, auxotrophic mutants, quantities of DNA and total protein and further subjected to RAPD and SDS-PAGE tests, respectively. It was found that the extract has potent lethal and mutagenic activities. Survival percentage decreased as the concentration or time of exposure increased. Frequency of auxotrophic mutants increased with increase in concentration or exposure time. Most auxotrophic mutants were amino acid requiring mutants. Quantity of DNA of each mutant was significantly less than in the corresponding control and so on for total protein. The RAPD results demonstrated polymorphic numbers of genetic bands, which were the electrophoretic products of PCR for all mutants compared with the wild type strain. SDS-PAGE results expressed a polymorphism of protein bands as well. All these results strongly point to the mutagenicity of the leaf extract of *R. stricta*.

INTRODUCTION

Rhazya stricta (Decne), a member of the Apocynaceae family, is a widely distributed plant in Saudi Arabia. Extract of its leaves is prescribed in folkloric medicine for the treatment of various disorders such as diabetes, sore throat, helminthiasis, inflammatory conditions and rheumatism [**Ageel *et al.*, (1987); Ali *et al.*, (1995) and Ali *et al.*, (1998)**]. The plant extract contains mainly alkaloids, glycosides, flavonoids, tannins and triterpenes [**Ahmad *et al.*, (1983) and Al-Yahya *et al.*, (1990)**]. Several studies on rats and mice reported that the leaf extract caused sedation, analgesia, decrease motor activity and have anti-depressant, anti-oxidant activities, complex effects on brain endogenous monoamine oxidase activity and centrally-mediated hypotension [**Ali *et al.*, (1998); Tanira *et al.*, (2000) and Ali *et al.*, (2000)**]. Another study has described the anticancer effects of the indole alkaloids of *Rhazya stricta* [**Mukhopadhyay *et al.*, (1981)**]. Moreover the genotoxicity of *Rhazya stricta* leaves aqueous extract was demonstrated for the first time by [**Baeshin *et al.*, (2005)**], against *Saccharomyces cerevicae* auxotrophic mutant test. However more investigations are required to establish its genotoxic effect. Thus the aim of this study is to evaluate the genotoxic effects of the aqueous leaves extract of *Rhazya stricta* using the *Aspergillus terreus* auxotrophic mutant test and by RAPD and SDS-PAGE tests.

MATERIALS AND METHODS

A. Strains

1. Wild type strain:

The haploid phase of the wild type strain of *Aspergillus terreus* was the test organism used in the present study. It was obtained from the Biology Department, Faculty of Sciences, King Abdul-Aziz University, Jeddah, where it had been maintained for several years.

2. Mutant strains:

The induced mutant strains of *A. terreus* were obtained by exposure to different concentrations of *Rhazya stricta* leaf extract, kept and maintained on Potato Dextrose Agar medium (PDA). The nutritional requirements of the mutants were determined. The mutants were characterized at the molecular level using random amplified polymorphic DNA (RAPD) technique and their total celluler proteins were banded

using polyacrylamide gel (SDS-PAGE). Growth medium supplies were purchased from Difco laboratories.

B. Media

1. Complete medium: Potato Dextrose Agar medium (PDA) was employed as the complete medium.
2. Minimal Medium: Difco Czapek Dox agar medium (DOX) was used as the minimal medium either alone for the isolation of auxotrophic mutants or supplemented with the nutritional requirements for further work.

C. Preparation of the extract

Leaves of *Rhazya stricta* were collected during the last week of December of 2005 from naturally growing plants located along the roadsides of Jeddah – Makkah highway. Collected leaves were kept in plastic bags in the field then transferred to a fridge and kept most of the time overnight before being subjected to extraction in the following day. The leaves were washed well with running water to get rid of dust and sand, hand-minced into small pieces, mixed with sterilized distilled water (3, 6, 12, 24 g/l) and further blended in a blending machine. The blended mixtures were left for 24 hours at room temperature with mild hand-shaking at regular intervals. The mixture was then filtered and the filtrate (the extract) was either used directly in the experiment or kept in the fridge for no longer than 3 days for future use. Four concentrations of this stock extract were prepared (3, 6, 12, 24 g/l) to be tested for genotoxic activities; the recommended dose prescribed in folkloric medicine being 6 g/l.

D. Chemicals

Standard concentrations of hydrolysed casein (CAS), mixed vitamins solution (VITS), hydrolysed yeast ribonucleic acid (RNA), individual amino acids, individual vitamins and individual purines and pyrimidines were used as supplements to the minimal media as indicated in Table (I). All the chemicals used in the present study were purchased from Sigma-Aldrich, except DNA polymerase (purchased from Perkin-Elmer) and the dNTPs (purchased from Boehringer Mannheim). DNA extraction and purification reagents and agarose were purchased from Qiagen. Oligonucleotides (random primers) were purchased from Operon, UK. The DNA Marker was purchased from Gibco BRL. The

loading dye solution was purchased from Fermentas, Lithvuania. Protein assay reagents, electrophoretic reagents, and protein marker were purchased from Bio-Rad.

Table (1): Concentrations of medium supplements for nutritional requirements of the mutants of *A. terreus*.

Supplement	Conc. of stock solution (mg/ml)	Conc. used in media (mg/ml)
Individual amino acids	10	10^{-1}
Individual purines and pyrimidines	4	2×10^{-2}
Aneurin	5	5×10^{-4}
Biotin	0.02	2×10^{-6}
Choline chloride	20	2×10^{-3}
Inositol	40	4×10^{-3}
Nicotinic acid	10	10^{-3}
Pyridoxine	5	5×10^{-4}
P-aminobenzoic acid	1	10^{-4}
Pantothenic acid	20	2×10^{-3}
Riboflavin	10	10^{-3}
Hydrolysed casein	60	3
Hydrolysed yeast ribonucleic acid	10	5×10^{-1}
Mixed vitamins	1 ml of each of the individual vitamin solutions mixed, and 0.2 ml of this mixture added to 200 ml media (0.2 ml 1/10 dilution/plate).	

E. Test of Genotoxicity

1- Auxotrophic mutation

Induction of auxotrophic mutants and biochemical genetic activities of leaf extract were tested according to the method described by **[Baeshin (1976)]**. A dense conidial suspension of *A. terreus* was made and number of conidia/ml was estimated using a hemocytometer. 5 ml of this suspension was immediately added to 5 ml of the *R. stricta* leaf extract and one ml sample of this mixture was immediately diluted in 9 ml sterile distilled water to serve as untreated (positive) control. Subsequent samples were taken at regular intervals (every 15 minutes

over one hour time of exposure) and serially diluted in sterile distilled water to halt the mutagenic treatment. Samples of the final dilutions containing about 100 conidia were spread on PDA plates and incubated for 4 days at 28°C. This was repeated for each of the four different concentrations of the leaves extract 3, 6, 12, 24 g/l.

Mutants were isolated according to the method described by **[Fincham *et al.* (1979)]**. At each time interval, a monoconidial inoculum was inoculated in each of 26 loci/plate containing PDA and served as a template. The template was in turn replicated on the minimal medium (DOX) to detect auxotrophic mutants. All replicates were incubated for 5 days at 28°C. Auxotrophic mutants were those which failed to grow on DOX after incubation for 5 days at 28°C. All mutant colonies were isolated on PDA templates and replicated on the following supplemented media for the determination of their nutritional requirements:

1. DOX
2. DOX + CAS + VITS – RNA
3. DOX + CAS - VITS + RNA
4. DOX - CAS + VITS + RNA
5. DOX + CAS + VITS + RNA

A colony which failed to grow on any of the media numbered 2, 3 or 4 requires the chemical missing from that medium. The auxanographic technique of **[Pontecorvo (1949)]** was used to specify the particular nutritional requirement of each mutant. One ml of a dense conidial suspension of the mutant was mixed with cooled molten DOX (45°C) in dishes and left to solidify. A few crystals of the nutrients to be tested were placed at marked positions around the periphery of the agar plate. Each mutant grew after 5 days incubation in the immediate vicinity of the nutrient required.

2- RAPD-DNA profile

Auxotrophic mutants were incubated for 5 days at 28°C in broth PDA media, then harvested and frozen in liquid nitrogen, ground with mortar and pestle and incubated in 1.5 ml of extraction buffer (100 mM Tris-HCl pH 8, 100 mM NaCl, 50 mM EDTA pH 8, 2% SDS and 0.1 mg/ml proteinase K) for 1.5 h at 37°C. DNA was extracted twice with chloroform:isoamyl alcohol (24:1) mixture, precipitated from the aqueous phase with 2 vol. cold 90° ethanol at -20°C during 48 h. DNA pellet was harvested by centrifugation, washed several times in 70°

ethanol, air-dried and dissolved in deionized water. RNase treatment was performed according to [**Scott *et al.*, (1991)**]. DNA was analyzed using agarose gel electrophoresis.

RAPD-PCR was carried out in 25 ul containing 30 ng DNA, 3 mM $MgCl_2$, 20 pmol of the primer (primer sequences are shown in Table 2), 1 U of AmpliTaq DNA polymerase, 250 puM of each of dCTP, dGTP, dATP, and dTTP in 10 mM Tris HCl (pH-8.3), 50 mM KCl, covered with a drop of mineral oil. A Perkin-Elmer TC480 thermal cycler was used for 40 cycles of amplification (94°C for 1 min, 36°C for 1 min, 72°C for 2 min) and a final extension cycle at 72°C for 7 min in RAPDs with 10-nucleotide-long primers. PCR amplification products were analyzed in 2.5 % (w/v) agarose gel in 1XTBE buffer and detected by staining with ethidium bromide (0.5 mkg/ml). DNA ladder 100 bp was used as a marker. Electrophoresis was carried out at 80 V for 3.5 h, and then the results were visualized under UV light and photographed.

Table (2): Primer sequences.

	Sequence	% G+C
OPA-16	5 TGC CTT GCA G,3	60%
OPB-03	5 CAT CCC CCT G`3	60%
OPA-04	5 GTC GAA CGA G`3	60%
OPA-02	5 AGC CTT CGC T`3	60%

3- Protein profile

Total extracted proteins were separated by denatured polyacrylamide gel electrophoresis (SDS-PAGE), with 8% (w/v) stacking gel and separation gel 12% (w/v). Protein samples were solidilized in loading buffer (1% SDS, 1% mercaptoethanol, 20% glycerol, 0.02% bromophenol blue, 0.01 M Tris-HCl, pH 8.0) at 100°C for 5 min before electrophoresis. Electrophoresis was performed at room temperature for approximately 2 h, and the system was programmed to a two-step mode with applying constant current 10 mA in stacking gel and 20 mA in the separation gel. Gels were stained with silver nitrite stain.

F. Statistical Analysis

Data of survival percentage and frequency of auxotrophic mutants were subjected to linear regression analysis for the detection of linear relationship between concentration of the extract or time of exposure and

survival percentage or auxotrophic mutants frequency using Microsoft Excel 2003 for MS-Windows.

RESULTS

The survival percentage and recovery of auxotrophic mutants among survivors of *Aspergillus terreus* cultured cells, as a function of treatment with different concentrations of leaves extract of *Rhazya stricta*, are shown in the figures from 1 to 7. As apparent, the increase in concentration and exposure time led to a decrease in survival percentage and an increase in auxotrophic mutation percentage as confirmed by linear regression calculation.

The highest possible percentage of mutation (1.7%) was achieved with the dose of 6 g/l of leaves extract of *Rhazya stricta* at exposure time of 45 minutes, which is the optimal dose for the induction of auxotrophic mutants. This dose is similar to the prescribed dose in folklore medicine.

A list of auxotrophic mutants recovered from *Rhazya stricta* leaves extract treated conidia of *Aspergillus terreus* is presented in table (3). All (fourteen) mutants were amino acid –requiring ones. Two of the mutants restore growth with either arginine or proline. They are probably leaky auxotrophic mutants; they fail to completely prevent the action of a gene and permit some residual functions.

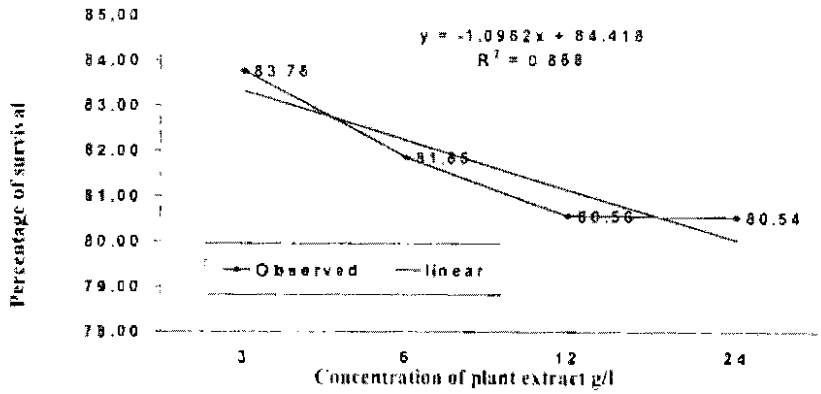


Fig. (1): Relationship between concentration of the leaves aqueous extract of *Rhazya stricta* and average of percentage of survival in *Aspergillus terreus*.

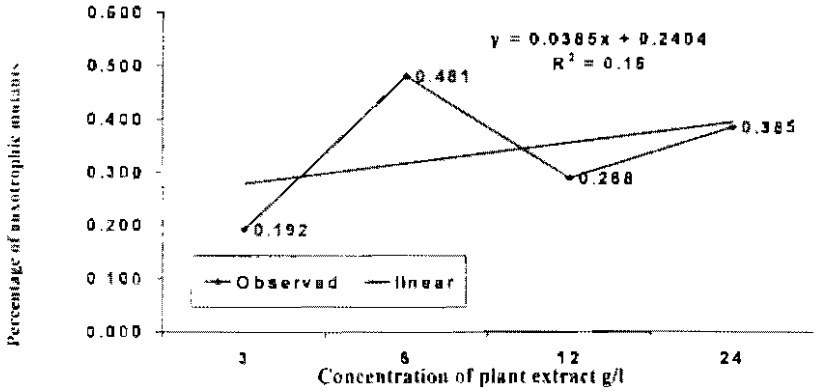


Fig. (2): Relationship between concentration of the leaves aqueous extract of *Rhazya stricta* and average of percentage of auxotrophic mutants in *Aspergillus terreus*.

Results of the molecular analysis for all auxotrophic mutants are shown in table (4) and figures (3-4). DNA quantity decreased significantly ($P > 0.05$) compared to the wild type which is a consequence of molecular changes in the genetic material of *A. terreus*. The RAPD-PCR results illustrated in table (5) and figure (16) show polymorphic numbers of the genetic bands, which were the electrophoretic products of PCR for all mutants, compared with the wild type. Table (5) shows that the highest number of polymorphic bands (80 DNA bands) among mutants was generated in reactions with the primers OPA-16, and the lowest number of polymorphic bands was obtained with primer OPB-03 (48), whereas the number of polymorphic bands of both primers OPA-02 , OPA-04 were 65 and 50, respectively. The percentage of polymorphic bands to source of polymorphism are 32.92, 19.75, 20.58 and 26.75 for OPA-16, OPB-03, OPA-04 and OPA-02, respectively.

The results of total protein profile and SDS-PAGE technique are shown in table (4) and figures (6-7). These results express a decrease in the total protein in all treatments, compared to wild type, and a polymorphism of protein bands. All these results strongly point out to mutagenicity of the leaves extract of *R. stricta*.

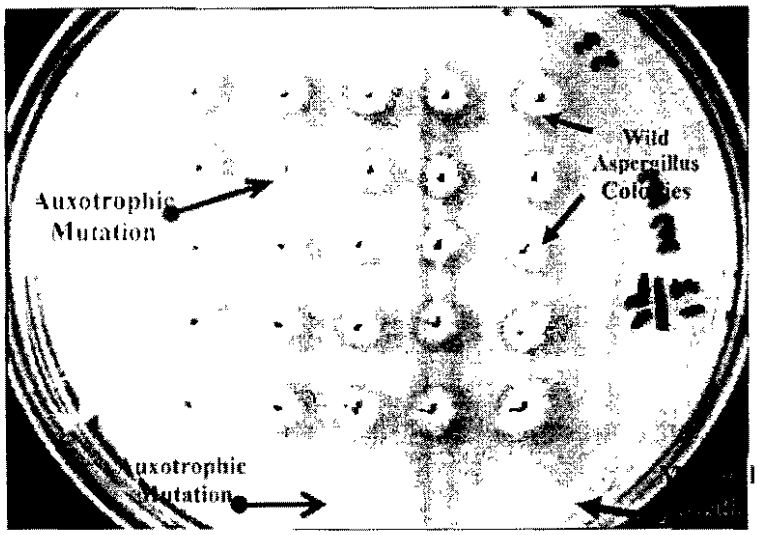


Figure (3): Auxotrophic mutants in *Aspergillus terreus* caused by leaves aqueous extract of *Rhazya stricta*.

Table (3): List of auxotrophic mutants recovered from *Rhazya stricta* leaves extract treated conidia of *Aspergillus terreus*.

Code No.	Extract concentration (g/l)	Treatment (min)	Requirement
AMR3T45	3 g/l	45min.	Cystine
AMR6T60A	6 g/l	60 min.	
AMR3T60	3 g/l	60 min.	Lysine or histidine
AMR6T30	6 g/l	30 min.	
AMR6T45A	6 g/l	45 min.	Tyrosine
AMR12T60B	12 g/l	60 min.	
AMR6T60B	6 g/l	60 min.	Glutamine
AMR24T30	24 g/l	30 min.	
AMR12T60A	12 g/l	60 min.	
AMR12T45	12 g/l	45 min.	Alanine
AMR6T45B	6 g/l	45 min.	Arginine
AMR24T45	24 g/l	45 min.	Proline or Arginine
AMR24T60A	24 g/l	60 min.	
AMR24T60B	24 g/l	60 min.	Methionine or Proline

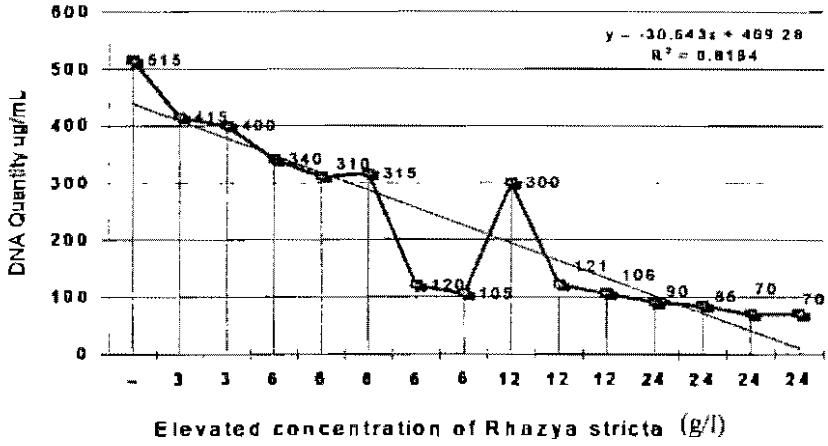


Fig. (4): Relationship between concentration of the leaves aqueous extract of *Rhazya stricta* and DNA quantity of mutants.

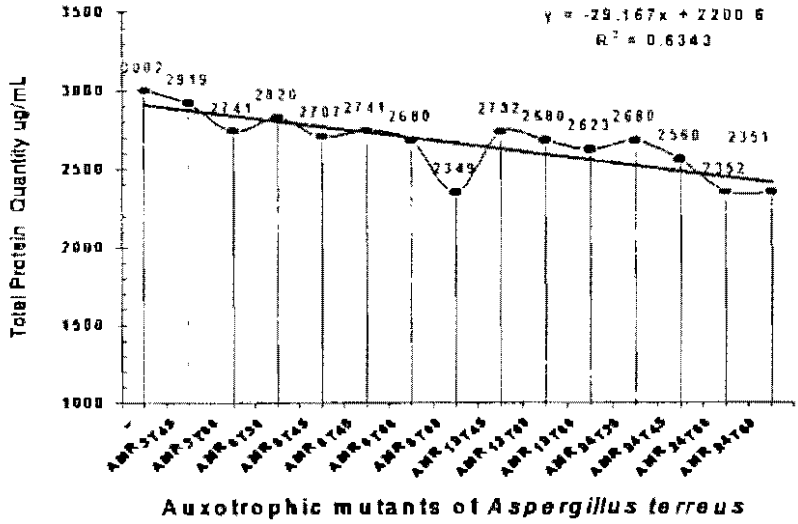


Fig. (5): Relationship between auxotrophic mutants and percentage of protein quantity of all mutants; compared to wild type.

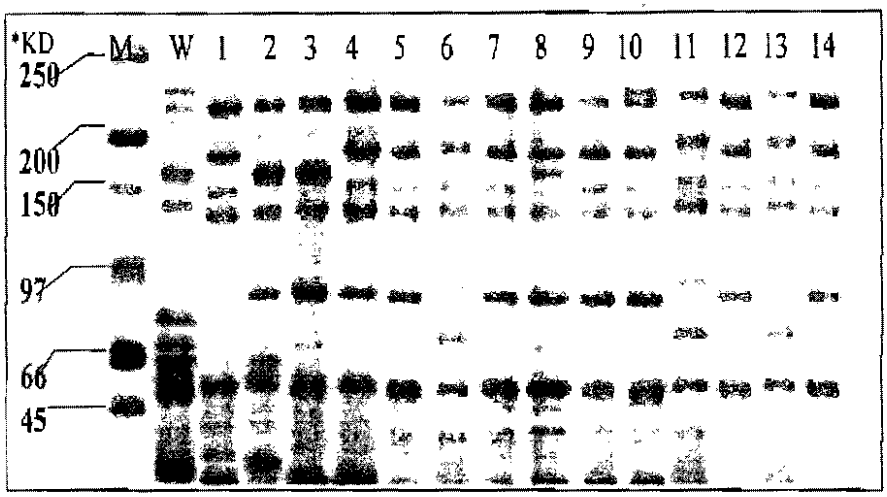
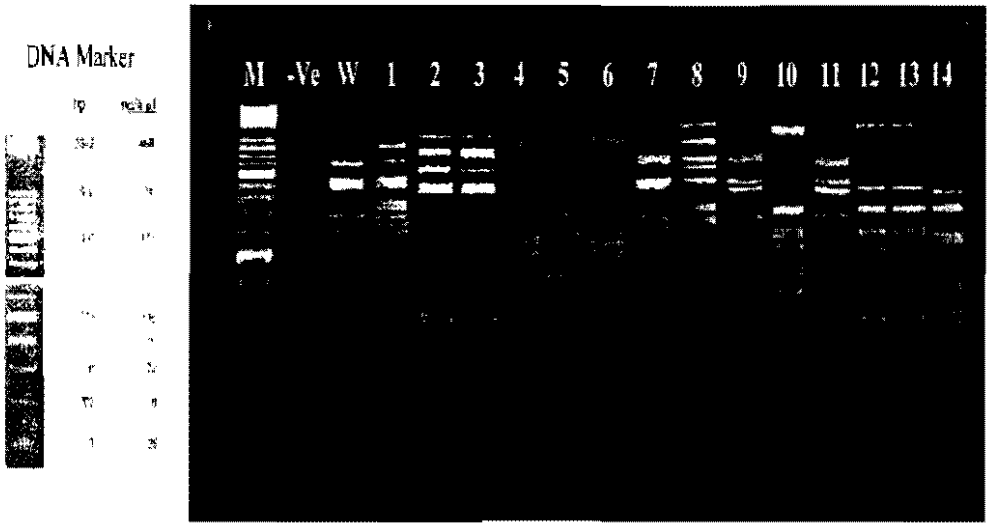


Fig. (6): Protein bands of auxotrophic mutants in *Aspergillus terreus* treatment with leaves aqueous extract of *Rhazya stricta*.

Table (4): Quantities of DNA and Total Protein isolated from wild type and auxotrophic mutants of *Aspergillus terreus*.

Strains	Treatments		DNA quantity ug/mL	Total protein quantity ug/mL	% decreasing of total protein
	Time (minutes)	Con. (g/l)			
WT	–	–	515	3002	0
AMR3T45	45	3	415	2919	2.76
AMR3T60	60	3	400	2741	8.69
AMR6T30	30	6	340	2820	6.06
AMR6T45A	45	6	310	2707	9.83
AMR6T45B	45	6	315	2741	8.69
AMR6T60A	60	6	120	2680	10.73
AMR6T60B	60	6	105	2349	21.75
AMR12T45	45	12	300	2732	9.66
AMR12T60A	60	12	121	2680	10.73
AMR12T60B	60	12	106	2623	12.62
AMR24T30	30	24	90	2680	10.73
AMR24T45	45	24	85	2560	14.27
AMR24T60A	60	24	70	2352	21.65
AMR24T60B	60	24	70	2351	21.68



14	13	12	11	10	9	8	7	6	5	4	3	2	1
(AMR24T60B) الطفرة	(AMR24T60A) الطفرة	(AMR24T45) الطفرة	(AMR24T30) الطفرة	(AMR12T60B) الطفرة	(AMR12T60A) الطفرة	(AMR12T45) الطفرة	(AMR6T60B) الطفرة	(AMR6T60A) الطفرة	(AMR6T45B) الطفرة	(AMR6T45A) الطفرة	(AMR6T30) الطفرة	(AMR3T60) الطفرة	(AMR3T45) الطفرة

Fig. (7): RAPD-PCR product profile of auxotrophic mutants of *Aspergillus terreus* treated with leaves aqueous extract of *Rhazya stricta* for OPA-04 primer.

Table (5): Polymorphic bands of genetic primers and percentage polymorphism in auxotrophic mutants of *Aspergillus terreus*.

Primers	Total bands	Monomorphic bands to control* bands No.	Polymorphic bands to control* bands No.	% Polymorphic bands to total bands of primer	% Polymorphic bands to source of polymorphism	% Polymorphic bands to total bands
OPA-16	129	49	80	62.02	32.92	19.42
OPB-03	91	43	48	52.74	19.75	11.65
OPA-04	98	48	50	51.02	20.58	12.14
OPA-02	94	29	65	69.15	26.75	15.78
Total	412	169	243	----	----	----

* Control = wild type of *A. terreus*

DISCUSSION

We found that dose and exposure time to *Rhazya stricta* leaves extract are inversely proportional to survival percentage, which means hat an increase in the dose and exposure time are met by a decrease in survival percentage, whereas mutation percentage increased as dose and exposure time increased. Our results are in general agreement with the rule mentioned by [**Fincham *et al.*, (1979)**], who stated that by using chemical mutagens, there was a constant relation between the dose and mutation percentage which increases to a certain limit with the increase in dose. This provides additional evidence for the mutagenicity of this extract demonstrated, for the first time, by [**Baeshin *et al.*, (2005)**] in *S. cerevicae*.

The strong mutagenic activities of *Rhazya stricta* leaf extract are comparable to the potent chemical mutagenic agent Nitrosoguaniline (NTG). It was found by [**Baeshin & Sabir (1987)**] that NTG produced 3.8% of auxotrophs in *A. terreus* with the optimal dose of 0.0075 g/10 ml at 70 min. exposure, whereas *Rhazya stricta* leaves extract in the present study gave a percentage of 1.7% of auxotrophic mutants with the dose of 6 g/l at 45 min. of exposure.

Most of the auxotrophic mutants obtained from treatment of the wild type strain of *A. terreus* by *Rhazya stricta* leaves extract, were amino acids-requiring mutants, specifically Arginine – Proline or Cystine – Methionine requiring mutants, suggesting the selected target locus of this extract at the molecular level. Similar results were obtained by [**Tayl**

(1975); Baeshin (1976); Baeshin & Sabir (1987) and Sabir (2005)]. This observation led to a molecular study of DNA and protein of the auxotrophic mutants compared to wild type. The investigated DNA and protein quantities revealed a significant decrease in all auxotrophic mutants compared to the wild type, suggesting molecular changes as a deletion in one or more loci which affect gene expression and interruption in biochemical pathways of DNA and protein synthesis, consequently as alkaloids in *Rhazya stricta* leaves extract often do. These results are consistent with the results obtained by **[David *et al.*, (1997); Adam *et al.*, (2000) and Morita *et al.*, (2005)].**

Polymorphic DNA bands were obtained in mutants comparing to wild type with four RAPD primers used. This observation provides good evidence to the ability of *Rhazya stricta* extract to induce point mutation as a result of change sequencing of at least one nucleotide as revealed by the polymorphic of many DNA bands, as compared with wild type. Some of the components of *R. stricta* may act as intercalation agent that generates free radicals which interact with DNA to account for the observed deletions, as suggested by similar results obtained by **[Ansah et *al.*, (2005)]** in their study with *Cryptolepis sangvinolehta*. The obtained results of SDS-PAGE- protein profile also suggest the ability of the leaf extract of *R. stricta* to induce frame shift mutation in *A. terreus*.

REFERENCES

Adam, S. E. A., Al–Farhan, H. and Al–Yahya A. (2000): Effect of combined *Citrullus colocynthis* and *Rhazga stricta* use in Najdi sheep. American Journal of Chinese Medicine, 28(3-4): 385–90.

Ageel, A. M., Mossa, J. S., Al-Yahya, M. A., Tariq, M. and Al-Said, M. S. (1987): Plants Used in Saudi Folk Medicine. King Saud University Press, Riyadh, Saudi Arabia.

Ahmad, Y., Fatima, K. and Le-Quesne, P. W. (1983): Further alkaloidal constituents of the leaves of *Rhazya stricta*. Phytochem., 27: 1017-1019.

Ali, B. H., Al-Qarawi, H., Mousa, H. M. and Bashir, A. K. (2000): Concentration of amino acids in brains of mice treated with the traditional medicinal plant *Rhazya stricta* (Dence). Indian J. Pharmacol., 32: 253-254.

Ali, B. H., Bashir, A. K. and Tanira, M. O. (1998): The effect of *Rhazya stricta*, a traditional medicinal plant, on the forced swimming test in rats. Pharmacol. Biochem. Behav., 59: 547-550.

Ali, B. H., Bashir, A. K., Tanira, M. O. and Banna, S. (1995): Some nervous system actions of *Rhazya stricta* in mice. Clin. Exp. Pharmacol. Physiol., 20: 496-502.

Al-Yahya, M. A., Al-Meshal, I. A. Mossa, J. S., Al-Badr, A. A. and Tariq, M. (1990): Saudi Plants: A Phytochemical and Biochemical Approach. King Saud University Press, Riyadh, Saudi Arabia.

Ansah, C., Khan, A. and Gooderham, N. J. (2005): *In vitro* genotoxicity of the West African anti-malarial herbal *Cryptolepis sanguinolenta* and its major alkaloid cryptolepine. Toxicology, 1(1):141-147.

Baeshin, N. A. (1976): The Genetics of *Nectria cosmariospora* Ces. & De-Not. Ph. D. Thesis, The University of Dundee, Dundee, Scotland, U.K.

Baeshin, N. A. and Sabir, J. M. (1987): Some genetic studies on *Aspergillus terreus* Thom. Researches Sci., K.A.U., 222: 215-224.

Baeshin, N. A., Twaty, N. and Al-Hebshi, A. (2005): Evaluation the genotoxicity of *Rhazya stricta* leaves extract by the *Saccharomyces cerveisiae* auxotrophic mutats test. Egyp. J. Natu. Toxi., 2: 87-100.

David, B., Sevenet, T., Thoison, O., Awang, K., Pais, M., Wright, M. and D. Guenard (1997): Hemisynthesis of rhazinilam analogues: structure–activity relationships on tubulin–microtubule system. Bioorganic & Medicinal Chemistry letters, 7(17): 2155–2158.

Fincham, J. R. S., Day, P. R. and Radford, E. (1979): Fungal Genetics. University of California Press. Berkeley and Loss Angeles.

Morita, H., Awang, K., Hadi, A. H., Takeya, K., Itokawa, H. and Kobayashi, J. (2005). Conformational analysis of rhazinilam and three-dimensional quantitative structure-activity relationships of rhazinilam analogues. Bioorg. Med. Chem. Lett., 15(4): 1045-1050.

Mukhopadhyay, S., Handy, G. A., Funayama, S. and Cordell, G. A. (1981): Anticancer indole alkaloids of *Rhazya stricta.* J. Nat. Prod., 44(6): 696-700.

Pontecorvo, G. (1949): Auxanographic technique in biochemical genetics. J. Gen. Microbiol., 3: 122 - 127.

Sabir, J. S. M. (2005): Assaying the single and combined mutagenicity of *Calotropis procera* Ait. latex and Chlorcyrin in *Aspergillus terreus*. J. Agri. Res., Tanta Univ., 31 (4-B): 820-839.

Scott, D., Galloway, S. M., Marshall, R. R., Ishidate, M. Jr., Brusick, D., Ashby, J. and Mybr, B. C. (1991): Genotoxicity under Extreme Gultue Conditions. Mutat. Res., 257: 147–204.

Tanira, M. O., Ali, B. H., Bashir, A. K., Dhanasekaran, S.; Tibirica, E. and Alves, L. (2000): Mechanism of the hypotensive action of *Rhazya stricta* in rats. Pharmacol. Res., 41(3): 369-387.

Tayl, A. A. (1975): Genetical studies with *Nectria comariospora* Ces. & De-Not., Ph. D. Thesis, The University of Dundee, Scotland, U. K.

تقييم كيموحيوي وجزيئي للتاثير الوراثي للمستخلص المائي لأوراق نبات الحرمل *Rhazya stricta* باستخدام فطر اسبرجلس تيريس *Aspergillus terreus*

نبيه عبدالرحمن باعشن – سمير حسن قاري* – جمال صابر محمد صابر – أحمد محمود الهجن**

قسم الأحياء – كلية العلوم – جامعة الملك عبدالعزيز ص.ب ٨٠٢٠٣ ، جدة ٢١٥٨٩ – المملكة العربية السعودية

*قسم الأحياء – كلية المعلمين جامعة أم القرى بمكة المكرمة ص.ب ٢٢٠٣ – المملكة العربية السعودية بريد الكتروني : sqarinet@gmail.com

**قسم الأحياء الطبية – كلية الطب –جامعة الملك عبدالعزيز ص.ب ٨٠٢٠٣ ، جدة ٢١٥٨٩ – المملكة العربية السعودية

تمت معاملة معلقات كونيدية كثيفة من فطر اسبرجلس تيريس بتركيزات متصاعدة من المستخلصات المائية لأوراق نبات الحرمل البري. وقد اخذت العينات على فترات منتظمة لكل معاملة لرصد النسبة المئوية للبقاء وطفرات العوز الغذائي بالإضافة إلى اجراء اختبارات وراثية جزيئية شملت البصمة الوراثية من خلال تقنية الـ RAPD-PCR والبصمة البروتينية من خلال تقنية الـ SDS-PAGE.

أشارت النتائج الى أن للمستخلص تأثير مطفر وإبادى قوى. فقد انخفضت النسبة المئوية للبقاء بزيادة التركيز أو بزيادة مدة التعريض لكل تركيز. كما أن معدل حدوث طفرات العوز الغذائي زاد بازدياد التركيز أو بزيادة مدة التعريض. وقد كانت جميع طفرات العوز الغذائي تسبب الاحتياج لأحد الأحماض الأمينية ، وبعزل الحمض النووي لكل طفرة على حده لوحظ انخفاض معنوى لكمية الدنا مقارنة بالسلالة البرية ، وكذلك الحال بالنسبة لكمية البروتين الكلي فقد حدث لها انخفاضاً معنوياً. وقد أظهرت نتائج كل من المضاعفة العشوائية لقطع متباينة من الدنا RAPD والتفريد الكهربي للبروتين تبايناً للحزم الوراثية وحزم البروتين على التوالي مقارنة بالسلالة البرية. مما يؤكد أن مستخلص أوراق نبات الحرمل مستحدث للطفرات الجينية.