The EuroBiotech Journal

RESEARCH ARTICLE
Plant Biotechnology

Greenhouse study on the influence of natural biostimulators and fertilizers on improving bean plants growth and microbial activity in oil-polluted soil

Matei Gabi-Mirela[1*], Matei Sorin[1], Drăghici Elena Maria[2] and Stoian Marian[2]

Abstract
The presence of oil hydrocarbons and salts in soils has as consequence the poor growth of plants due to the low availability of nutrients caused by unappropriate water regime, increased soil toxicity and the deterioration of soil structure. The present research has as main purpose the improvement of the bean plants (cultivar UNIDOR) growth and to stimulate microbial activity in rhizosphere using various natural stimulators and fertilizers (AMALGEROL,VERMIPLANT, POCO, IGUANA and FORMULEX) in greenhouse experiments on oil-polluted soil from Icoana farm, Olt county. The total counts of microorganisms (heterotrophic aerobic bacteria and filamentous fungi) were estimated by dilution plate method. The global microbial activity was measured as soil respiration by substrate-induced respiration method. Total bean plants biomass accumulation significantly increased under the influence of natural stimulators and fertilizers added (excepting IGUANA) when compared to untreated control. The best results were recorded for VERMIPLANT. Natural products induced activation of physiological activities of soil microbiota reflected in increased values of CO_2 released by respiration, lower levels of colonization with phytopathogenic species, the domination of fluorescent pseudomonads, actinomycetes and cellulolytic fungi, too. The particular aspect of paper circular chromatograms reflected qualitative differences between rhizosphere soils determined by the effect of treatments with natural stimulators and fertilizers.

Keywords: soil pollution, oil hydrocarbons, beans, soil microorganisms, natural biostimulators

[1]National RD Institute for Soil Science, Agrochemistry and Environment, Bucharest, Romania

[2]University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Horticulture, Bucharest, Romania

*Corresponding author: M. Gabi-Mirela
E-mail: mirelamatei61@yahoo.com

DOI: 10.2478/ebtj-2018-0051

Introduction
The main cause of poor growth of plants in oil-polluted soils is the low availability of nutrients caused by inappropriate water regime, the increase of soil toxicity, the deterioration of soil structure due to the presence of oil hydrocarbons and salts.

Bioremediation plays an important part in solving these problems by the capacity of various microorganisms to degrade hydrocarbons by their enzymatic equipment and to utilize them as source of carbon and energy. Literature cites the utilization of inorganic or organic fertilizers for stimulating biodegradative activity of microbiota and creating conditions for plants growth (1), as well as the advantages and disadvantages of various methods of application of microorganisms in bioremediation (2).

The purpose of the present research was to improve the bean plants growth and microbial activity in rhizosphere using various natural stimulators and fertilizers in greenhouse experiments on oil-polluted soil from Icoana farm, Olt county.

Materials and Methods
Soil in greenhouse experiment: Surface soil (0-20cm) from Icoana farm, Olt County, polluted with total petroleum concentration (TPH previously determined by gravimetric method) of 72.87 g total petroleum hydrocarbon x kg^{-1} dry soil.

Test plants: Bean plants (*Phaseolus vulgaris* L. cultivar UNIDOR)

Natural stimulators and fertilizers: AMALGEROL, VERMIPLANT, POCO, IGUANA and FORMULEX (**Table 1**).

Table 1. Main characteristics of natural products utilized in experiment and their origin

No.	Products	Characteristics/Source
1	**AMALGEROL**	Natural product with vegetal oils and hormones that stimulates plant growth, mycorrhizal symbiosis, N_2-fixing, microbial activity, vegetal debris decomposition, improves soil structure and fertility
2	**VERMIPLANT**	Natural nutrients enriched product (biofertilizer) from earthworms containing microelements (barium, iron, zinc, manganese) and amino acids that stimulate microbial activity and plant growth
3	**POCO**	Natural product of herbs and plant extracts (utilized for pollution control), stimulating and accelerating the growth and metabolic activity of microorganisms by micro-nutrients and trace elements
4	**IGUANA**	Natural product of algae with macro and microelements for improving soil conditions and plant growth stimulating
5	**FORMULEX**	Natural organic complete, balanced and stabilized product of macro and microelements for optimum plant growth and rooting in horticulture

Total plant biomass (grams) accumulated was determined by weighing fresh plants (shoots and roots) cultivated in polluted soil (non-treated control) and variants treated with natural stimulators and fertilizers (2%).

Microbiological analysis of soil samples was performed by plating soil decimal dilutions on specific solid culture media (Topping for heterotrophic bacteria, potato dextrose agar-PDA for fungi and Stapp with filter paper for cellulolytic microorganisms).

After incubation at dark, microbial colonies were counted and their density was reported to gram of dry soil.

Taxonomic identification of bacteria was done according to Bergey's manual of determinative bacteriology (3).

Fungi identified according to Domsch and Gams (4) and Watanabe (5) determinative manuals.

The global physiological activities of microflora were determined by substrate induced respiration method (SIR) and expressed as mg CO_2 x 100 g^{-1} soil (6).

All assays were carried out in triplicate.

Statistical analysis

Results were interpreted by one-way analysis of variance (ANOVA). The value $p<0.05$ was considered statistic significant (Student test).

Results

Total plant biomass accumulated significantly increased in variants with natural stimulators and fertilizers (excepting IGUANA) when compared to untreated control, the best results being of 176.25 g, recorded for the variant with VERMIPLANT (**Fig. 1** and **Fig. 2**).

Natural products improved physiological activities of microbiota reflected in increased values of CO_2 released by respiration (**Fig. 3**), increased number of bacteria, especially of *Pseudomonas fluorescens* and actinomycetes (**Fig. 4** and **Table 2**), lower levels of colonization with fungi and with potential plant pathogenic species of *Fusarium*, the domination of cellulolytic fungi (**Fig. 5** and **Table 2**).

Figure 1. The aspect of bean plantlets in experimental variants.

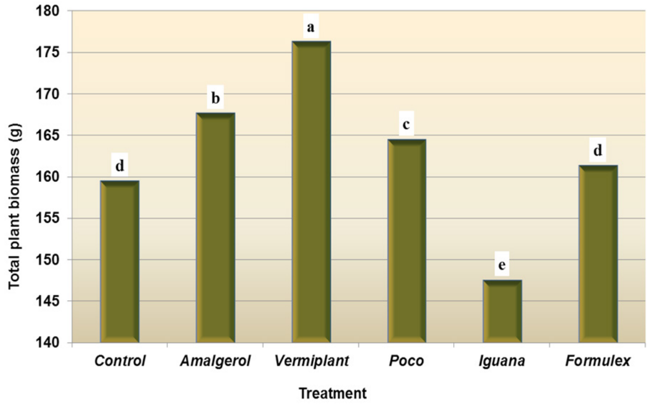

Figure 2. Influence of natural stimulators and fertilizers on plant biomass. Values followed by the same letter are not significantly different for p<0.05 (Student test).

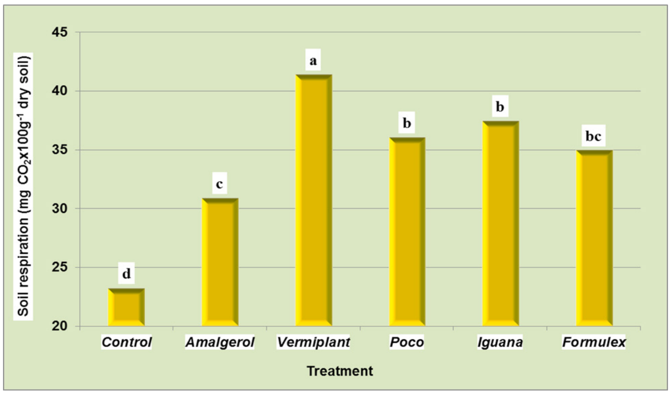

Figure 3. Influence of natural stimulators and fertilizers on soil respiration. Values followed by the same letter are not significantly different for p<0.05 (Student test).

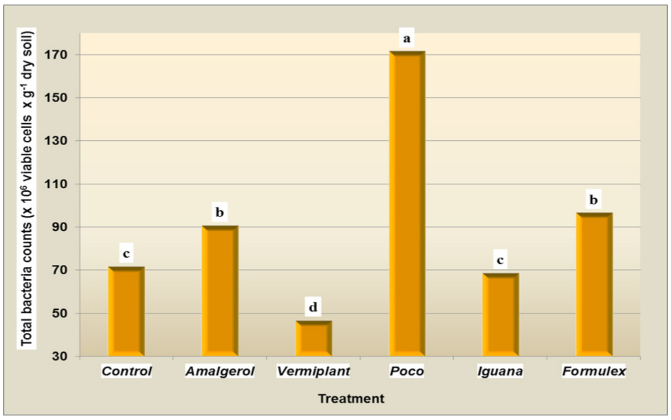

Figure 4. Influence of natural stimulators and fertilizers on soil bacteria. Values followed by the same letter are not significantly different for p<0.05 (Student test).

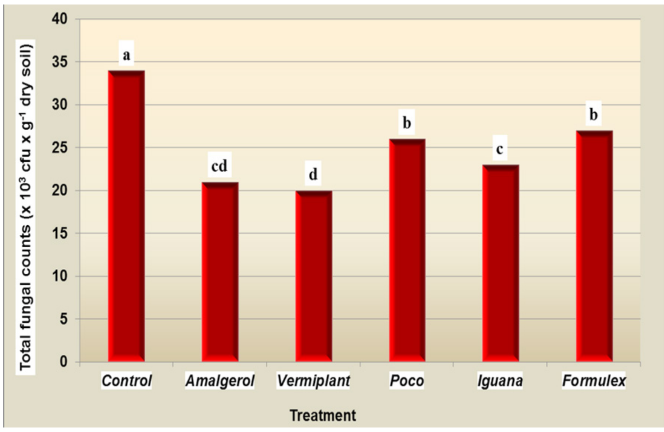

Figure 5. Influence of natural stimulators and fertilizers on soil fungi. Values followed by the same letter are not significantly different for p<0.05 (Student test).

Effect of natural stimulators and fertilizers was evidenced by creating conditions for development of microbial species characteristic for the non-polluted soil in the area (e.g. bacteria *Bacillus cereus* var. *mycoides*, fungal species *Paecilomyces marquandii*, *Aspergillus niger*, *Trichoderma viride*, *Penicillium* sp.) previously investigated (7), especially under the influence of Vermiplant, Iguana and Poco.

Excepting for the variant with Vermiplant, all fungal communities from the variants with natural stimulators and fertilizers were dominated by *Cunninghamella elegans.*

The qualitative differences between rhizosphere soils determined by the treatments with natural stimulators and fertilizers were evidenced on the particular aspect of paper circular chromatograms (**Fig. 6**).

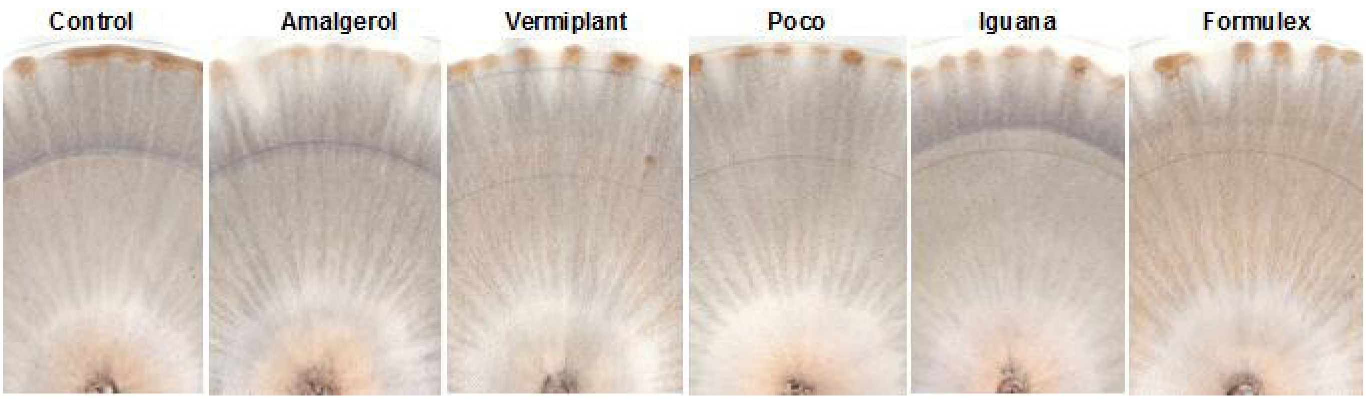

Figure 6. Paper circular chromatograms of soil from experimental variants.

Specific radiation, number, color and shape of pike-like formations, differentiation in inner, middle and outer zones, ring formations between the middle and outer zone and at the edge of the outer zone, textures indicate different degrees of soil vitality, health, intensity of biotic activity, complexity of organic matter or mineralization degree.

Table 2. Taxonomic composition of bacterial and fungal microflora

Experimental variants	Bacterial species (Topping)	Fungal species/cellulolytic microflora (Czapek/Stapp)
Control	*Pseudomonas fluorescens*, *Bacillus polymixa*, *Bacillus circulans*, *Bacillus cereus* Actinomycetes Series Albus, Fuscus	*Fusarium verticillioides*, *Aspergillus ochraceus*, *Fusarium avenaceus*, *Aspergillus flavus*, *Aspergillus terreus*, *Fusarium* sp., White sterile mycelia, *Rhizopus stolonifer*, *Eurotium herbariorum*, *Fusarium sporotrichioides*
Amalgerol	*Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus polymixa*, *Bacillus megaterium*, *Bacillus circulans*, Actinomycetes Series Albus, Ruber	*Cunninghamella elegans*, *Rhizopus stolnifer*, *Emericella nidulans*, *Fusarium sporotrichioides*, *Fusarium* sp., *Aspergillus versicolor*
Vermiplant	*Pseudomonas fluorescens*, *Bacillus circulans*, *Pseudomonas* sp. Actinomycetes Series Griseus, Albus, Ruber	*Fusarium* sp., *Fusarium oxysporum*, *Fusarium sporotrichioides*, *Paecilomyces marquandii*, *Mucor racemosus*
Poco	*Pseudomonas fluorescens*, *Pseudomonas sp.*, *Bacillus circulans*	*Cunninghamella elegans*, *Fusarium oxysporum*, *Fusarium* sp., *Aspergillus niger*, *Rhizopus stolnifer*, *Myrothecium roridum*
Iguana	*Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Bacillus polymixa*, *Alcaligenes* sp., *Serratia marcescens*, *Arthrobacter globiformis*, *Bacillus* sp., Actinomycetes Series Albus, Luteus, Ruber	*Cunninghamella elegans*, *Fusarium culmorum* var. *roseum*, *Paecilomyces marquandii*, *Aspergillus versicolor*, *Aspergillus flavus*, *Penicillium* sp., *Trichocladium* sp., *Aspergillus terreus*
Formulex	*Pseudomonas fluorescens*, *Bacillus megaterium*, *Bacillus mesentericus*, *Bacillus circulans*, *Pseudomonas aeruginosa*, Actinomycetes Series Albus, Luteus, Fuscus, Ruber	*Cunninghamella elegans*, *Rhizopus stolnifer*, *Aspergillus fumigatus*, *Fusarium* sp., *Fusarium culmorum*, *Trichocladium* sp., *Aspergillus flavus*, *Paecilomyces marquandii*, *Penicillium* sp.

Discussions

The results of the greenhouse experiments carried out demonstrated that natural fertilizers and biostimulators coupled with improved aeration conditions in the soil by dilluting oil-polluted soil with perlite stimulated microbial activity plant growth, yields and resistence to pathogens.

Our results are in concordance with data from literature (8) that reported growth and yields stimulation of cucumber plants, as well as higher protein contents and increased peroxidase activity with beneficial effects on plant resistance to pathogens when organic fertilizers were combined with biological gels in environment friendly technologies.

The use of digestate and composts stimulated soil microbial activity, proved to be beneficial for lettuce crops and avoided environmental problems caused by agrochemicals (9).

Results obtained from previous field experiments on soil polluted with petroleum residues and salts evidenced the favorable influence of using perlite in proportion of 50%to 75% in phytoremediation processes involving potato cultures by stimulating biodegradative activity of microbiota, including halo tolerant species (10)

There are research showing that microbial strains or consortia of microorganisms from oil-polluted soils are able to degrade various hydrocarbons by efficient metabolic processes, with positive effect on improving soil conditions and making it less restrictive for plants growth (11).

Similar results were reported for biosurfactant-producing strains of microorganism such as *Pseudomonas putida* (12), *Bacillus subtilis* (13), *Micrococcus* sp., *Streptomyces griseus* (14), that contributed to soil bioremediation by increased capacity to degrade oil hydrocarbons.

Other research showed that metabolites from culture filtrates of *Pseudomonas aeruginosa* strain PG1 presented hydrocarbon-degrading capacity and antagonistic properties against plant pathogenic bacteria and fungi (15).

Recent research (16) showed that fungal isolates of *Trichoderma, Polyporus, Aspergillus*, *Fusarium* were able to metabolize certain water pollutants with polycyclic structure, process mediated by enzyme laccase, known also as responsible for antimicrobial properties of microorganisms able to produce it.

Research on various species from genus *Trichoderma* evidenced the role of certain metabolites in plant protection and plant growth promoting, stimulating of soil microbiota and improving soil aeration ("biological tillage") (17, 18, 19, 20, 21).

Other research (22) reported that biodynamic paper circular chromatography and scanning electron microscopy proved to be very useful in evaluating comparatively the quality of various variants of biofertilizers produced by earthworms and microorganisms.

Present work evidenced that the natural products were stimulators for rhizosphere microbiota and its metabolic activity and ensured an adequate nutrition of plants, recommending them for utilization in bioremediation of oil-polluted soils under the cultures of horticultural plants.

Conclusions

Total plant biomass accumulation significantly increased in variants with natural stimulators and fertilizers (excepting IGUANA) when compared to untreated control, the best results being recorded for VERMIPLANT.

Natural products also improved physiological activities of microbiota reflected in increased values of CO_2 released by respiration, lower levels of colonization with potential plant pathogenic species of *Fusarium*, the domination of cellulolytic fungi, *Pseudomonas fluorescens* and actinomycetes.

The qualitative differences between rhizosphere soils determined by the treatments with natural stimulators and fertilizers were evidenced on the particular aspect of paper circular chromatograms.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number PN-III-P2-2.1_PTE-2016-0084, (40-PTE), acronym ECOREMTEH.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

1. Nduka JK, Umeh LN, Okerulu IO, Umedum LN, Okoye HN. Utilization of different microbes in bioremediation of hydrocarbon contaminated soils stimulated with inorganic and organic fertilizers. J Pet Environ Biotechnol 2012; 3: 116. doi:10.4172/2157-7463.1000116.
2. Abatenh E, Gizav B, Tsegaye Z, Wassie M. Application of microorganisms in bioremediation-review. J Environ Microbiol 2017; 1(1): 2-7.
3. Bergey DH, Holt JG. Bergey's manual of determinative bacteriology 9. Williams & Wilkins Eds., Baltimore 1994.
4. Domsch KH, Gams W. Fungi in agricultural soils. T&A Constable Ltd. Edinburg, London 1970.
5. Watanabe T. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species 2nd ed. 2002; CRC PRESS.
6. Matei S. Determination of soil respiration and microbial biomass. In: Dumitru M, Manea A(coord.). Methods of chemical and microbiological analysis (utilized in soil monitoring system), (in Romanian). Ed. SITECH, Craiova 2011; p. 283-288.
7. Matei GM, Matei S. Preliminary study of microbial communities in soil contaminated with oil hydrocarbons from Icoana. Sci Papers Agron 2017; 60(1):85-90. Ed. „Ion Ionescu de la Brad", Iași, http://www.revagrois.ro/volum/vol-60-1_2017.pdf
8. Petre S, Pele M, Draghici EM, Postamentel M. Influence of fertilizers on cucumber fruit quality. Rev Chim 2016; 67(7):1360-1362.
9. Gomez Sanchez E., Solis Oba MM, Delgado Flores J, Montalvo Aguillar KH, Solis Oba A. Evaluation of the use of organic fertilizers in lettuce (*Lactuca sativa*) crop. J Biotechnol 2018; 280S:S82. https://doi.org/10.1016/j.jbiotec.2018.06.270.
10. Drăghici EM, Scarlat V., Pele M, Postamentel M, Somăcescu C. Usage of perlite in polluted sandy soils for potato crop. Rev Chim 2016; 67(11):12281-2286.
11. Nisha P, Nayana M, Viji V. Degradation studies on diesel oil using bacterial consortium isolated from oil polluted soil. Adv Biotech 2013; 13 (2)
12. Tuleva BK, Ivanov RG, Christova NE. Biosurfactant production by a new *Pseudomonas putida* strain. Z Naturforsch 2002; 57:356-360.
13. Waogu LA, Onyeze GOC, Nwabueze RN. Degradation of diesel oil

in a polluted soil in using *Bacillus subtilis*. Afr J Biotechnol 2008; 7(12): 1939-1943.

14. Santhini K, Myla J, Sajani S, Usharani G. Screening of *Micrococcus* sp. from oil contaminated soil with reference to bioremediation. Bot Res Int 2009; 2(4): 248-252.
15. Patowary K, Patowary R, Kalita MC, Deka S. Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. Front in Microbiol 2017; 8: 279.
16. Iordache O, Popa G, Dumitrescu I, Rodino S, Matei A, Cornea CP, Diguta C, Varzaru E, Ionescu I. Evaluation of decolorization abilities of some textile dyes by fungal isolates. Ind Text 2016; 67(2):181-188.
17. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M. A novel role for *Trichoderma* secondary metabolites in the interaction with plants. Physiol Mol Pl Pathol 2008; 72: 80-86.
18. Viterbo A, Landau U, Kim S, Chernin L, Chet I. Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. Fems Microbiol Lett 2010; 305: 42-48.
19. Vinale F, Nigro M, Sivasithamparam K, Flematti G, Ghisalberti EL, Ruocco M, Varlese S, Marra R, Lanzuise S, Eid A, Woo SL, Lorito M. Harzianic acid: a novel siderophore from *Trichoderma harzianum*. Fems Microbiol Lett 2013; 347: 123-129.
20. Vinale F, Strakowska J, Mazzei P, Piccolo A, Marra R, Lombardi N, Manganiello G, Pascale A, Woo SL, Lorito M. Cremenolide, a new antifungal, 10-member lactone from *Trichoderma cremeum* with plant growth promotion. Nat Prod Res 2016; 30: 2575-2581.
21. Oancea F, Răuț I, Șesan T, Doni M, Popescu M, Zamfiropol-Cristea V, Jecu L. *Trichoderma* strains as plant biostimulants in high residues farming systems. In: *Trichoderma* spp. - applications in agriculture and horticulture, Chapter 7, Ed University, Bucharest 2017; p. 336-383.
22. Kumar DS, Kumar PS, Rajendran NM, Kumar VU, Anbuganapathi G. Evaluation of Vermicompost maturity using scanning electron microscopy and paper chromatography analysis. J Agr Food Chem 2014; 62: 2738-2741.