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THE USE OF CHEMICALS, PLANT MATTER AND ANIMAL SOURCES TO DEVELOP BIOFERTILISERS

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Abstract: *The article concerns the effect of glycine, glucose, gelatin (powdered and granulated), meat and bone meal, peas and some other plant matters on the growth of Pseudomonas fluorescens AP-33 and Bacillus subtilis 26-D microorganisms used in biological fertilizers. The article considers the influence of some of the plant matters, such as wintergreen (pipsissewa), burnet, Centella asiatica, on the growth of Pseudomonas fluorescens bacteria, while some plants - nettle, buckthorn, bearberry - inhibit bacteria growth. We found that chemicals (glycine), vegetable matter (pea fibre) and animal sources (meat and bone meal) were the most effective additives among those studied in increasing the abundance of Pseudomonas fluorescens AP-33 by a factor of 2.5; 5.0 and 5.8, respectively. Bacillus subtilis 26-D abundance increased 3. 3 times with the addition of pea fibre and 6. 7 times with the addition of meat and bone meal.*

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Introduction

As the population grows, more attention is focused on increasing the amount of food. Land depletion and plant diseases have led to the use of chemical pesticides. The introduction of chemical pesticides into agricultural practices has consequently increased crop and food production. The global production of pesticides is increasing by 11% per year and has reached about 5 million tonnes [1].

However, pesticides widespread use has led to chemical pollution of soil, groundwater and air in almost all countries, with not only environmental but also economic and social consequences.

Some pesticides contain heavy metals: mercury, zinc, copper. Mineral fertilizers extracted from rocks contain heavy metal impurities. Phosphate fertilizers contain the highest amounts of these substances, e.g. superphosphate may contain lead, cadmium, copper, zinc, chromium, cobalt, nickel, vanadium [2].

Superphosphates, as well as potash fertilizers, can contain uranium, strontium, radium, thorium as impurities, when ingested by humans and animals with plant food can cause internal irradiation.

The use of mineral fertilizers causes acidification of the soil, dissolution of calcium, magnesium, manganese, zinc and copper, which can reduce the plant disease resistance.

The most important factor for increasing crop productivity and improving soil fertility is the scientifically proven use of fertilisers [3].

The increasing amount of mineral fertilizers applied in order to improve the soil fertility causes a disbalance of the main biogenic elements, affection by phyto-pathogens, the occurrence of plants stress response. Reductancy of nitrates along with increasing doses of nitrogen fertilizers causes their accumulation in plants and grain [4-6].

Recently, the agricultural practice of the bionomic ways of increasing crop productivity, namely the use of bio-fertilisers, have attracted more and more attention. Bio-fertilizers regulate the pollutants from the environment, limit the growth of phytopathogens and increase the crop productivity of agricultural plants. Rhizosphere bacteria in symbiosis with the plant contribute the adaptation of plants to stress [7-10].

The aim of this work is to use chemicals, plant matter and animal sources to develop biofertilisers.

The effects of biopreparations used in agricultural practice have not yet been thoroughly studied. In field conditions in different climatic regions and at different doses of mineral fertilisers they may exibit different efficiency [7, 8].

The market for biological crop protection products offers products based on live microbial cultures. New pesticides are being developed all over the world, producing biogenic biofungicides, bioinsecticides and growth regulators. The authors [11] predict an increase in the use of biologicals to replace/supplement existing pesticides over the next 5-10 years, and in the long term, their dense adoption in agricultural practices.

The literature is replete with papers on the use of different strains of *Bacillus subtilis* bacteria.

Bacillus subtilis can dissolve phosphorus in the soil, enhance nitrogen fixation and produce siderophores, which promote its growth and inhibit the growth of pathogens. *Bacillus subtilis* increases host plant stress tolerance by inducing the expression of stress response genes, phytohormones and stress-related metabolites [12].

The work dwells on the [13] the effect of the bacteria *Bacillus subtilis 2*, applied to the soil along with different doses of nitrogen fertilizers, on the physiological status of the plant-microbe system. The study shows the increasing of plant adaptation to high doses of nitrogen fertilizer in associative symbiosis with micro-organisms. *Bacillus subtilis 26D* and *Bacillus subtilis 11 BM* strains exhibited an attenuating effect on toxic cadmium ions. Each strain behaved individually depending on the plant species [14].

A review [15] examined the molecular mechanisms of regulatory systems and their physiological significance in the mutually beneficial interactions between *Bacillus subtilis* and plants.

Bacillus subtilis 26D strain was applied after damage by the Colorado potato beetle. A rapid recovery of shoot growth, wet and dry plant root mass after pest damage was noted [16].

Bacillus subtilis BZR 336g and *BZR 517* strains, selected from natural sources, showing antifungal effect against phytopathogenic fungi *Fusarium graminearum*, *F. culmorum*, *Microdochium nivale* and *Pyrenophora tritici-repentis*, and also promoting growth and development of plants were used to protect winter wheat against harmful diseases [17].

Recently, studies have been appearing on the use of the bacterium *Pseudomonas fluorescens* for plant protection.

Pseudomonas fluorescents is a common gram-negative bacillus.*Pseudomonas fluorescens* has simple nutritional requirements and grows well in a medium with mineral salts supplemented with any of a sufficient number of carbon sources [18].

Some species of *Pseudomonas fluorescens* have been shown to be potential biocontrol agents inhibiting plant diseases by protecting seeds and roots from fungal infections [19]. The use of *Pseudomonas fluorescens 9 and 10* isolates can increase horse bean (Viciafaba) growth and crop productivity [20].

Pseudomonas fluorescens-based preparations, such as Planriz and others, are approved for use against diseases of potatoes (phytophthora, etc.), sugarbeet (cauliflower rot), cabbage (blackleg), spring barley (helminthosporiosis root rot) and other crops. *Pseudomonas fluorescens* strain AP-33 exhibits a fungistatic effect.

Studying the conditions of accelerated growth of microorganisms *Bacillus subtilis* and *Pseudomonas fluorescens* when different substances are added to the nutrient medium will contribute to expanding the range of microbial biopreparations in the Russian market of environmentally safe plant protection products.

Experimental part

In order to make biopreparations cheaper, research on the effects of different substances on the growth of microbial populations is relevant one.

Various chemicals are used to cultivate *Pseudomonas fluorescens*: carbohydrates, amino acids, proteins, etc. In this research, *Pseudomonas fluorescens AP-33* was made submerged cultivation in nutrient media containing molasses, potassium phosphate, iron (III) sulphate, ammonium nitrate, magnesium sulphate, ground peas, plant extract and water. Plant dry raw materials in amount of 10 g per 1 litre of each extract were added into autoclave after sterilization. The pH value was kept between 7.5 and 7.6. The sterilization time was 30 min at 1.0 Bar. After autoclaving, the nutrient medium was cooled down to room temperature and filtered. We cultivated the microorganisms at (28±2) °C with forced aeration at speed of 125 rpm in an "Innova 44" shaker incubator for 20-24 hours. We repeated the experiments for three times. The bacteria count was assessed by standard dilutions of ten times and sown in sterile conditions into Petri dishes on agarised medium of the above composition. The results are presented in Table 1.

Table 1. Effect of plant extracts on the count of *Pseudomonas fluorescens AP-33* during cultivation

№	Plant preparation		CFU/ml ·10^{-8}
1	Wintergreen (pipsissewa)	*Chimáphila umbelláta*	60±10
2	Burnet	*Sanguisorba officinalis*	60±10
3	Great nettle	*Urtíca dióica*	40±10
4	Bearberry (leaf)	*Arctostáphylos úva-úrsi*	40±10
5	Buckthorn	*Frángula álnus*	30±10
6	Gotu Kola	*Centella asiatica*	60±10
7	Without added plant extracts		50±10

Table 1 shows that some of the plants, such as wintergreen, burnet and Centella asiatica, promote bacterial growth, while others, such as bearberry, common nettle and buckthorn, inhibit bacterial growth.

The search for microbial growth stimulants led to the use of various chemical, plant and animal substances added plating medium of *Pseudomonas fluorescens AP-33.*

In order to study the effect of chemical, plant and animal substances on the growth of *Pseudomonas fluorescens AP-33*, we used nutrient media of the following composition: molasses, potassium phosphate, iron (III) sulfate, ammonium nitrate, magnesium sulfate, burdock, horsetail and water to 1 litre. We added substances (glycine, glucose, etc.) in an amount of 5 g per 1 litre of each extract into autoclave before sterilization. The pH value was kept between 7.5 and 7.6. The sterilization time was 30 min at 1.0 Bar. After autoclaving, the nutrient medium was cooled down to room temperature and filtered. We cultivated the microorganisms at (28±2) °C with forced aeration at a speed of 125 rpm in an "Innova 44" shaker incubator for 20-24 hours. We repeated the experiments three times. The count of micro-organisms was assessed by standard dilutions of ten times and sown in sterile conditions into Petri dishes on agarised medium of the above composition. The results of the effect of chemical, plant and animal substances on the growth of *Pseudomonas fluorescens AP-33* can be seen in Table 2.

In order to study the effect of chemical, plant and animal substances on the growth of *Bacillus subtilis 26-D* we used nutrient media of the same composition as for *Pseudomonas fluorescens AP-33*. We added substances (glycine, glucose, etc.) in an amount of 5 g per 1 litre of each extract into autoclave before sterilization. The methodology used is similar to described above for *Pseudomonas fluorescens AP-33*. The results of the effect of chemical, plant and animal substances on the growth of *Pseudomonas fluorescens 26-D* can be seen in Table 2.

Table 2. Effect of chemical, plant and animal substances on the growth of *Pseudomonas fluorescens AP-33* and *Bacillus subtilis 26-D*

Addition to the nutrient medium	CFU/ml·10^{-6}	
	Pseudomonas fluorescens AP-33	*Bacillus subtilis 26-D*
Glycine	30±5	5±1
Glucose	15±1	10±1
Gelatin (powder)	10±1	10±1
Gelatin (granulated)	5±1	20±1
Peas (fibre)	70±10	50±10
Meat and bone meal	60±5	100±10
Monitoring	12±1	15±1

Table 2 shows the positive growth of *Pseudomonas fluorescens AP-33* influenced by the addition of glycine, increasing it 2.5 times, meat and bone meal 5.0 times and peas (fibre) 5.8 times. *Bacillus subtilis 26-D* count increased 3.3 times with the addition of pea fibre and 6.7 times with the addition of meat and bone meal.

Results and Discussion

We have studied the effect of chemicals, plant matter and animal source on the growth of *Pseudomonas fluorescens AP-33* and *Bacillus subtilis 26-D* microorganisms. By the results of the

experiments, some of the plants, such as wintergreen, burnet, Asian centella, promote bacterial growth, while others, such as common nettle, buckthorn and bearberry, inhibit their growth.

Chemicals (glycine), plant matter (pea fibre) and animal source (meat and bone meal) were the most effective additives studied in increasing the population of *Pseudomonas fluorescens AP-33*. The *Bacillus subtilis 26-D* count increased with the addition of plant matter and animal source, such as meat and bone meal and pea fibre.

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