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GENETICAL ASSESSMENT OF 2-NITROPROPANE VIA BATTERY OF SHORT SYSTEMS

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ABSTRACT: The mutagenecity of the 2-Nitropropane (2-NP) was assayed through a battery of short systems. These systems included prophage induction, transduction assays and survival percentages. The mutagenecity showed a positive mutagenic effect depending on the concentrations of 2-NP. However, the concentration of 2-NP, which gave the highest response, was 25 µg/ml. The induction of prophage F116 in 3 lysogenic bacterial cells were three or more fold increases than those observed in control. However, concentration 25µg/ml of 2-NP gave the highest fold increase in most lysogen strains ranged from 6.4 to 8.9, and gave the highest number of clear plaques in most strains ranged from 267 to 306 x 10^{13} pfu/ml. Transduction frequency dropped from 9.04 x 10^{-5} to 1.2 x 10^{-6} upon using 100µg/ml of 2-NP. No transductants were detected at 140µg/ml. The survival percentages of the tested strains were greatly affected when treated with 2-NP. At high concentration 140 µg/ml, it reached 2 - 3 %. It seem that 2-NP had a toxic effect on bacterial cells. Acridine orange and vitamin E were used as positive and negative control comparing with 2- NP. Fruit juice as a source of vitamins had a remarkable effect in reducing the mutagenic activity of 2-NP.

Key words: 2-Nitropropane, *Pseudomonas aureginosa*, prophage, lysogen, mutagenecity.

INTRODUCTION

2-Nitropropane is a colorless, oily liquid slightly soluble in water [17milliliter (mL)/Liter (L)] at 20°C. It has a mild odour and remains liquid over a relatively broad temperature range i.e. -93 to 120 °C. 2-NP is flammable, and volatile, its vapour forms flammable or explosive mixture with air. Its boiling point is 88.6 °C (Woo *et al.,* 1985).

There is no evidence that 2-NP and other nitroaliphatic compounds are produced by biological processes, although a related organic compound, β-nitropropionic acid, has been isolated from plants and microorganisms (Goldwhite, 1965).

Hoffmann and Rathkamp, (1968) reported that 1.1-1.2 microgram (ug) of 2-NP is present in the smoke from a single 85 millimeter United States of America blended non-filter cigarette.

However, Fiala *et al.,* (1987) found that dimethyl sulfoide (hydroxyl radical scavenger) caused a concentration-dependent decrease in mutagenecity in strain TA102 when assays were carried out at a constant level of 28 micromol (2-NP nitronate)/plate in Ames *Salmonella* assay.

In addition, 2-NP has a number of minor uses. These include a medium for chemical reactions, an intermediate for the manufacture of propane derivatives and a component of explosives, propellants and fuels for internal combustion engines. Although the addition of 2-NP to fuel improves diesel engine performance, it is not used commercially as a diesel fuel additive since superior alternatives are available (Anon, 1996).

General population exposure to 2-NP appears to be very low and is derived from cigarette smoke (1.1 to 1.2 mg / cigarette), from residues in coatings such as beverage can coating, adhesives and print, and from vegetable oils fractionated with 2-NP. (Finklea, 1997).

2-NP has been reported to be mutagenic in several strains of *Salmonella typhimurium* using the Ames test, both with and without an exogenous activity system S9 (Kawai *et al.,* 1997).

The importance of 2-NP as an industrial chemical stems mainly from its desirable and occasionally

unique characteristics as a solvent. It is excellent solvent or cosolvent for a variety of fats, waxes, gums, resins, dyes and other organic compounds, including vinyl, acrylic, polyamide and epoxy resins, chlorinated rubbers and organic cellulose esters (IARC, 1999).

Ascorbic acid protected *Escherichia coli* and *Shigella* against inactivation by peroxidase systems significantly reduced the ascorbic acid content of juices. (Opstal *et al.,* 2006).

The aim of this study is to assess the mutagenic activity of 2-NP in prophage induction, transduction and survival percentages assays. Moreover, some vitamins and processed fruit juices have been also investigated to detect their anti-mutagenic activity against 2- NP.

MATERIALS AND METHODS

This investigation was carried out at the Microbial Genetic Lab., Genetics Dept., Fac. Agric., Zagazig Univ.

Materials

2-Nitropropane

2-NP is a colorless with mild odour liquid, moderately volatile. Its density is 0.988. (Sigma Aldrich Chemical Co., Milwaukee).

Acridine Orange

Orange powder a cationic cytochemical stain specific for cell nuclei, especially DNA. It is used as a supravital stain and in fluorescence cytochemistry. Acridine orange (AO) induced mutation in *Escherichia coli.* . (Arshad *et al.,* 2006).

α-Tocopherol Acetate

It is clear slightly greenish yellow oil. Its form is soft gelatin capsules. Each capsule contains 400 mg vitamin E., produced by Pharco Pharmaccuticals, Alexandria.

Growth Media

The nutrient agar (NA) and nutrient broth (NB) media were used. Soft agar (0.8% w/v agar) was prepared in distilled water and kept at 45°C on water bath. Phosphate buffer was prepared from 1/15M potassium phosphate (KH_2PO_4) and 1/15M disodium phosphate ($Na_2HPO_4.\ 2H_2O$). Streptomycin (12 mg/ml) was added as sterilized solutions by

filtration through 0.2 µm filter membrane to the media after autoclaving.

Bacteriophage and Bacterial Strains

The generalized transducing F116 phage and bacterial strains of *Pseudomonas aeruginosia* (PAol, PU21 and MAM2) that used in this study were obtained from M. Day, University of Wales, Cardiff, UK. These bacterial strains are lysogen with F116 phage and resistance to streptomycin.

Fruit Juice

In this study, manufactured fruit Orange and Guava juices of Juhayna Company were used.

Methods

Prophage Induction from Lysogenic Strains

The lysogen cells were inoculated into 10 mls of Nutrient broth (NB), then placed in a 150 rpm shaker incubator LAB- LINE overnight, at 30°C followed by centrifugation REMI Model.R 32 A at 5000 rpm for 30 min. The supernatant was removed, a few drops of chloroforme were added and passed through a sterile filter membrane (0.2 µm, Whatman) and titred.

Phage Titration

Serial hundred- fold dilutions of phage were prepared in phosphate buffer (PH 7.0). Phage titer was determined by mixing equal volumes (0.1 ml) of a phage dilution with host cells (growing overnight in NB at 30°C), adding soft agar, and pouring immediately onto an NA plate (Gulig *et al.*, 2002). Plates were incubated at 30°C for 24h., and plaque-forming unit (pfu/ml) was recorded.

Survival Percentage of Bacterial Strains

The overnight bacterial cells was prepared, two ml of each individual strain (PAo1, MAM2 and PU21) were added to each individual volume from 2-NP before and after treatment with vitamin or juice. After incubation at 30°C for Overnight, serial dilutions were prepared; 0.1 ml of each dilution was spreaded onto NA plates. The plates were incubated at 30°C for 24h. The colonies were counted and the survival was calculated as colony forming units (cfu /ml). Survival percentage (S%) was calculated by dividing cfu/ml of each treatment on cfu/ml of control, and killing percentage (K%) = 100- S%.

Mutagenic Activity of 2-NP

1-A loop of different lysogen strains from *Pseudomonas aeruginosa* to phage (F116) was inoculated into 10 mL nutrient broth and incubated at 30 °C for 24h.

2-From this culture, 0.1 mL was taken and added into fresh nutrient broth (5 mL) each of which was supplemented with different concentrations of 2-NP (5, 10, 20, 25, 30, 35, 100 and 140ug/mL)., filtered with 0.45um millipore to be free from bacteria and incubated at 28°C for 24h. At the same time, a loop of the sensitive strain was inoculated into 10mL nutrient broth and incubated at 30 °C for 24h.

3-The phage lysate particles were filtered through 0.45um millipore filter.

4- 0.1 mL of the phage lysate particles was added to semisolid nutrient agar, kept at 46°C, containing 0.1 mL of *P. aeruginose* sensitive culture. Then the semisolid nutrient agar poured into nutrient agar plates and incubated at 30 °C for 24h.

5- 0.1 mL of *P. aeruginose* strain culture after filtration (containing the bacteria after phage induction) was added to semisolid nutrient agar. Then the semisolid nutrient agar poured into nutrient agar plates and incubated at 30 °C for 24h.

6-All assays were carried out in triplicates. The pfu/mL and cfu/mL were calculated, fold increase (F.I.) was calculated by dividing pfu/ml of each concentration on pfu/ml of control, Mutagenic index (M.I.) was detected according to Heinemann, (1971).

7-All these steps were carried out under complete sterile conditions.

Treatment with α-Tocopherol

α-Tocopherol was emulsified in water by using 2% Tween 80 then filtered with 0.45um millipore to be free from any bacteria. The same steps of mutagenic activity of 2-NP were performed, except that in step 2, where 2-NP 25ug/mL was used only. The α-tocopherol was added to the nutrient broth containing *Pseudomonas aeruginosa* at different concentrations (5, 10, 20, 25, 30, 35, 100 and 140ug/mL).

Treatment with Fruit Juice

Fruit juice was used after filtration with 0.45um millipore to be free from any bacteria. The same steps of mutagenic activity of 2-NP were performed, except that in step 2, where 2-NP 25ug/mL was used only. The fruit juice was added after and before treatment to the nutrient broth containing *Pseudomonas aerurginosa* at different concentrations (17, 34, 51, 68, 85 and 102 ug/mL).

RESULTS AND DISCUSSION

Survival Percentages of *Pseudomonas aureginosa* Strains upon Exposure to 2-NP

The survival percentages of the three tested strains were greatly affected when treated with 2-NP (Table 1). At high concentration 140 µg/ml, S% reached 2-3 % (cfu/ml = 0.10 $x10^{13}$- 0.14 $x10^{13}$) seeming that 2-NP had a toxic effect on bacterial cells .

Mutagenic Activity of 2-NP on Prophage Induction

The induction of prophage F116, were increased by using different concentrations of 2-NP (Table 2). Three or more fold increases than those observed in control were detected.

Table 1. Survival percentages of *P. aureginosa* lysogenic strains with phage F116 upon exposure to 2-NP

Con. µg/ml	PAo1 F116			MAM2 F116			PU21 F116		
	cfu / mL x 10^{13}	S %	K%	cfu / mL x 10^{13}	S %	K%	cfu / mL x 10^{13}	S %	K%
Zero	4.90	100	0	3.40	100	0	3.10	100	0
5	4.65	94	6	3.31	97	3	2.97	95	5
10	4.60	93	7	3.30	97	3	2.80	90	10
20	4.23	86	14	3.00	88	12	2.55	82	12
25	3.30	67	33	2.62	76	24	2.41	77	23
30	3.31	67	33	2.60	76	24	2.40	77	23
35	2.40	48	52	2.30	67	33	2.20	70	30
100	1.45	29	71	1.10	32	68	1.05	33	67
140	0.14	2	98	0.10	2	98	0.10	3	97

S%: Survival percentage K% : Killing percentage

Table 2. Effect of 2-NP on prophage F116 induction from different lysogens

Con. µg/ml	PAo1 F116						MAM2 F116						PU21 F116					
	Pfu/ml x 10^{13}	plaques		F.I	I.P	M.I	Pfu/ml x 10^{13}	plaques		F.I	I.P	M.I	Pfu/ml x 10^{13}	plaques		F.I	I.P	M.I
		T	C					T	C					T	C			
Zero	0.40	40	0	1	0	-	0. 40	40	0	1	0	-	0.51	51	0	1	0	-
5	1.22	29	93	3.1	0.82	+	1.32	22	110	3.3	0.92	+	1.61	31	130	3.2	1.10	+
10	2.40	36	204	6.0	2.00	+	2.45	35	210	6.1	2.05	+	2.09	42	167	4.1	1.58	+
20	2.98	35	263	7.5	2.58	+	3.05	51	254	7.6	2.65	+	2.53	50	203	5.0	2.02	+
25	3.46	40	306	8.7	3.06	+	3.51	52	299	8.9	2.55	+	3.25	58	267	6.4	2.74	+
30	2.15	39	176	5.4	1.75	+	2.10	40	170	5.0	3.11	+	3.19	56	263	6.3	2.68	+
35	1.20	32	88	3.0	0.80	+	1.23	33	90	3.1	1.70	+	1.75	31	144	3.4	1.24	+
100	0.20	8	12	-	-	-	0.36	12	24	-	-	-	0.28	6	22	-	-	-
140	0.08	4	4	-	-	-	0.08	3	5	-	-	-	0.05	2	3	-	-	-

T: Turbid plaques C: Clear plaques F.I. Fold increase
I.P. Induced phage M.I. Mutagenic Index
+ = Fold increase > 3 - = Fold increase < 3

According to Heinemann (1971), any compound cause 3 fold increases in prophage induction than control should be mutagenic agent. However, concentration 25µg/ml gave the highest fold increase in most lysogen strains. At high concentrations 100-140 µg/ml, no fold increase in the induction mechanism was noticed. This might due to the toxic effect of high concentration of 2-NP in bacterial cells as shown in Table 1.

The assay could test very small samples as in complex mixture fractionation. So this assay should prove useful in situations where sample size is limiting (Rossman *et al.,* 1991).

When comparing this assay with other microbial system such as *Salmonella* Ames test, the concordance for the two systems was 71%. However, the sensitivity of prophage assay in detecting carcinogens was 76% compared with 58% for *Salmonella* assay (Rossman *et al.,* 1991).

Data obtained in this work showed that the prophage induction assay, to assess the mutagenic activity of 2-NP; is a rapid and convenient microbial sensing system based upon the induction of the prophage from the lysogenic bacterial cells (Lee *et al.,* 1992).

Moreover, the mutagenicity of many compounds might escape from *Salmonella* test, but gave positive mutagenic activity by using prophage assay. Thus, the prophage induction assay was proven to be more appropriate methodology than the *Salmonella* to test the mutagenicity (Vargas *et al.,* 2001).

Moreover, the bacteriophage was template phage, so it produced only turbid plaques with *Peseudomonas* bacteria. However, upon treated the lysogen with 2-NP, clear plaques were formed. Again concentration 25µg/ml gave the highest number of clear plaques in most strains ranged from 267 to 306 x 10^{13} pfu/ml in prophage F116.

The formation or increasing the rate of forming clear plaques appeared to be another proof to the mutagenic activity of compounds. Data in Table 3 showed the percentages of clear plaques that are formed among the whole number of plaques. The percent of clear plaques increased by increasing the concentration of 2-NP up to 25µg/ml followed by a decrease in the percentage.

Table 3. Percentages of clear plaques produced when lysogenic strains of phage F116 treated with 2-NP

Con. µg/ml	% of clear plaques in lysogens		
	PAo1 F116	MAM_2 F116	PU21 F116
5	76.2	83.3	80.8
10	85.0	85.7	80.0
20	88.3	83.3	80.2
25	88.4	85.2	80.2
30	81.9	80.9	82.2
35	73.3	73.2	82.4
100	60.0	66.7	82.3
140	50.0	62.5	78.6

Wild type bacteriophage F116 seemed to be temperate phage since they displayed turbid plaques only on *Pseudomonas aureginosa* bacterial strains. The exposure of the prophage in the lysogen by 2-NP, might change of the life cycle of the bacteriophage from the lysogenic.

Influence of 2-NP on bacteriophage F116

Ability to form clear plaques

Bacteriophage particles, F116 were treated with 2-NP. The ability to produce clear plaques was assessed (Table 4). The number of clear plaques was increased followed by decrease in turbid plaques. At 25µg/ml of 2-NP, 270 x 10^{13} clear plaques were formed in phages F116. However, number of turbid plaques was dramatically decreased reached to 3 x 10^{11} upon using 140 µg/ml of 2-NP. These data showed that 2-NP had a powerful influence in the stability of bacteriophage F116 .This might due to the action of 2-NP on the viva DNA upon treated with 2-NP.

Ability to transduce

Data in Table 5 presented the transduction frequency of streptomycin resistance gene upon treated F116 bacteriophage. A drop in transduction frequency was observed when the phages was treated with 2-NP. Transduction frequency dropped from 9.04 x10^{-5} to 1.2 x 10^{-6} for phage F116 upon using 100µg/ml of 2-NP. No transductants were detected at 140µg/ml. This decrease in transduction frequency might due to a decrease in number of

Table 4. Ability of bacteriophage F116 treated with 2-NP in forming clear plaques

Con. µg/ml	Phage F116	
	T. plaques x 10^{13}	C. plaques x 10^{13}
Zero	260 x 10^{13}	-
5	55 x 10^{13}	155 x 10^{13}
10	28 x 10^{13}	220 x 10^{13}
20	21 x 10^{13}	277 x 10^{13}
25	18 x 10^{13}	270 x 10^{13}
30	18 x 10^{13}	190 x 10^{13}
35	11 x 10^{13}	100 x 10^{13}
100	8 x 10^{12}	89 x 10^{12}
140	3 x 10^{11}	21 x 10^{11}

T: Turbid plaques C: Clear plaques

Table 5. Ability of bacteriophage F116 treated with 2-NP in transducing streptomycin resistance gene

Con. / µg/ml	Phage F116	
	No. of transductant	No. of transduction frequency
Zero	3.71 x 10^{6}	9.04 x 10^{-5}
5	3.63 x 10^{6}	8.85 x 10^{-5}
10	3.51 x 10^{6}	8.56 x 10^{-5}
20	3.40 x 10^{6}	8.29 x 10^{-5}
25	2.10 x 10^{6}	5.12 x 10^{-5}
30	1.70 x 10^{6}	4.14 x 10^{-5}
35	0.90 x 10^{6}	2.19 x 10^{-5}
100	0.50 x 10^{6}	1.20 x 10^{-6}
140	-	-

Recipient cells cfu/ml = 4.10 x 10^{10} .

transducing particles that responsible for transduction mechanism when phage was treated with 2-NP.

Influence of 2-NP on recipient strain

The recipient strain in transduction experiment (PAo1 Str^r) was treated with 2-NP. Data in Table 6 showed that a low frequency in transduction was detected. Transduction frequency at zero concentration was 9.67 x 10^{-5} for F116 reached up to 5.2 x 10^{-6} when using 140µg/ml of 2-NP. This might due to the inducing mutants in the recipient which prevent any step in transduction mechanism because of treating the recipient with 2-NP.

This agreed with Li *et al.*, (2007) whereas transduction frequency with phage P1 had been observed to be very low in *Escherichia coli* K12 mutants

Using Acridine Orange as Positive Control

Effect on survival percentages

Lysogens, PAo1F116 was treated with acridine orange, the strong mutagenic agent, as positive control using the same concentrations of 2-NP. Results are shown in Table 7. S% was dropped up to 2% upon using 100µg/ml of acridine orange. No colonies have been survived at 140µg/ml.

Table 6. Transduction frequency using treated recipient strain with 2-NP

Con. / µg/ml	Phage F116	
	Transductant x10^6	Transduction frequency
Zero	3.87	9.67 x 10^{-5}
5	3.12	7.80 x 10^{-5}
10	3.10	7.75 x 10^{-5}
20	3.01	7.52 x 10^{-5}
25	2.34	5.85 x 10^{-5}
30	2.11	5.27 x 10^{-5}
35	1.99	4.97 x 10^{-5}
100	0.41	1.02 x 10^{-5}
140	0.21	5.20 x 10^{-6}

Transduction frequency was calculated per recipient counts at zero concentration (4.0 x 10^{10} cfu/ml).

Table 7. Survival percentages of lysogen PAo1F116 treated with acridine orange

Con .of AO µg/ml	PAo1F116		
	cfu / mL x 10^{13}	S %	K %
Zero	4.90	100	0
5	4.35	88	12
10	4.00	81	19
20	3.59	73	27
25	3.20	73	27
30	2.90	70	30
35	2.00	48	52
100	0.10	2	98
140	-	-	-

S% : Survival percentage. K% : Killing percentage.

Effect on prophage induction

Data in Table 8 showed the effect of acridine orange in the induction of prophage, F116. All the concentration used in this study had a mutagenic activity in prophage induction. When comparing these results with those showing the effect of 2-NP, it is clear that 2-NP had a mutagenic and toxic response.

Effect on transduction

Data in Table 9 exhibited the effect of treated phage particles and recipient cells on transduction process. Number of transductants and subsequently transduction frequency were affected when treated with acridine orange. This due to the mutagenic activity of acridine orange.

Using Vitamin E as Negative Control

Effect on survival

Vitamin E had no toxic effect on the survival of lysogenic bacterial cells PAo1F116 (Table 10).

Effect on prophage induction

Table 11 showed the influence of vitamin E on prophage induction process. No mutagenic activity was observed with the used concentrations. Science M.I. was less than 3. Moreover, no clear plaques were detected. So, vitamin E played a role, protecting bacterial DNA to be damaged. There for, vitamin E might be used as antioxidant agent.

Table 8 . Effect of acridine orange in induction of prophage F116

Con .of AO µg/ml	F116					
	Pfu/ml x 10^{13}	Plaques		F.I	I.P	M.I
		T	C			
Zero	0.43	43	0	1	0	-
5	1.88	23	165	4.4	1.45	+
10	2.73	43	230	6.3	2.30	+
20	3.20	20	300	7.4	2.77	+
25	4.00	31	369	9.3	3.57	+
30	2.80	14	266	6.5	2.37	+
35	1.72	15	157	4.0	1.29	+
100	1.31	5	126	3.1	0.88	+
140	-	-	-	-	-	-

T: Turbid plaques C: Clear plaques F.I. Fold increase
I.P. Induced phage M.I. Mutagenic Index
+ = Fold increase > 3 - = Fold increase < 3

Table 9. Transduction by phage F116 and recipient cells treated with acridine orange

Con .of AO µg/ml	Pfu / ml x 10^{13}	T	C	Cfu /ml recipient x 10^{12}	No. of Transductant x 10^{6}	Transduction frequency
Zero	3.60	360	0	4.13	3.91	0.95
5	2.23	63	160	3.61	4.20	1.16
10	1.74	40	143	3.12	4.73	1.51
20	1.23	28	95	2.64	4.63	1.75
25	1.00	19	81	2.22	4.10	1.85
30	0.90	20	70	1.97	3.22	1.63
35	0.54	11	43	1.54	2.00	1.29
100	0.16	4	12	0.70	0.54	0.77
140	-	-	-	-	-	-

T: Turbid plaques C: Clear plaques

Table 10. Effect of vitamin E on survival of bacterial lysogenic cells

Con .of Vitamin E	PAo1F116		
	cfu / mL x 10^{13}	S %	K %
Zero	4.90	100	0
5	4.94	100	0
10	4.94	100	0
20	4.96	101	-
25	4.95	101	-
30	4.99	102	-
35	5.04	103	-
100	5.09	104	-
140	5.13	105	-

S% : Survival percentage. K% : Killing percentage.

Table 11. Effect of vitamin E on prophage induction process

Con. of vitamin E	F116					
	Pfu/ml x 10^{13}	Plaques		F.I	I.P	M.I
		T	C			
Zero	0.40	+	-	1	0	-
5	0.43	+	-	1.1	0.03	-
10	0.46	+	-	1.2	0.06	-
20	0.48	+	-	1.2	0.08	-
25	0.51	+	-	1.3	0.11	-
30	0.53	+	-	1.3	0.13	-
35	0.56	+	-	1.4	0.16	-
100	0.60	+	-	1.5	0.20	-
140	0.63	+	-	1.6	0.26	-

T: Turbid plaques= + C: Clear plaques= - F.I. Fold increase
I.P. Induced phage M.I. Mutagenic Index

Effect on transduction

Table 12 illustrated the influence of vitamin E in transducing streptomycin resistance gene using treated phage. No decrease in transduction frequency was observed, however, an enhancement in number of transductants was detected.

Effect of Manufactured Fruit Juice

Orange and guava juices products of Juhayna manufactory were chosen in this study. To evaluate the mutagenic activity of the manufactured fruit juices, 25µg/ml of 2-NP was added into each concentration of the juice. Data in Tables 13 and 14 showed the survival of lysogen up on adding the 2-NP.

The S% ranged between 91-92 % upon using the two juices. However, Tables 15 and 16 showed the anti-mutagenic properties of fruit juices in prophage induction.

No mutagenic activity was observed. Fold increase than control was less than 3 F.I. at 102 µg/ml of the juice ranged from 1.3 to 1.4 only.

Transduction frequencies using bacteriophage are shown in Tables 17 and 18. NO remarkable enhancing in number of transductants was detected. These results showed that manufactured fruit juices had no mutagenic activity and could be used as antioxidant agents.

Table 12. Effect of vitamin E on transduction using F116 phage

Con .of Vit. E µg/ml	Pfu /ml x 10^{13}	T	C	Cfu/ml x 10^{12}	No. of Transductant x 10^{6}	Transduction frequency
Zero	3.60	+	-	4.13	3.87	0.93
5	3.63	+	-	4.14	3.89	0.93
10	3.65	+	-	4.15	3.91	0.94
20	3.65	+	-	4.17	4.00	0.95
25	3.66	+	-	4.17	4.09	0.98
30	3.66	+	-	4.19	4.12	0.98
35	3.68	+	-	4.21	4.21	1.00
100	3.69	+	-	4.23	4.47	1.05
140	3.69	+	-	4.32	4.80	1.11

T: Turbid plaques = + C: Clear plaques = -

Table 13. Survival percentages upon adding 25µg/ml of 2-NP to different concentration of juhayna orange juice

Con. Of vita. C in orange juice µg/ml.	PAo1F116		
	cfu / mL x 10^{13}	S %	K %
Zero	4.90	100	0
17	4.85	98	2
34	4.80	98	2
51	4.71	95	5
68	4.68	95	5
85	4.61	94	6
102	4.50	92	8

S% : Survival percentage.

K% : Killing percentage.

Table 14. Survival percentages upon adding 25µg/ml of 2-NP to different concentration of juhayna guava juice

Con. of vita. C in Guava juice µg/ml.	PAo1F116		
	cfu / mL x 10^{13}	S %	K %
Zero	4.90	100	0
17	4.82	98	2
34	4.71	96	4
51	4.66	95	5
68	4.65	95	5
85	4.60	94	6
102	4.52	92	8

S% : Survival percentage.

K% : Killing percentage.

Table 15. Anti-mutagenic activity of juhayna orange juice in prophage induction assay

	F116			
Con. Of vita. C in orange juice µg/ml.	**Pfu/ml x 10^{13}**	**F.I**	**I.P**	**M.I**
Zero	0.40	1	0	-
17	1.04	2.6	0.64	-
34	1.03	2.5	0.63	-
51	0.90	2.2	0.50	-
68	0.89	2.2	0.49	-
85	0.68	1.7	0.28	-
102	0.56	1.4	0.16	-

F.I. Fold increase I.P. Induced phage M.I. Mutagenic Index
- = Fold increase > 3

Table 16. Anti-mutagenic activity of juhayna guava juice in prophage induction assay

	F116			
Con. of vita. C in Guava juice µg/ml.	**Pfu/ml x 10^{13}**	**F.I**	**I.P**	**M.I**
Zero	0.40	1	0	-
17	1.05	2.6	0.65	-
34	1.01	2.5	0.61	-
51	0.97	2.4	0.57	-
68	0.85	2.1	0.45	-
85	0.63	1.6	0.23	-
102	0.51	1.3	0.11	-

F.I. Fold increase I.P. Induced phage M.I. Mutagenic Index
- = Fold increase > 3

Table 17. Transduction frequency upon adding 25µg/ml of 2-NP to different concentration of juhayna orange juice

Con. Of vita. C in Orange juice µg/ml.	Pfu / ml F116 x 10^{13}	Cfu /ml recipient x 10^{12}	No. of transductant x 10^{6}	Transduction frequency
Zero	3.60	4.13	3.86	0.93
17	3.61	4.14	3.85	0.92
34	3.61	4.18	4.08	0.97
51	3.63	4.22	4.07	0.96
68	3.63	4.26	4.03	0.94
85	3.65	4.32	4.04	0.94
102	3.65	4.34	4.07	0.94

Table 18. Transduction frequency upon adding 25µg/ml of 2-NP to different concentration of juhayna guava juice

Con. of vita. C in Guava juice µg/ml.	Pfu / ml F116 x 10^{13}	Cfu / ml recipient x 10^{12}	No. of transductant x 10^{6}	Transduction frequency
Zero	3.60	4.13	3.86	0.93
17	3.60	4.13	3.89	0.94
34	3.62	4.16	3.86	0.93
51	3.63	4.17	4.00	0.96
68	3.63	4.18	4.00	0.96
85	3.65	4.21	4.10	0.97
102	3.65	4.22	4.10	0.97

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التقدير الوراثى لمادة ٢- نيتروبروبان من خلال عدة انظمة قصيرة

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اختبرت القدرة الطفرية لمادة ٢ - نيتروبروبان من خلال عدة أنظمة قصيرة. هذة الأنظمة تتضمن **Prophage induction, Transduction and survival percentage**. حيث تم الحصول على نتائج ايجابية تعتمد على التركيزات المختلفة لمادة ٢ - نيتروبروبان. و كان التركيز ٢٥ ميكروجرام /مل من ٢ - نيتروبروبان هو أعلى تركيز أعطى استجابة عالية للـ **prophage induction** للـ **F116** فى ثلاث انواع من البكتريا الليسوجينية. والذى أظهر زيادة ثلاث مرات أو أكثر عن الكنترول والتى تراوحت ما بين ٦,٤ الى ٨,٩ عند تركيز٢٥ ميكروجرام /مل ، كذلك أعطت اعلى عدد من مناطق التحلل الرائقة مع سلالات كثيرة والتى تراوحت ما بين 267 الى 306×10^{13} pfu/ml. كما هبط معدل نقل الجينات من 9.04×10^{-5} الى 1.2×10^{-6} وذلك عند أستخدام تركيز ١٠٠ ميكروجرام / مل من ٢-نيتروبروبان. وتوقف تماما عند تركيز ١٤٠ ميكروجرام / مل. كانت نسبة ال **survival** للسلالات المختبرة متأثرة كثيرا بالمعاملة بـ ٢- نيتروبروبان. حيث وصلت عند تركيز ١٤٠ ميكروجرام / مل الى ٢ - ٣ % مما يبدوا أن للـ ٢- نيتروبروبان تأثير سمى على الخلايا البكتيرية. الأكريدين أورنج وفيتامين E أستخدموا كـ **Positive and Negative** كنترول للمقارنة بالـ ٢- نيتروبروبان. عصير الفواكة كمصدر للفيتامينات كان له تأثير رائع فى تخفيض التأثير الطفرى للـ٢- نيتروبروبان.