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# EFFECT OF PHENACYLPYRIDINIUM SALTS ON BACTERIAL POPULATION IN LIGHT OLIVE-BROWN SOIL OF BUNDELKHAND REGION (U.P.) INDIA

**R.K. GUPTA*, VANDANA GUPTA[2], NEEL RATAN, MANISHA MISHRA[3], MANOJ GUPTA[2] AND K. C. GUPTA[1]**

Department of Botany,
[1]Department of Chemistry
D.V. College, ORAI -285001 (U.P.)
[2]Department of Applied Chemistry
BIET, JHANSI-284001 (U.P.)
[3]Department of Chemistry
Govt. D.B. Girls (P.G.) College
RAIPUR-492001 (C.G.)
*Corresponding Author
Email : ramkishorepahariya@rediffmail.com

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## ABSTRACT

Effect of phenacylpyridinium bromide (1a) and p-chlorophenacylpyridinium bromide (1b) was investigated on the soil bacterial population by plate dilution method. Based on the average total bacterial count at different concentrations of salts, it was observed that salt 1b was more bactericidal than salt 1a and deleterious effectivity increased with the increasing level. The greater antibacterial effect of salt 1b may probably be attributed to the presence of chlorine atom attached to benzene ring.

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KEY WORDS : Bacterial population, Pyridinium salts

## Introduction

In order to cope with tremendously increasing human demands for food owing to multiple population buldge, crops of high yielding varieties have been introduced for intensive cultivation. But crop production can not go up by mere planting new high yielding varieties, putting more fertilizers and pumping more water unless crop is protected against damages cuased by insects, pests, plant diseases, weeds and others. Therefore, the modern technique of crop protection comprises the use of insecticides, fungicides and herbicides which in mono cultivation have considerable influence in agriculture [1-2].

The pyridinium salts and phosphonium salts find their extensive application in the synthesis of a wide range of heterocyclic compounds; but surprisingly the biological activities of these salts have not been studied so far. However, the effect of pyridinium salts on germination behaviour and radicle growth of *Cajanus cajan* and *Phaseolus radiatus* have been studied[3-5]. In view of above context, the present work has been undertaken to observe the effect of phenacylpyridinium bromide (1a) and p-chlorophenacyl-pyridinium bromide (1b) on bacterial population.

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**TABLE-1 : Variation in soil bacterial population with different concentrations of salts (1a-1b). (Mean value ± S.D.)**

| Salt | Mean total bacterial count with different ppm solution | | | | | |
|---|---|---|---|---|---|---|
| | Control | 50 ppm | 100 ppm | 200 ppm | 500 ppm | 1000 ppm |
| 1a | 490 ± 43 | 353 ± 32 | 255 ± 25 | 236 ± 26 | 116 ± 22 | 90 ± 20 |
| 1b | -do- | 305 ± 35 | 230 ± 20 | 200 ± 25 | 105 ± 26 | 75 ± 24 |

**TABLE-2 : Average total bacterial number of different concentrations (ppm) of salts (1a-1b).**

| Salt | Total bacteria No. (x $10^5$/g of soil with different ppm solution | | | | | |
|---|---|---|---|---|---|---|
| | Control | 50 | 100 | 200 | 500 | 1000 |
| 1a | 49 | 35.3 | 25.5 | 23.6 | 11.6 | 9.0 |
| 1b | -do- | 30.5 | 23.0 | 20.0 | 10.5 | 7.5 |

## Materials and Methods

All the reagents were obtained from commercial sources (B.D.H., E. Merck, SISCO *etc.*). Phenacylpyridinium bromide (1a) and p-chlorophenacylpyridinium bromide (1b) were prepared by reaction of phenacyl bromide and p-chlorophenacyl bromide with pyridine in anhydrous benzene solution at reflux temperature reported in literature[6-10].

The plate count method is based on the assumption that each viable organism develops into a colony. The counting of bacteria using Thornton's medium gives a good representation of bacterial population in the soil. This medium is favourably chosen because it supresses the development and spreading of other microorganisms which over grow the bacterial colonies. It supplies nourishment both in organic and inorganic forms.

**Chemical composition of Thornton's agar medium**

The following chemicals were required for the preparation of medium :

| | | |
|---|---|---|
| 1. | Potassium hydrogen phosphate ($K_2HPO_4$) | 1.0 g |
| 2. | Magnesium sulphate ($MgSO_4.7H_2O$) | 0.2 g |
| 3. | Calcium chloride ($CaCl_2.2H_2O$) | 0.1 g |
| 4. | Potassium nitrate ($KNO_3$) | 0.5 g |
| 5. | Sodium chloride (NaCl) | 0.1 g |
| 6. | Ferric chloride ($FeCl_3$) | 0.002 g |
| 7. | Aspargin | 0.5 g |
| 8. | Mannitol | 1.0 g |
| 9. | Agar | 20.0 g |

The pot. hydrogen phosphate, pot. nitrate, and aspargin were dissolved in 500 ml of distilled water by boiling. Then magnesium sulphate, calcium chloride and sodium chloride were dissolved. Later on, agar and mannitol were dissolved and volume is made upto 1000 ml. The pH was adjusted to 7.4 by bromothymol blue.

Five concentrations (50, 100, 200, 500, 1000 ppm) of salts (1a-1b) were prepared.

**Dilution of soil solution**

As the soil contained innumerable bacteria, the number of colonies that develop in the petri dishes, were too large to be counted in known standards and among the three, the third solution was found to be the best for the study purpose.

**I Solution** : 10g of soil was dissolved in distilled water and volume was made upto 100 ml

Strength of solution= 0.1 g/ml

**II Solution** : 10ml of $I^{st}$ solution was diluted upto 100 ml by distilled water

Strength of solution= 0.01 g/ml

**III Solution** : 1ml of $II^{nd}$ solution was diluted upto 100 ml by distilled water

Strength of solution = 0.0001g/ml

$= 10^{-4}$ g/ml

1.00ml of IIIrd solution was placed in each sterilized petri dish. Before starting the dilution, agar was kept for melting in a water bath. When agar was melted and then transferred to another water bath at $45^0C$ (the temperature when agar did not solidity and did not kill the bacteria). Agar was poured from tube to the petridishes after flaming the neck of the tube. The petridishes were rotated 5 times in clockwise and anticlockwise so that the medium spread uniformly. The medium was now allowed to set, the petridishes were then inverted and kept for inoculation at $30\text{-}35^0C$ for 3-5 days. The number of bacterial colonies was counted. Similar procedure was adopted with treated sets of salt solutions (1a-1b).

## Results and Discussion

As evident from Table 1 the development of bacterial colonies was maximum in both the cases of salts (1a-1b) after 4days. The mean bacterial population decreased with the increasing concentration of salt 1a-b. The 1000 ppm solution had maximum bactericidal effect. Table 1 revealed that salt 1b was more effective bactericide than salt 1a because the total bacteria number in case of salt 1a was slight higher than salt 1b in each concentration of solution (ppm). The greater bactericidal activity of salt 1b might be due to the chlorine atom present in salt 1b.

In Table 2, the mean total bacteria number per gram of soil with different concentrations of salts has been reported. The total bacteria number ranges from $49 \times 10^5$ to $9 \times 10^5$/g of soil in case of salt 1a. This indicated that the bactericidal activity decreased with the increasing concentration of salt 1a. Similar results were also seen with salt 1b but the bactericidal activity was greater than 1a.

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