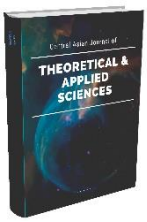

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# Bacterial Reverse Mutation Test for Two Salmonella Typhimurium (TA100, TA98) Strains to Detect Mutant and Carcinogenic Effect of Some Insect Growth Regulators

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***Summary.*** *This study has aimed to investigate the imutant influence of three insect growth regulators (IGRs): Neporex and Apploud, which belong to chitin synthesis inhibitors (CSIs), and Admiral, which belong to juvenile hormone analog JHAs. In Iraq , Kerbala Governorate of December 2022 . These IGRs are used to insect control by using biological methods including the study of reversal mutation for two strains of Salmonella typhimurium (TA100, TA98) in two ways: The plate incorporation method and the fluctuation method. The results showed that the CSIs (Neporax, Applaud) have mutant influence in bacteria cells for all used concentrations( which are prepared after determining the minimal inhibition concentration for each growth regulator). The plate incorporation method, It has been noticed that the numbers of reversal colonies in each strain increased with increasing concentrations compared with spontaneous reversal colonies in negative control. In using the fluctuation methods, It has been noticed increasing the turbid hole numbers which shows the material effect on bacteria mutation. The result referred to the positive relationship between concentration and the number of holes when testing Admiral (JHIs) by plate incorporation and fluctuation methods, the result has shown there is no mutant effect on Salmonella typhimurium. The statistical analysis has shown there are no significant differences between the numbers of reversal colonies and unclear holes as a result of different concentration treatments of JHIs with negative control of both strains (TA100, TA98)under testing.*

***Keywords:*** *Ames; Carcinogenic; Insect growth regulators; and Mutation.*

## INTRODUCTION

Insect growth regulators (IGRs) also called third-generation insecticides are considered the most population chemicals used in pest control. Interfering with growth development are reproduction in insect, Recently they have more intention because their high efficiency in insect pest control .in addition to their high specifity they are considered safe for most target organism.( Mulla, **1925 ; Gada et al., 2021)** The resistance of IGRs because of their use is rare . These material include groups of chemical that differ in

 

chemical nature and are the same in their on metamorphosis and reproduction in insect (Kornuta and Nedopitanskaya, 1996 ). Hormone analoges and chitin synthesis inhibitors are group of IGRs. Although of their importance in controlling the pest insect (**Chandi** and **Kaur, 2022**), they are chemical poisones materials inter the environment ,So their side effects must be investigated continuously ,especially these of bad use ,In addition to the probable damage caused by its long use and indirected exposure (Batazing , 2002; Dulout et al., 1985) .This cause dangerous diseases as cancer teratogenic disorder. In general, pesticide are considered an active biological material which can interact with DNA and damage strand or change the structure.Also, it can make coavellant bound with different centers in cells and nucleus and cause change or damage DNA molecule (Lucero et al., 2000; Dulout et al., 1985). Pesticides are con - sedered as mutagenesis chemical material (Bolognesi et al., 1997) .Many researchs have shown that theses material ave ability to chromosomal changes and cause defecate in DNA strain (**Rawi** and **Bakhoum, 2022** , **Wilhelm** et al., 2020) , geneic poisons caused by using chemical material can be noticed by using many technique such as : bacterial reverse *Salmonella typhimurium* mutagenesis assay or what is called" Ames test" which tests the chemical material that cause mutation and carcinogenesis because of accumulation through long exposure (Gatehouse , 1978).This study aims to investigate the probable existence of the mutant effect of three IGRs Admiral, Applaud and Neoporx which are widely used as an insecticide to control the pest insect by using bacterial reverse mutagenesis assay of *Salmonella typhimurium*

## MATERIAL AND METHODS

### I/ Culture media preparation :-

**1) Nutrient agar media (N.A.) :-** prepare this media by soluble 9 gram with 1Liter distilled water ( according to instructions from fluke company -Germany ) then sterilize in autoclave under temperature 121° c pressure 15 pound /inch$^2$ for 15 minute. Then pour of petri dishes or glass tube as needed .use the media for activation bacteria strains used in testing.

**2) Nutrient Broth (N.B.):-** prepare this media by soluble 13 gram with 1Liter distilled water (according to instructions of Himedia Laboratories –India) pour in glass tubes with cover tightly secrocup tube. sterilize in Autoclave under temperature 121° c pressure 15 pound /inch$^2$ for 15 minute. save with fridge to used after cold . Use the medium for activation bacteria strains used in testing. And prepare to minimal inhibition concentration (MIC) of IGRs.

**3) Bacterial** Minimal Medium (BMM):- A method (OECD, 1997)was used to prepare this medium and as follows:-

**A) Davis –Mingioli salt solution (D.M.) :-** prepare this solution by soluble matrials ( 14 gm/l $k_2Hpo_4$ , 6gm /l $KH_2po_4$ , 0.89 gm/l ,0.2 gm/l $Na_3$-citrate-$3H_2o$ , 2gm/l $(NH_4)_2$ $SO_4$) in distilled water . and sterilize solution in autoclave under 110°c ,10 pound/inch$^2$ for 20 minute.

**B) Glucose solution 40% w/v:-** prepare by soluble 40 gm glucose in 100ml in distilled water .sterilize under temperature 121° c pressure 15 pound /inch$^2$ for 15 minute. save with fridge to used after cold .

**C) Agar-Agar:-** prepare by soluble 12 gm from media in 1Liter distilled water. To prepare Minimal medium in solid state mixed solution A with C and sterilize autoclave under temperature 121° c pressure 15 pound /inch$^2$ for 15 minute. Then add to each 400ml of the mixture 1ml glucose solution 40% ,0.1ml from histedine solution in 10 Mg/ml. then pour in petri dishes.

In the case of the lowest nutritional status of the liquid state, it is prepared without adding a medium agar-agar which only the solution D.M. with glucose and histedine and sterility in autoclave. For the purpose of obtaining Top agar BMM a semi-solid medium is added from0.6 gm agar-agar to D.M. of the record

by100 ml distilled water then the sterilized autoclave and pour into sterile, (2ml each tube) sealed tubes and keep in the refrigerator until use.

**II// Minimal inhibition concentration (MIC) of IGRs:** I attended a series of concentric concentrations of an insect growth regulator under test 8-6 concentrations and concentrations were added to a sterile tube container on 9 mL of central nutrient broth N.B. sterilized with $10^5$ of bacteria cells of each ml .incubator on 37◦c for 24-hour .take from each test tube 0.1 ml distribute on Petri dishes contain N-agar incubator on 37◦c for 24 hour. certain growth bacteria and determine the lowest concentration of chemical material that's not allowed growth bacteria named its minimal inhibitions concentration (Ames et al., 1975).

**III// use bacterial strains:**

Use in testing that's related to searching two strains of *Salmonella typhimurium* (TA100, TA98)

1)Testing methods:- directed test of IGRs by using Ames test with two methods:- The plate incorporation method (OECD, 1997) and the fluctuation test method ( Lucero et al., 2000) each of the two methods prepares to positive control and Negative control all testing in three replicates each test.

**IV// Statistical analysis:-**

1- The results of the experiments were analyzed using the plate incorporation method based on the analysis of variance analysis ANOVA TABLE and the comparison of the averages using the least significant difference L.S.D. and the probability level 0.05

2- By using fluctuation methods The results were analyzed by the test $X^2$ equation they developed by (Liddel, 1976) according to:-

$X^2= 2n\ (t – c - {}^1/_2) / (t +c)\ (2n- t-c)$

That's: n= the total number of holes per plate

t = Total number of turbid growth of the treated plate

c = Total number of turbid growth of the negative control treatment.

Compare the results to a value $X^2$ below a probability level of 0.05

3- Calculate the standard deviation for each transaction in the test

**Results and discussion:-**

**I/ Neporex 10DC (CSIs):**

Table 1 shows the mutant effect of Neoporex (CSIs) through the positive relationship between the concentration used and the growth of bacterial strains. In the plate incorporation test using TA100 strain, the number of reverse colonies was 612±23.3 in 0.5 ppm concentrate. With the increasing concentration, the number of reverse colonies increased to 960±31.5 in 1.5 ppm, compared with 58±1.4 colonies in the negative control treatment (NCT) and 1327±36.2 colonies in the positive control treatment (PCT). The same thing happened with the used TA98 strain. The number of reverse colonies has increased with the Neporex concentration increasing to 38 ±1.4 and 51 ±1.4, respectively, by using 0.5 and 1 ppm subsequently. The number of colonies had increased to 65±2.8 in 1.5 ppm concentrate. By using the last concentration, the mutant effect strength occurred. The statistical analysis showed that there are no significant differences in the number of reverse colonies (67±1.8). In the fluctuation methods, the results were the same as in the plate incorporation method, which resulted in an increasing mutant effect of *Salmonella typhimurium for* both strains with increasing Neoporex concentration. In Table 1, the results refer to the increasing number of unclear holes (indicating bacterial growth after exposure to CSIs). For TA100, the average number of unclear holes in the negative control was 26±1.4 while using low

concentration (0.5 ppm). The number of unclear holes was 7.7±1.4 increased to 90±1.4 in the use of 1.5 ppm compared with 94±1.4 in the positive control treatment. The statistical analysis results referred to non-significant differences in the number of unclear holes between the treatment in the 1.5 ppm concentration and the positive control. In T A98 strain test (table 1) showed an increasing mutant effect of Neoporex in the used concentration increasing the unclear holes numbers were 58±2.8,60±1.4, 72±2.8 in 0.5,1,1.5 concentration subseguently. The statistical analysis showed that there were significant differences in unclear hole numbers among all concentrations used.

**Table 1. the mutant effect of Neoporex (CSIs) through the positive relationship between the concentration used and the growth of bacterial strains**

| L.S.D. 0.05 | Positive control | 1.5 | 1 | 0.5 | Negative control | Con/ strain | Neoporex |
|---|---|---|---|---|---|---|---|
| 81.6 | 1327 ± 36.2 | 960 ± 31.5 | 855 ± 18.2 | 612± 23.3 | 58 ±1.4 | TA100 | Plate incorporation |
| 12.3 | 67±1.8 | 65±2.8 | 51±1.4 | 38±1.4 | 22±2.8 | TA98 | |
| 7.5 | 94±1.4 | 90±1.4 | 81±2.8 | 77±1.4 | 26±1.4 | TA100 | fluctution |
| 1.6 | 92±4.2 | 72±2.8 | 60±1.4 | 58±2.8 | 22±2.8 | TA98 | |

2(C6H15N2O2)+ 2I3-H2O iodine coordination complex is a new pharmaceutical substance that has high antibacterial activity against both sensitive and multidrug-resistant strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Mutagenicity of the 2(C6H15N2O2)+2I3-H2O iodine coordination complex was assessed using the Salmonella/microsome (Ames) test. The test strains used were Salmonella typhimurium TA98, TA100, TA1535, TA1537, and Escherichia coli WP2uvrA. The mutagenic activity was determined both in the absence or presence of the S9 mixture. The study has shown that the test item did not cause any increase in the number of his+ revertants in S. typhimurium and trp+ revertants in E. coli WP2uvrA strains in the presence or absence of absence of S9-mix, compared to the controls. Under the conditions of this test and according to the criteria set For the evaluation of the test results, the 2(C6H15N2O2)+2I3-H2O iodine coordination complex did not show mutagenic activity in the *S. typhimurium* and *E. coli* plate incorporation assay (Jumagaziyeva et al., 2020).

**II/ Applaud (CSIs):-**

From the results in Table (2), Applaud has clearly effect in causing mutation by noticing the reverse colony numbers in both bacteria strains, In the plate incorporation method, the a positive relationship between the reverse colony numbers and under-testing concentration. By using TA100 in 1,2,3 ppm concentration, the numbers of reverse colonies were 580±36.6 .790±4.3,100±23.1respectvely, compared with 63±1.4 in negative control treatment and 1520±41.5 in positive control treatment. The statistical analysis results showed significant differences in reverse colony numbers in all used concentrations in the experiment. By using TA98 in the test, the reverse colonies number in negative control was 18±1.4 while it was 41±21.6 in 1ppm concentration. The number of reverse colonies had increased with increasing concentration to be 62±1.4 in 3ppm concentration compared with 63±1.8 reverse colony in positive control treatment. The statistical analysis results showed that there were no significant differences between 3ppm concentration and positive control treatment These results were the same as those by using the fluctuation methods in the mutant effect of CSIs Applaud. In Table (2) it was obvious that the positive relationship between the average of unclear holes numbers and the used concentration for both T100 and T98 strains, the number of unclear holes in the negative control treatment was 27±1.4 while in 3ppm concentration was 92± 2.8 compared with 94±1.4 in the positive control treatment. Also by using T98, the average of unclear hole numbers in negative control was 21±1.4 while it was 89±2.8 in 3ppm concentration compared with 91±2.8 hole in positive control in using 3ppm concentration for both strains

| L.S.D. 0.05 | Positive control | Concentration | | | Negative control | strain | Applaud |
|---|---|---|---|---|---|---|---|
| | | 1.5 | 1 | 0.5 | | | |
| 65.1 | 1520±41.5 | 1000±23.1 | 790±4.3 | 580±36.6 | 63±1.4 | TA100 | Plate incorporation |
| 4.3 | 63±1.8 | 62±1.4 | 55±2.8 | 46±21.6 | 18±1.4 | TA98 | |
| 8.2 | 94±1.4 | 92±2.8 | 84±1.4 | 67±1.4 | 27±1.4 | TA100 | fluctution |
| 2.3 | 91±2.8 | 89±2.8 | 70±1.4 | 54±2.8 | 21±1.4 | TA98 | |

the average of unclear hole numbers didn't differ significant to those previously obtained in positive control.

**Table 2. the mutant effect of Applaud and noticing the reverse colony numbers in both bacteria strains**

UV filters, or ultraviolet absorption compounds, are frequently used in personal hygiene products to shield human skin and hair from UV radiation harm. Little is known about the genotoxicity consequences of these compounds, even though they are released into the environment during the production and consumption processes. Three species of aquatic organisms exposed to benzophenone-type UV filters showed acute toxicity, according to our earlier research. In this work, the Salmonella typhimurium/reverse mutation assay (Ames assay) was used to test for mutagenesis caused by benzophenone (BP) and benzophenone-1 (BP-1). For both compounds, all of the positive reverse mutations happened without the S9 liver extract system. Positive mutation effects at dosages of 0.05 μg and 0.5 μg/plate were found in the TA102 strain from BP. Positive mutation consequences on the BP-1 (Wang et al., 2018).

**III//Admiral (JHIs):** in table (3) the results showed there was no mutant effect of admiral (JHIs) in *Salmonella typhimurium* of both T100 and T98 strains. By using the plate incorporation, the average reverse colonies numbers from 60±2.8 to 62±1.4 to three concentrations (0.1,0.5,1ppm)compared with 61±2.8 colony in negative control treatment. The statistical analysis results indicated that there were no significant differences in reverse colony numbers in all the mentioned treatments above. The average of reverse colonies number in positive control treatment was 1100±38.7 reverse colony by using T98 strain in the test, the average reverse colonies numbers were (19±1.4 in 0.1ppm) and (21±1.4 in ppm) concentration spontinous reverse compared with 20±1.4 in negative control treatment. The statistical analysis results showed that there were no significant differences between the negative control treatment and the treatments in( 0.1,0.5,1ppm) concentration of JHIs while in the positive control treatment, the average reverse colonies numbers 64±2.8 which statistically differed from the rest of the treatment for TA98 strain. In the fluctuation method, it was noticed in Table (3) that there were no significant differences in average reverse colony numbers of all used concentrations. by using TA100 in the test, the average unclear holes number ( from 26±1.4 in 0.1ppm concentration to 29±1.4 in 1ppm concentration compared with 27±1.4 in the negative control treatment. The average unclear hole numbers in testing by TA98 were from 20±1.4 to (21± 1.4) for all used concentrations compared with 22±1.4 holes in the negative control treatment. The average unclear hole numbers in the positive control treatment were 94±1.4, and 91±5.1 for both TA100 and TA98 respectively.

**Table 3. The mutant effect of admiral (JHIs) in *Salmonella typhimurium* of both T100 and T98 strains**

| L.S.D. 0.05 | Positive control | Concentration | | | Negative control | strain | JHIS |
|---|---|---|---|---|---|---|---|
| | | 1.5 | 1 | 0.5 | | | |
| 2.8 | 38.7±2.8 | 60±2.8 | 61±1.4 | 62±1.4 | 61±2.8 | TA100 | Plate incorporation |
| 2.1 | 64±2.8 | 21±1.4 | 20±1.4 | 19±1.4 | 20±1.4 | TA98 | |
| 3.1 | 94±1.4 | 29±1.4 | 27±1.4 | 26±1.4 | 27±1.4 | TA100 | fluctution |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2.1 | 91±1.4 | 20±1.4 | 21±1.4 | 21±1.4 | 22±1.4 | TA98 | |

N-nitrosamines are present in low concentrations in humans due to many factors. N-nitrosamines are recognized mutagenic carcinogens that belong to the cohort of concern mentioned in the ICH M7 guideline. They have lately been found as contaminants in several marketed medications. The validity of the bacterial reverse mutation assay and the use of induced rat liver S9 as the external source of metabolism to assess their mutagenic potential are still being debated, despite their well-known mutagenic qualities. Thus, we have examined in vitro under varied settings the mutagenic potential of N-nitroso dimethylamine, N-nitroso diethylamine, N-nitrosodipropylamine, and N-nitrosodibutylamine. Our research demonstrated that the bacterial reverse mutation assay, which uses Salmonella typhimurium strains TA100 and TA1535 and E. coli WP2 uvrA, is appropriate when utilizing plate integration or preincubation methods (Brinqezu and Simon, 2022).

## CONCLUSIONS

1. Insect growth inhibitors (IGRs) They are also called third generation insecticides are considered the most population chemical used in pest control. It was high specificity that they are considered safe for most target organism
2. Hormone analoges and chitin synthesis inhibitors are a group of IGRs. Although of their importance in controlling the pest insect
3. They are chemically poisonous materials in the environment, So their side effects must be investigated continuously, especially those of bad use, In addition to the probable damage caused by their long use and indirect exposure
4. Pesticides are con -sedered as mutagenesis chemical material, Many researchs have shown that theses material have ability to chromosomal changes and cause defecate in DNA strain , geneic poisons caused by using chemical material can be noticed by using many technique such as : bacterial reverse *Salmonella typhimurium* mutagenesis assay or what is called" Ames test" which tests the chemical material that cause mutation and carcinogenesis because of accumulation through long exposure
5. This study aims to investigate is to investigate the probabible exsistarce of mutant effect of three IGRs Admiral ,Applaud and Neoporx which are widely used as insecticide to contraol the pest insect by using bacterial reverse mutagenesis assay of *Salmonella typhimurium* **.**

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