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THE MUTAGENIC EVALUATION OF THE MEDICINAL PLANT BLACK NIGHTSHADE SEEDS EXTRACTS

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ABSTRACT: The mutagenicity of the medicinal plant, black nightshade (*Solanum nigrum L.*) was assayed through a battery of short systems. These systems included prophage induction, transduction assays, and chromosomal abnormalities in *Vicia faba L.* root tips. The two bacteriophages F_{116} and, AMS that are used in 4 lysogenic bacterial cells, showed a positive mutagenic effects depending on the concentrations of the seed extract. This was correlated with high increase in phage induction and percent of clear plaques. Transduction frequencies of streptomycin and tetracycline resistance genes were also affected. However, by using 10% of the seed extract with different concentrations of the powerful mutagenic agent EMS, the extract showed a remarkable anti-mutagenic response against EMS in prophage induction and transducing ability. These results agreed with the cytological examination of *Vicia faba L.* root tips that treated with seed extract. The mitotic index was reduced and many chromosomal abnormalities have been observed. The abnormalities included compact and non-compact nuclei, formation of binucleate and chromosomal aberrations such as fragments and bridges.

Key words: Microbiology, cytology, solanaceae, mutagenicity, aberrations, nightshade.

INTRODUCTION

Solanum nigrum L. is a low branching annual 1 to 2 m tall with triangular stems that bear oral thin-textured, alternate leaves with wavy margins. The tiny white flowers borne in drooping cluster on lateral stalks between the leaves. The berry fruits is green when immature, purplish black when ripe (Kothekar, 1987). *S. nigrum L.* belongs to the solanaceae family and is considered an important weed among more than 30 crops in several countries.*S. nigrum L.* is shown to be diploid (2n =24) with 12 bivalents , most of which are closed, and having a regular meiosis (Andrada *et al.*, 2003). It may be seen by the wayside and it often found on rubbish, but also among growing crops and in damp and shady places, it also often occurs in cultivated ground (Dafni and Yaniv, 1994).

Solanum nigrum L. has a high nitrate content (39g NO_3/kg DM) (Vogal and Gutzwiller, 1993). The *S. nigrum L.* has a high protein value as well as low fibre content which could make it suitable for the extraction of leaf proteins for use as a low cost source of proteins (Awasthi and Tandon,1988).The plant has neuropharmacological property on experimental animals and prolonged pentobarbital induced sleeping time, produced the exploratory behaviour pattern, and suppressed aggressive behaviour(Perez *et al.*, 1998). The plant can decreas the ulcer index significantly (Akhtor *et al.*, 1989). *S. nigrum L.* extracts inhibit also the formation of nitrosodiethanolamine which induces tumours (Aruna *et al.*, 1991). However, the presence of leaf extacts of *S. nigrum L.* in the reaction mixture containing calf thymus DNA and a free radical generating system can protect DNA against oxidative damage to its deoxirybose sugar moiety (Sarwat *et al.*, 1995). The historical use of *S. nigrum L.* in primitive anesthesia was reported (Carter, 1996). *S. nigrum L.* plants have a hepatoprotective, antiseptic, antispasmodic, immunomodulating anticonvulsant and antiinflammatory activities (Nadeem and Hussain, 1996). Moreover, *S. nigrum L.* is a molluscicidally active against biomphalaria alexandring, bulinus truncatus and lymnaea caillandi (Kloops *et al.*, 1987 and Shoeb *et al.*, 1990).

Although *S. nigrum L.* has been used extensively as a medicinal plant, but few studies have been carried out in order to evaluate its mutagenic activity. The aim of this study is to determine the mutagenicity of *S.nigrum L.* using two different models. The prokaryotic and eukaryotic models, prophage induction and transduction assays and root tips of *Vicia faba L.* have been employed in this investigation.

MATERIALS AND METHODS

The investigation was conducated during 2002-2004 at Microbial Genetics Lab and Cytology Lab, Dept. Genetics, Fac. Agric., Univ.Zagazig.

A. Materials

1. *Solanum nigrum L.* seed extract

A 250g of seeds dried materials was added to 60% alcohol, then set up for 2-3 days at room temperature then filtered. The unfiltered material retreated again using 60% alcohol, then evaborated using a rotary evaborator. The solvent,dimethyl sulphonate (DMS), added to the dried material in 0.25% to obtain the aqueous extract of *S. nigrum L.* The seeds were obtained from Prof. E. Sammour,National Researsh Center, Doki, Giza.

2. Growth media

Nutrient agar (NA) and nutrient broth (NB) media were prepared according to manufactures' instruction. NA composition was (g/l) as follow: pepton (3); yeast extract (5); glucose (15). Nutrient broth composition was (g/l): pepton (3); yeast extract (5); and glucose (10). Soft agar (0.8% w/v agar) was prepared in distilled water and kept at 48°C on water bath. The antibiotics were added as sterilized solutions by filtration through (0.2 um, Whitman) to the media after autoclaving. The concentration of streptomycin was (12 mg/ml) and for tetracycline was (200 ug/ml). The two antibiotics were dissolved in distilled water.

3. Bacteriophage and bacterial strains

Two phages were used in this study, phage F_{116} as a generalized transducing phage (Holloway *et al.,* 1960; Pemberton, 1973, Moller *et al.,* 1974) and AMS phage was isolated through our lab. The two phages propageted on *P. aureginosa H.*

bacterial strains and lysogenized most of them. The phages were also generalized transducing particles.

Pseudomonas aeruginosa H. bacterial strains, which have been used in this study, are listed in Table 1. The bacteriophage F116 and *P. aeruginosa H.* bacterial strains have been obtained from Prof. M. Day, Applied Biology Dept. Wales University,UK.

Table 1: Bacterial strains of *Pseudomonas aeruginosa* H.

Strains	Characteristics
PAO1	Prototrophic,str.senstive,tet.senstive
PU21	Ouxotrophic,str.senstive,tet.senstive
MAM2	Ouxotrophic,str.senstive,tet.senstive
Lys.1*(PAO1/F116)	Ouxotrophic,str.senstive,tet.resistant
Lys.5 (PU21/F116)	Prototrophic,str.senstive,tet.senstive
Lys.7(MAM2/F116)**	Ouxotrophic,str.resistant,tet.senstive
Lys.9 (PAO1/AMS)	Ouxotrophic,str.senstive,tet.resistant

Str = streptomycin Tet= Tetracycline
* Lys.1 = Tetracycline resistant gene doner
** Lys.7 = streptomycin resistant gene doner

4. Seed material for cytological system

The variety Giza 3 (G3) of *Vicia faba L.* was used in the present study. The *Vicia faba L.* seeds were obtained from Prof. Fayed, A.H., Genetic Dept., Fac. Agric. Zagazig Univ.

B. Methods

1. Treatment of *S. nigrum L.* seed extracts on prophage induction

NB flasks with different concentrations of the seed extract were prepared, inoculated by lysogen cells and incubated at 30°C for 24h. These cultures were contrifugated at 5000 rpm for 30 min, a few drops of chloroform were added, filtrated and recentrifuged at 12.000 rpm, for 20 min. The supernatants were assayed and Pfu/ml (Plaque forming unit per ml) for each concentration was calculated. Pt/Pc percent has been calculated by dividing Pfu/ml in each concentration (Pt) by Pfu/ml of control (Pc) as a percentage. The pfu/ml of each concentration (Pt) was divided by Pfu/ml of the back ground release of lys.1 (Pk), as a percentage resulting in pt/pkpercent.

Recipient cells were grown in NB flasks at overnight, washed 2-3 times by phosphate buffer (PH 7.00) and then resuspended. Viable counts of the recipient strains were calculated. Equal volume (0.1ml) of phage lysate and recipient cell suspensions were mixed and kept for 15-30 min at room temperature, to allow phage adsorption.

2. Treatment of *S. nigrum L.* seeds extract on transducing streptomycin and tetracycline resistance genes

Phage lysate, recipient cells and different concentrations of seed extracts were mixed together. Using the selective media, the transductants were counted and the transduction frequency was calculated. The number of observed transductants per each concentration was divided by number of transductants that observed in control in order to calculate Tt(transductants in each concentrations) / Tc(transductants in control) Tt/Tc percent.

3. Treatment of *S. nigrum L.* seed extract on *Vicia faba L.* root tips

The seeds of *Vicia faba L.* were soaked in distilled water for 15 hours. The soaked seeds were germinated on damp blotting paper in petri-dishes at room temperature 20°C in dark for 5 days. Apices of young primary roots were cut and then the seedlings were placed again in petri-dishes under the previously mentioned conditions to obtain the lateral roots. When laterals were about 2cm long, whole seedlings with their root systems were used to treat with the different concentrations of *S. nigrumL.* seed extract. The whole seedlings with their root systems were treated for 12 and 24 hours at 20°C with freshly prepared solutions of seven different concentrations of the plant extract. The concentrations were 0.25, 0.50, 1.00, 1.50, 2.00, 4.00 and 10.00%.

For each treatment, 100 seedlings were taken in flask to which and excess of *S. nigrum L.* seed extract was added. After each treatment period, seedlings were washed thoroughly in running tap water for 1 hour and then cultivated in petri-dishes for recovery periods of 12 and 24 hours. For each treatment as well as its correspondent control, the lateral roots were cut and pretreated for 2 hours with 0.02% solution of colchicine and fixed in ethanol glacial acetic acid (3:1).

After 24 hours they were transfer to 70% ethyl alcohol and stored. The aceto-carmine staining method was used to stain the root-tip cells for all investigated points.

The percentage of mitotic index (MI) was estimated by counting the total number of dividing cells to the total cells examined for each treatment. The percentage of each mitotic phase was calculated by dividing the number of cells in this phase on the total number of dividing cells per treatment. The number of micronuclei was determined in interphase cells as well as in dividing cells. These estimations included the two different types of micronuclei, compact and non-compact. The total number of chromosomal aberrations were estimated in dividing cells. The percentage of aberrations was calculated by dividing the number of cells with chromosomal aberrations on the total number of dividing cells. The aberration included fragments, bridges and binucleat cells.

4. Statistical analysis

For microbial system , the data were statistically analysed using standard deviation. Percentage of transducing particles was calculated by dividing number of transduction on number of plaques and S (survival cells) percent by dividing cfu/ml (cell forming unit per ml) of each dose on cfu/ml of zero dose as a percentage .Each value is the mean of three replica. For cytological system, the data were statistically analysed using Chi-square test by means of 2x2 contingency table.

RESULTS AND DISCUSSION

1. Mutagenic Activity of Seed Extraction of *S. nigrum L.* on Prophage Induction

The mutagenic activity of *S. nigrum L.* seed extraction was evaluated using the microbiol model of prophage induction assay. Two bacteriophases, F_{116} and AMS, were employed for this purpose using 4 lysogenic bacterial cells, (data are shown in Table 2 a,b,c and d). All the used concentrations of seed extraction seemed to have a mutagenic activity ranged from moderate to high response. This was correlated with observed increasing in the induced phages. The induced phage ranged from 35.2 up to 80.1 in the four lysogens when (con.10%) of the seed extract was tested.

2. Effect of *S. nigrum L.* Seed Extraction on Transduction

The influence of seed extract of *S. nigrum L.* on transducing two different genes, streptomycin and tetracycline resistances genes, was assessed .Results are shown in Tables 3 and 4 for the both genes. All the used concentrations (0.25% up to 10%) were found to have a stimulating influence on transducing the two genes . Transduction frequencies ranged from 1.6 x 10^{-5} up to 8.9 x 10^{-5} and from 1.96 x10^{-5} up to 14.3x 10^{-5} for streptomycin and tetracycline resistance genes respectively. Moreover, the clear plaques were produced at the high concentrations only (1 up to 10 %). The ratio of clear to turbid plaques was nearly constant for prophage F_{116} (24.7 to 26.1) at concentration 10 %. It seems that this ratio may be dose- dependent for both bacteriophages.

3. Effect of EMS on Prophage F_{116} Induction and Transducing Streptomycin Resistance Gene

Ethylmethansulfonate (EMS) has been reported to have a powerful mutagenic activity. So the same concentrations of seed extraction have been used with EMS in prophage F_{116} induction (Table 5) and transducing streptomycin resistance geve (Table 6) as a positive control. 10 % of EMS resulted in dramatically increase in prophage induction (11.3 – fold increase) and in transduction frequency (15.1- fold increase) than those observed on control. However, the fold increase in prophage induction and transduction frequencies concentrations (2.0 – 10%) were observed to have a mutagenic response. Although a fold increase in transduation frequency was observed in the all used concentrations of the plant seeds.

4. Anti-mutagenic Activity of *S.nigrum L.* Seeds Extract Against EMS in Prophage Induction and Transduction

The highest dose of seeds extract (10%) used is this study was added to the same concentrations of EMS in order to asses the antimutagenic activity of seed extraction. The two microbial models, prophage induction and transduction assay, were also employed. The prophage indution of phage F116 has been dropped from 11.3 up to 3.7 (Table 7) whereas the frequency of transducting sterptomycin resistance gene has also been dropped from 15.1 up 4.1 (Table 8).

These results clearly showed that seeds of *S. nigrum L.* appeared to have an antimutagenic activity against the powerful mutagenic agent EMS. This effect might be due to compounds contained in this plant.

The ability of *S. nigrum L.* plant to inhibit the mutagenicity of mutagen agents was reported. Since, *S. nigrum L.* extracts showed a strong inhibitory effects towards the mutagenicity of 2 – amino, 3 methyl – imidazo, 4 – 5 quinoline, NQNO, and 4 – nitroquinoline- N – oxide *Salmonella typhimurium.* However, the extract of *S. nigrum L.* showed cytoxicity to *S. typhimurium.* Moreover, the immature fruit extracts of the plant exhibited strong cytotoxicity with dose dependence and induced significant DNA damage in human lymphocytes. However the plant is consumed as leafy edible vegetable in Taiwan (Yen *et al.*, 2001). Ethanol extract of *S. nigrum L.* had a cytoprotective role against gentamicin induced kidney cell damage in vitro. The extract also exhibited significant hydroxyl radical scavenging potential activity, so it has been suggested that the extract of the plant could be used as anti-oxidant and even anti-tumar agent Moreover, many plants extracts showed an antioxidant activity (Son *et al.*, 2003).

5. Effect of Seed Extract on Mitotic Index (MI) and Mitotic Abnormalities

The root tips of *Vicia faba L.* plants were grown in different concentrations of *S. nigrum L.* seed extract for 12 and 24 hrs in order to evaluate the mutagenic activity of this medicinal plant. The mitotic index was decreased from 12.18 up to 9.42 and from 11.51 up to 8.90 when using 10% of the seed extract for 12 and 24 hrs respectively (Table 9). However, the number of divided cells and frequency of mitotic phases increased espically when the root tips were exposed for 12 hrs.

Distribution of micronuclei either compact or non – compact was also enhanced all over the used concentrations (Table 10 and Figs. 1-3). The number of compact nuclei increased from 12 up to 53 at 12 hrs. . The influence of 12 hrs was more obvious than the influence of 24 hrs. It seems that the soaking time had no observed effect. Although the dose effect was clear. The reduction of the mitotic index might refect the mutagenic activity of the tested plant. This might due to the reduction of the number of cells enterring mitosis. However, the reduction in mitotic index could be interpreted also to the inhibition of nuclear protein synthesis essential in the cell cycle or due to inhibiting the formation of various metabolic events necessary for mitosis (Abdelsalam *et al.,* 1997).

The number of cells having chromosomal aberration including fragments and bridges (Fig. 4) was also seriously affected upon exposure to different concentration of seed extract. The fold increase reached 6 and 5 folds than those observed in control at 12 and 24 hrs. in addition, the number of cells with bionucliate (Fig. 5) was also influenced. Table 11 shows the Chi-square (X^2) values to evaluate the significant level of all treatments (concentrations and time of treatment) on these parameters.

The increase of mitotic abnormalities might reflect the mutagenic activity of the seed extract of *S. nigrum* It has been suggested that compact micronuclei might be formed from chromosomal fragments whereas the non- compact might result from the eliminated chromosomes during the mitosis cell division (Heseman and Fayed 1982). Chromosomal bridges could be attributed to the general stickiness of chromosome or may be due to chromosomal breakage and reunion (Hassan and Soliman, 1998).

Micronuclei might be produced from laggards or centric fragment. The induction of micronuclei indicates a mutagenic effect which may lead to loss of genetic materials (Hassan and Soliman, 1998).

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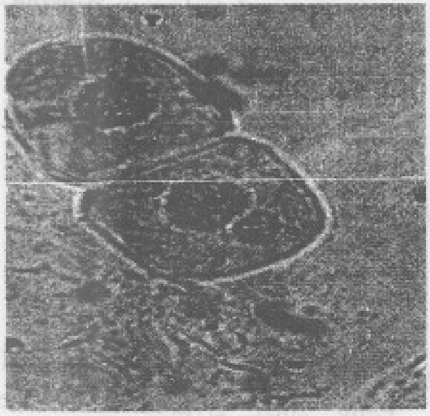

Fig. 1: Interphase cell with non-compact micronuleus

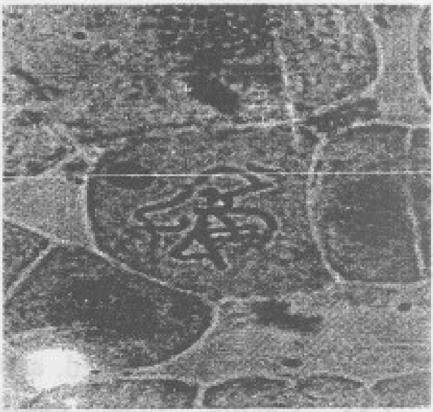

Fig. 2: Metaphase cell with non-compact micronuleus

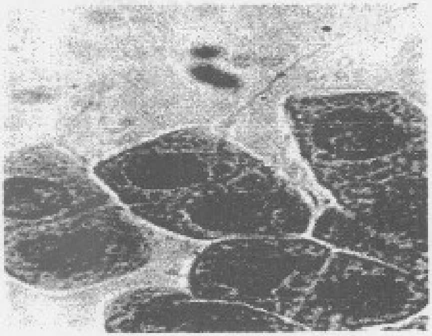

Fig. 3: Interphase cell with compact micronuleus

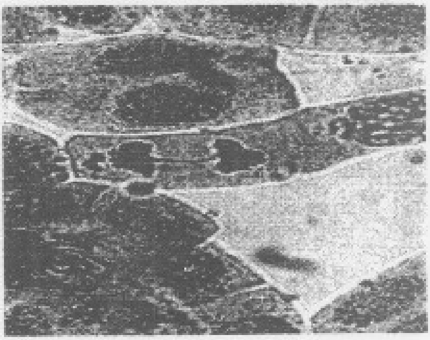

Fig. 4: Anaphase cell with bridge

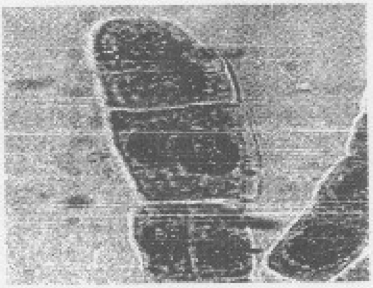

Fig. 5: Binucleate cell

Table 2 : Prophages induction upon exposure to seed extraction of *S.nigrum L.*

Concentrations %	T. plaques (pfu/ml × 10^5)	Induced phage	Mutagenic Response	C. plaques (pfu/ml × 10^5)	C/T %
a: Prophage F_{116} from Lys.1					
0.00	7.3±0.1	0.0	-	0.0	0.0
0.25	9.2±0.2	1.9	-	0.0	0.0
0.50	10.8±0.3	3.5	+	0.0	0.0
1.00	14.5±0.2	7.2	+	1.3±0.07	8.96
1.50	19.4±0.07	12.1	+ +	3.7±0.1	19.1
2.00	27.5±0.1	20.2	+ +	5.2±0.08	18.9
4.00	45.9±0.2	38.6	+ +	10.4±0.3	22.6
10.00	60.4±0.3	53.1	+ +	14.9±0.2	24.7
b : Prophage F_{116} from Lys.5					
0.00	5.1±0.2	0.0	0.0	0.0	0.0
0.25	8.5±0.1	3.4	-	0.0	0.0
0.50	11.4±0.2	6.3	-	0.0	0.0
1.00	13.2±0.3	8.1	-	0.0	0.0
1.50	14.7±0.09	9.6	-	2.2±0.03	14.96
2.00	19.2±0.2	14.1	+	3.5±0.1	18.2
4.00	25.7±0.08	20.6	+	5.4±0.07	21.01
10.00	40.3±0.1	35.2	+	10.1±0.2	25.06

Table 2: Continue

Concentrations %	T. plaques (pfu/ml × 10^6)	Induced phage	Mutagenic Response	C. plaques (pfu/ml × 10^6)	C/T %
c : Prophage F_{116} from Lys.7.					
0.00	6.3±0.1	0.0	-	0.0	0.0
0.25	9.2±0.2	2.9	-	0.0	0.0
0.50	11.7±0.1	5.4	+	0.0	0.0
1.00	13.4±0.3	7.1	+	0.0	0.0
1.50	17.2±0.09	10.9	+ +	0.0	0.0
2.00	22.4±0.2	16.1	+ +	5.1±0.07	22.8
4.00	31.9±0.3	25.6	+ +	7.9±0.1	24.8
10.00	60.1±0.3	53.8	+ +	15.7±0.2	26.1
d : Prophage AMS from Lys.9.					
0.00	8.7±0.2	0.0	-	0.00	0.0
0.25	11.4±0.3	2.7	-	0.00	0.0
0.50	17.9±0.2	9.2	+	0.00	0.0
1.00	19.2±0.3	10.5	++	0.00	0.0
1.50	22.7±0.2	14	+ +	5.5±0.2	24.2
2.00	27.3±0.3	18.6	+ +	9.1±0.08	33.3
4.00	61.4±0.4	52.7	+ +	21.5±0.2	35.01
10.00	88.8±0.3	80.1	+ +	37.2±0.3	41.9

T = Turbid C = Clear

- , + , and ++ no, moderate and high mutagenic response, respectively

Table 3: Effect of seed extraction of *S. nigrum L.* on transducing streptomycin resistance gene.

Concentrations %	No. ⚥ 10^4	⚥ / ♀	Fold increase	Mutagenic response
0.00	2.7±0.03	8.4×10^{-6}	0.0	0.0
0.25	5.4±0.07	1.6×10^{-5}	2	-
0.50	6.5±0.9	2.03×10^{-5}	2.4	-
1.00	7.1±0.1	2.2×10^{-5}	2.6	-
1.50	7.8±0.2	2.4×10^{-5}	2.9	-
2.00	18.5±0.3	5.8×10^{-5}	9.6	+
4.00	27.9±0.3	8.7×10^{-5}	10.3	+ +
10.00	28.4±0.4	8.9×10^{-5}	10.5	+ +

⚥ Transductants cells

♀ Recipient cells

- , + , and ++ no, moderate and high mutagenic response, respectively

Table 4: Effect of seed extraction of *S. nigrum L.* on transducing tetracycline resistance gene.

Concentrations %	No. ⚥ 10^4	⚥ / ♀	Fold increase	Mutagenic response
0.00	4.1±0.03	1.3×10^{-5}	0.0	-
0.25	6.3±0.07	1.96×10^{-5}	1.5	-
0.50	7.4±0.9	2.3×10^{-5}	1.8	-
1.00	8.9±0.6	2.8×10^{-5}	2.2	-
1.50	11.1±0.1	3.5×10^{-5}	2.7	-
2.00	19.5±0.1	6.1×10^{-5}	4.8	+
4.00	43±0.2	13.4×10^{-5}	10.5	+ +
10.00	45.8±0.3	14.3×10^{-5}	11.2	+ +

⚥ Transductants cells

♀ Recipient cells

- , + , and ++ no, moderate and high mutagenic response, respectively

Table 5 : Influence of EMS on prophage F_{116} induction.

Concentrations %	Pfu/ml 10^6	Mutagenic reponse	Fold phage	Fold increase
0.00	6.3±0.2	-	0.0	0.0
0.25	22.5±0.3	+	16.2	3.6
0.50	28.9±0.4	+	22.6	4.6
1.00	36.7±0.3	+	30.4	5.8
1.50	49.6±0.3	+	43.3	7.9
2.00	64.3±0.4	+ +	58	10.2
4.00	65.9±0.3	+ +	59.6	10.5
10.00	71.4±0.4	+ +	65.1	11.3

- , + , and ++ no, moderate and high mutagenic response, respectively

Table 6: Effect of EMS on transducing streptomycin resistance gene.

Concentrations %	No. ⚥ 10^4	Fold increase	⚥ / ♀	Mutagenic response
0.00	3.3 ± 0.03	0.0	0.8×10^{-5}	-
0.25	13.9 ± 0.07	4.2	3.6×10^{-5}	+
0.50	21.8 ± 0.09	6.6	5.6×10^{-5}	+
1.00	26.5 ± 0.1	8.0	6.8×10^{-5}	+
1.50	31.7 ± 0.2	9.6	$8.1. \times 10^{-5}$	+
2.00	40.8 ± 0.3	12.4	10.5×10^{-5}	++
4.00	43.6 ± 0.4	13.2	11.2×10^{-5}	+ +
10.00	49.9 ± 0.4	15.1	12.8×10^{-5}	+ +

⚥ Transductants cells

♀ Recipient cells

- , + , and ++ no, moderate and high mutagenic response, respectively

Table7: Antimutagenic activity of seed extraction (10%) against EMS in prophage F_{116} induction.

Concentrations %	Pfu/ ml 10^6	Fold increase	Mutagenic response
0.00	3.7 ± 0.07	0.0	-
0.25	4.2 ± 0.09	1.1	-
0.50	6.1 ± 0.09	1.6	-
1.00	7.0 ± 0.1	1.9	-
1.50	7.2 ± 0.3	1.9	-
2.00	11.3 ± 0.2	3.1	+
4.00	12.4 ± 0.4	3.4	+
10.00	13.7 ± 0.1	3.7	+

- , + , and ++ no, moderate and high mutagenic response, respectively.

Table 8: Antimutagenic activity of seed extraction(10%) against EMS in transuding Streptomycin gene.

Concentrations %	No. ⚥ 10^4	⚥ / ♀	Fold increase	Mutagenic response
0.00	4.5 ± 0.07	1.3 x 10^{-5}	0.0	-
0.25	5.7 ± 0.01	1.8 x 10^{-5}	1.3	-
0.50	6.3 ± 0.05	1.9 x 10^{-5}	1.4	-
1.00	7.2 ± 0.1	2.3 x 10^{-5}	1.6	-
1.50	8.4 ± 0.3	2.6 x 10^{-5}	1.9	-
2.00	14.7 ± 0.2	4.6 x 10^{-5}	3.3	+
4.00	17.3 ± 0.1	5.4 x 10^{-5}	3.8	+
10.00	18.6 ± 0.09	5.8 x 10^{-5}	4.1	+

⚥ Transductants cells

♀ Recipient cells

- , + , and ++ no, moderate and high mutagenic response, respectively

Table 9: Mitotic index (MI) and frequency of mitotic phases in *Vicia faba L.* root tips grown in seed extracts of *S. nigrum L.*

Soaking period	Concentrations %	No. of studied cells	No. of divided cells	MI %	Frequency of mitotic phases		
					Prophase	Metaphase	Ana-telophase
12 hrs	0.00	2971	362	12.18	207	51	104
	0.25	3440	410	11.91	234	66	110
	0.50	4051	458	11.30	261	77	120
	1.00	5549	606	10.92	342	108	156
	1.50	6304	674	10.69	380	124	170
	2.00	6870	718	10.45	404	135	179
	4.00	7650	785	10.26	440	157	188
	10.00	8501	801	9.42	443	166	192
24 hrs	0.00	1216	140	11.51	70	30	40
	0.25	1065	120	11.26	56	32	32
	0.50	2290	240	10.48	111	67	62
	1.00	2776	282	10.15	130	80	72
	1.50	3530	350	9.92	160	102	88
	2.00	4075	386	9.40	174	119	93
	4.00	4180	384	9.18	172	122	90
	10.00	3960	352	8.90	155	115	82

Table 10: Mitotic abnormalities in *Vicia fabaL.* root tips grown in seed extracts of *S.nigrum L.*

Soaking period	Concentrations %	No. of studied cells	No. of divided cells	Distribution micronuclei		No. of cells with chr.aberrations			No. of cells with Binucliate
				Compact	Non-compact	Fragment	Bridge	Total	
12 hrs	0.00	2971	362	12 (0.40)	2 (0.06)	-(-)	4 (1.10)	4 (1.10)	53 (14.60)
	0.25	3440	410	15 (0.43)	3 (0.08)	- (-)	5 (1.21)	5 (1.21)	62 (15.12)
	0.50	4051	458	18 (0.44)	4 (0.09)	1 (0.21)	6 (1.31)	7 (1.52)	71 (15.51)
	1.00	5549	606	26 (0.46)	6 (0.10)	2 (0.33)	9 (1.48)	11 (1.81)	95 (15.67)
	1.50	6304	674	32 (0.50)	7 (0.11)	3 (0.44)	12 (1.78)	15 (2.22)	109 (16.17)
	2.00	6870	718	36 (0.52)	8 (0.12)	4 (0.55)	15 (2.08)	19 (2.64)	118 (16.43)
	4.00	7650	785	45 (0.58)	10 (0.13)	5 (0.63)	17 (2.16)	22 (2.80)	132 (16.81)
	10.00	8501	801	53 (0.62)	12 (0.14)	6 (0.74)	18 (2.24)	24 (2.99)	140 (17.47)
24 hrs	0.00	1216	140	5 (0.41)	1 (0.08)	1 (0.71)	2 (1.42)	3 (2.14)	21 (15)
	0.25	1065	120	5 (0.46)	1 (0.09)	1 (0.83)	2 (1.66)	3 (2.50)	19 (15.83)
	0.50	2290	240	12 (0.52)	3 (0.13)	2 (0.83)	4 (1.66)	6 (2.50)	39 (16.25)
	1.00	2776	282	16 (0.57)	4 (0.14)	3 (0.06)	5 (1.77)	8 (2.80)	46 (16.31)
	1.50	3530	350	21 (0.59)	6 (0.16)	4 (1.14)	7 (2.00)	11 (3.14)	58 (16.57)
	2.00	4075	386	25 (0.61)	7 (0.17)	6 (1.55)	8 (2.07)	14 (3.62)	65 (16.83)
	4.00	4180	384	27 (0.64)	8 (0.19)	6 (1.56)	9 (2.34)	15 (3.90)	65 (16.92)
	10.00	3960	352	27 (0.68)	8 (0.20)	6 (1.70)	9 (2.55)	15 (4.26)	62 (17.61)

( ) Percentage

Table 11: X^2 Values in comparison between the effect of aqueous seed extract of *Solanum nigrum L.* with in each soaking period on MI and frequency of cells containing micronuclei and chromosomal abnormalities as well as binucleate cells on *Vicia faba L.* root tips

Typs of comparison		X^2 Values			
		MI	Micronuclei	Chr.aberrations	Binucleat cells
	Control/ 025%	0.120	0.070	0.020	0.022
	Conrrol/0.50%	0.534	0.160	0.240	0.080
	Control/1.00%	2.049	0.350	0.710	0.126
12 hrs	Control/1.50%	3.265	0.700	1.569	0.290
	Control/2.00%	4.054*	0.990	2.610	0.400
	Control/4.00%	5.200*	1.940	3.090	0.610
	Control/10.00%	6.240*	2.700	3.626	1.030
	Control/0.25%	0.082	0.040	0.030	0.020
	Control/00.50%	2.110	0.330	0.044	0.069
	Control/1.00%	3.630	0.660	0.160	0.080
24 hrs	Control/1.50%	6.200*	0.900	0.320	0.125
	Control/2.00%	8.100**	1.050	0.670	0.150
	Control/4.00%	8.260**	1.450	0.897	0.180
	Control/10.00%	7.320**	1.770	1.190	0.330

* Significant ** Highly significant

التقييم الطفرى لمستخلص بذور النبات الطبى عنب الديب

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أجرى هذا البحث بهدف تقدير القدرة الطفورية للنبات الطبى *عنب الديب* ، وذلك من خلال عدة أنظمة. هذه الأنظمة تتضمن التأثير على مقدرة الفاج على الإدمصاص داخل خلية العائل البكتيرى وكذلك مقدرته على نقل المادة الوراثية والتأثير على تكوين الشذوذ الكروموسومي فى القمم النامية لجذور الفول البلدى.

ولقد تم استخدام نوعين من البكتريوفاج وهما F116 و AMS مع أربع أنواع من الخلايا البكتيرية الليسوجينية، حيث تم الحصول على نتائج إيجابية تعتمد على التركيزات المختلفة لمستخلص البذور من نبات *عنب الديب* . والتى كانت متفقة مع الإنتاج العالى من الفاج وكذلك مع نسبة المناطق الرائقة الغير عكرة الناتجة من تحليل الفاج للخلايا البكتيرية.

ولقداجريت دراسة تأثير المستخلص على نقل جين المقاومة للإستربتومايسين والتتيراسيكلين باستخدام ١٠% من تركيز مستخلص البذور مع عدة تركيزات مختلفة من المادة ذات القدرة الطفورية العالية EMS. حيث أعطى المستخلص تأثير ضد القدرة الطفورية للمادة EMS. وقد اتفقت هذه النتائج مع الاختبارات السيتولوجية على القمم النامية لجذور الفول البلدى التى عوملت بالمستخلص النباتى. حيث لوحظ أن دليل الانقسام الميتوزى MI يزداد بزيادة تركيز المستخلص، وأيضاً لوحظ زيادة فى نسبة الشذوذ الكروموسومي بزيادة تركيز المستخلص.

وهذه الشذوذ الكروموسومية تضمنت تكوين الأنوية الصغيرة المصمتة والغير مصمته وأيضا تكوين خلايا ذات نواتين وشظايا كروسومية وأيضا تكوين قنطرة كروموسومية.