# STUDIES REGARDING THE RESISTANCE OF SOME BACTERIAL STRAINS FROM SOIL TO CHEMICAL FACTORS

## CERCETĂRI ASUPRA REZISTENŢEI UNOR TULPINI BACTERIENE IZOLATE DIN SOL, LA ACŢIUNEA UNOR FACTORI CHIMICI

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**Abstract.** *The use of different chemical substances for preventing or treating the plant diseases involved a special attention for their remanence in soil and plants. Because the soil microbiota is a very important factor for normal biochemical processes at this level, the chemical treatments should not influence in negative way the physiological activities of microorganisms. The aim of this paper was the established sensibility or resistance of soil bacteria against some chemical substances, frequently used in plant therapy (in bacterial or fungal diseases). The difusimetric method was use. The bacterial strains presented various degree of sensibility. The majority of them was sensible to streptomycin, resistant to copper sulphate (tested concentrations) and sensible to Mancozeb.*
**Key words*:*** microbiota, streptomycin, mancozeb

**Rezumat.** *Utilizarea diferitelor substanţe chimice în scopul prevenirii sau combaterii bolilor plantelor de cultură determină o atenţie deosebită pentru remanenţa acestor substanţe în sol şi în plante. Deoarece microbiota din sol este un factor esenţial pentru desfăşurarea normală a proceselor biochimice care au loc la acest nivel, tratamentele chimice aplicate nu trebuie să influenţeze negativ activitatea fiziologică a acesteia. Scopul acestei lucrări a fost evidenţierea gradului de rezistenţă sau sensibilitate a microorganismelor izolate din sol la acţiunea unor substanţe chimice care se utilizează frecvent în terapia bolilor bacteriene şi micotice la plante. Pentru determinarea sensibilităţii a fost folosită metoda difuzimetrică. Tulpinile bacteriene din sol au prezentat grade variate de sensibilitate. Majoritatea au fost sensibile la streptomicină, au prezentat rezistenţă faţă de concentraţiile testate de sulfat de cupru şi sensibilitate faţă de mancozeb.*
**Cuvinte cheie:** microbiotă, streptomicină, mancozeb

## INTRODUCTION

The soil microbiota is important for normal course of biochemical processes at this level. The applied chemical treatments for different plant cultures had to protect plants against pathogens, without adverse effects on microorganisms from soil microbiota.

In time, the phytopathogenic bacteria from soil, which are constantly in contact with different chemical substances, may develop resistance against these, and the treatments may become ineffective at a time.

The bacteria are most numerous and most active microorganism from soil, and Gram negative bacteria are more frequent than Gram positive bacteria. (Mihăescu, 2000). There are various category of bacteria in soil, from bacteria implicated in carbon, nitrogen, phosphorus, sulphur or iron cycle, with crucial role for plant nutrition and ensure soil fertility, to pathogenic bacteria (some of them pathogenic for plant, the other pathogenic for animals and humans).

The microorganisms influence plants through changes of soil structure and through decomposition of different toxic substances, accumulation in the soil which could lead to plants death (Zarnea, 1970).

The chemical treatments that are used for phytopathogenic bacteria control, involves the application of organic, inorganic or antibiotic solution. These solutions accede on plants and on soil, too.

The aim of this study was the established the sensibility or resistance level for some bacteria, isolated from soil, against some chemical substances (organic, inorganic or antibiotic, with varied concentrations), frequently used in plant treatments (in bacterial or fungal diseases).

## MATERIALS AND METHODS

In this paper were used 100 bacterial strains isolated from nine samples of soil (marked from P1 to P9), removed of vineyard plantation.

The soil samples were obtained from the rizosfera of vine plants at 10cm depth and were further processed in laboratory by making a suspension in sterile saline solution. Decimal dilutions of obtained suspensions ($10^{-6}$, $10^{-7}$, $10^{-8}$) were inoculated by incorporation in nutrient agar. Isolated bacterial colonies were obtained after five days at 22ºC. These colonies were used for bacterial strains selection.

Each bacterial strain was isolated in pure culture on the slant of nutrient agar.

The difusimetric method was use for testing bacterial sensitivity or resistance against three substances frequently used in plant treatments (bacterial and fungal diseases) - figure 1.

Those three substances were: streptomycin (antibiotic for human, animal and plant use), copper sulphate (inorganic copper substance, used as a Bordeaux mixture) and Dithane 45 (organic substance for plant protection, with 80% mancozeb, efficiency against some fungal pathogens in vineyards).

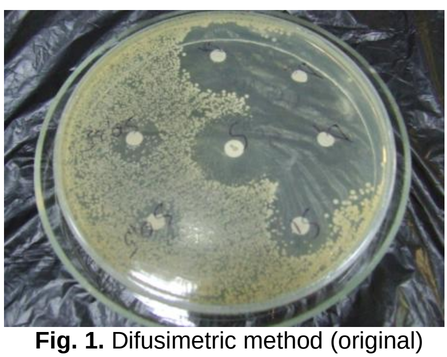

**Fig. 1.** Difusimetric method (original)

The antibiotics used in treatments of plant diseases acting systemically and remain in plants more than in animal organisms. The antibiotic dissemination in plant is not uniform (D. Pramer, 1954). In some countries the antibiotic treatments for plants are widely used. These have quite small efficiency, relatively high toxicity and large costs (P. McManus, 2002). Sometimes the effects obtained through antibiotic treatments against plants, like streptomycin, are similar to effects of very low temperatures (R. Yoshida et al., 1998), or treatments stain the fruits in green. Restricting use of antibiotics in plant treatments is possible through cultivation of resistant plants varieties or through biological control of plant pathogens. Depending on crops and the objective of treatment, recommended concentrations of streptomycin varies between 50 - 200µg/ml.

Streptomycin resistance was detected for almost all susceptible pathogen, especially *Erwinia amylovora*, *Erwinia carotovora*, *Pseudomonas lachrymans*, *Xanthomonas campestris*, (McManus et al, 2002).

The copper sulphate was selected from inorganic substances. This is the most common form to use copper for control plant diseases, the concentrations varies between 0,5 - 1%, (especially neutralized mixtures). The copper compounds begin to be used from 1882, when Professor A. Millardet (Bordeaux University) discovered the first efficient compound against grapevine downy mildew, the mixture of copper sulphate and hydrated lime (Bordeaux mixture, fr. *bouillie bordelaise*). Inextricably linked molecules of copper sulphate determined proteins coagulation, lock of fermentative breathing systems and inhibition of carbohydrate metabolism, but also phytoalexin formation in plants.

The organic plant protection products present high toxicity against plant pathogen and relatively low risk to humans and animals. The most popular antibacterial substance from organic group is mancozeb, a mixture to maneb and zineb, old fungicide and bactericide, with widely spectrum of antibacterial activity, but without phytotoxicity; it can correct deficiencies of manganese and zinc at treated plants, too. In Romania are approved these products: Dithane M-45 (mancozeb 80%, used in this paper), Mancozeb 800 (mancozeb 80%), Dacmancoz 80 WP (mancozeb 80%), Dithane 75 WG (mancozeb 75%).

We used in our experiments various concentrations: streptomycin 10µg (standard micro disks for antibiogram); aqueous solution of copper sulphate 0,25%, 0,5% and 1% (usual for treatment 0,5%); 0,1%, 0,2% and 0,4% aqueous solution of Dithane 45 (usual for treatment 0,2%), corresponding to dilutions 0,08%, 0,16%, 0,32% mancozeb.

Were used, for copper sulphate and mancozeb, sterile filter paper disks impregnated in corresponding solutions. These disks were placed on solid media (nutrient agar) in Petri dishes after uniform inoculation of bacterial suspension. The results were obtained after incubation at 37ºC for 24 hours.

Interpretation of the results consisted of classification in three degrees of sensitivity, depending on diameter of bacterial growth inhibition zone, determined by those three substances: sensitive strains (S), strains with intermediate sensitivity (SI), resistant strains (R).

- Streptomycin conc. 10µg/disk (S):
    - S: 21 – 50 mm
    - SI: 7 – 20 mm
    - R: 0 – 6 mm

- Copper sulphate ($S_1$, $S_{0,5}$ and $S_{0,25}$):
  - $S_1$ - conc. 1%:
    - S: 11 – 20 mm
    - SI: 7 – 10 mm
    - R: 0 – 6 mm
  - $S_{0,5}$ – conc. 0,5%:
    - S: 11 – 20 mm
    - SI: 7 – 10 mm
    - R: 0 – 6 mm
  - $S_{0,25}$ – conc. 0,25%):
    - S: 11 – 20 mm
    - SI: 7 – 10 mm
    - R: 0 – 6 mm
- Mancozeb (Dithane 45) ($D_1$, $D_2$ and $D_4$):
  - $D_1$ - conc. 0,08%:
    - S: 11 – 30 mm
    - SI: 7 – 10 mm
    - R: 0 – 6 mm
  - $D_2$ - conc.0,16%:
    - S: 11 – 30 mm
    - SI: 7 – 10 mm
    - R: 0 – 6 mm
  - $D_4$ – conc. 0,32%):
    - S: 11 – 30 mm
    - SI: 7 – 10 mm
    - R: 0 – 6 mm

## RESULTS AND DISCUSSIONS

The sensitivity, intermediate sensitivity and resistance of tested bacterial strains against streptomycin, can be observed in Figure 2. The most tested bacterial strains were sensitive or had intermediate sensitivity against streptomycin. Just two of soil samples presented a number of resistant bacterial strains against streptomycin at used concentration; for these, the diameter of the growth inhibition zone was maximum 6mm.

This antibiotic isn't used in Romania for treatment of grapevine bacterial diseases, so the soil bacteria not require resistance genes against streptomycin, which to be able to transmit to next generation and in bacterial population.

Regarding the action of copper sulphate, the tested bacterial strains were resistant against small concentrations, but the sensitivity and intermediate sensitivity increased with the growing concentration (Figure 3). Since 94% from tested bacterial strains were resistant at concentration of copper sulphate twice smaller than therapeutic dose, just 32% from all strains were resistant at concentration twice larger than therapeutic dose. In this case the majority of tested strains had intermediate sensitivity.

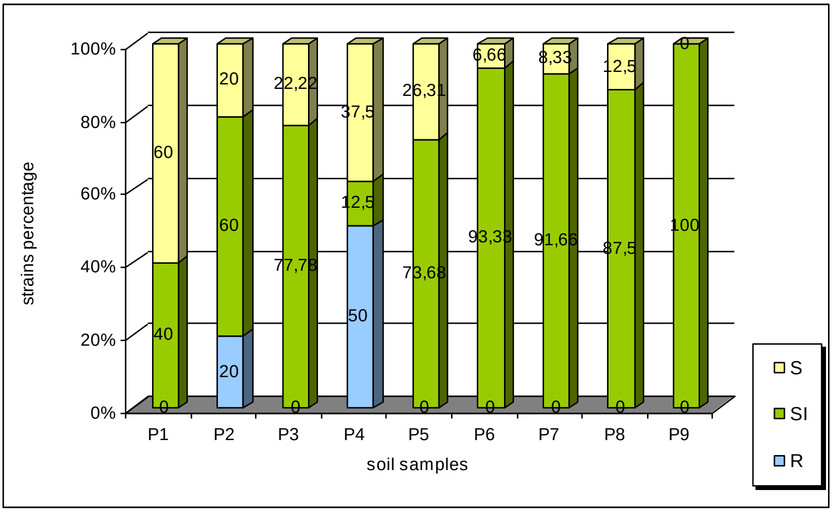


**Fig. 2.** The sensitivity spectrum of studied strains against streptomycin

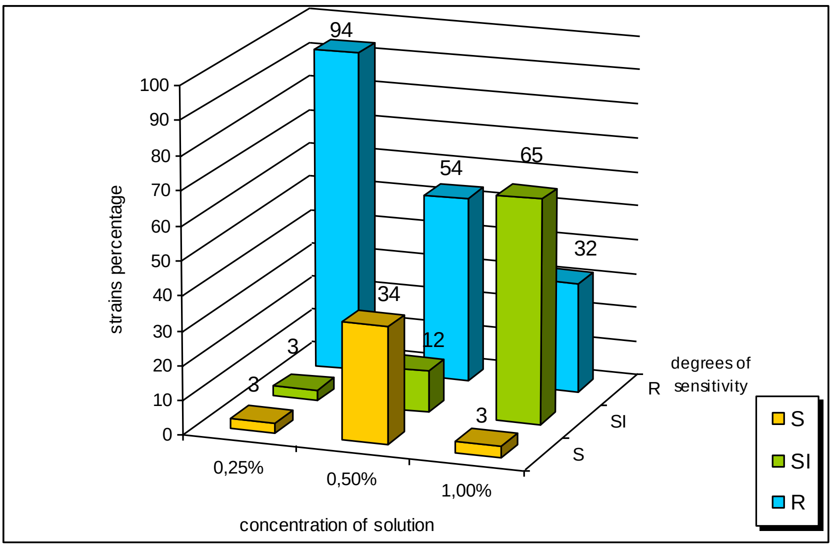


**Fig. 3.** The sensitivity spectrum of studied strains against copper sulphate

The bacterial strains tested against mancozeb had similar sensitivity with sensitivity against copper sulphate, the sensitivity and intermediate one increased with the growing concentration (figure 4). The percentage of resistant strains decreases from 53% against mancozeb with concentration 0,08% to 14% against mancozeb with concentration 0,32%.

The sensitivity and the intermediate sensitivity seems to be more obviously against mancozeb than copper sulphate, so a cautious approach in widely using the substance is required, because the tested bacterial strains were not pathogenic by all means.

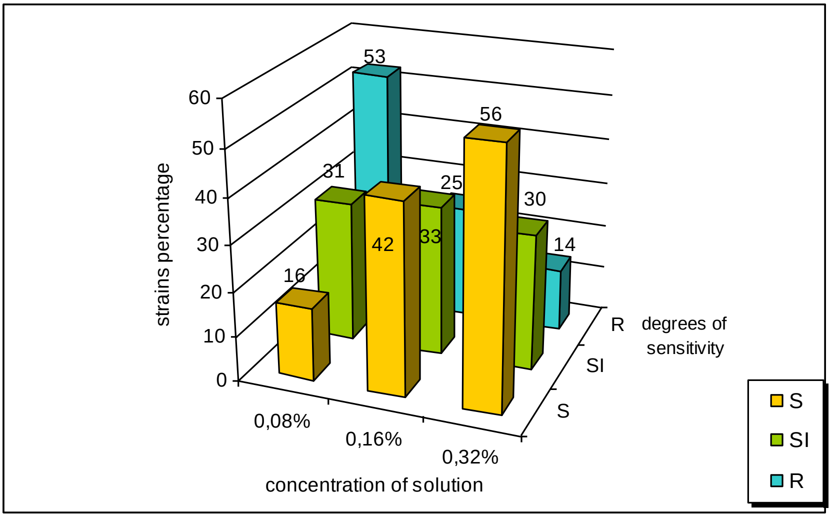


**Fig. 4.** The sensitivity spectrum of studied strains against mancozeb

## CONCLUSIONS

1. The bacterial strains isolated from soil samples presented various degrees of sensibility against substances frequently used in plant treatments (bacterial and fungal diseases).

2. The resistance against low concentration of streptomycin was observed for small percentage of bacterial strains, which means the absence of resistance genes against this antibiotic on soil bacteria and the absence of risk to transmit the resistance to pathogenic bacteria for animals and humans.

3. The copper sulphate, with well known effects against fungal plant diseases, don't influence in negative way the bacterial microbiota from soil, consist especially of saprophytic bacteria; almost 54% from tested strains against usually concentration of copper sulphate are resistant and smaller concentrations determined higher resistance.

4. The mancozeb action against soil bacteria is more pronounced than streptomycin or copper sulphate action, which means the mancozeb could have a bad influence on the sensitive microbiota from soil.

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