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IN VITRO STUDIES ON SIDE-EFFECT OF SOME INSECTICIDES ON THE GROWTH OF N_2-FIXING ROOT-NODULE BACTERIA

Mungunzaya Ganbat, Hosam E.A.F. Bayoumi Hamuda*

Sándor Rejtő Faculty of Light Industry and Environmental Protection Engineering/ Óbuda University, Budapest, Hungary, mungunzayaganbat@gmail.com, +36202597693
Sándor Rejtő Faculty of Light Industry and Environmental Protection Engineering/ Óbuda University, Budapest, Hungary
**Coresponding author*

Abstract: *Globally, the consumption of nitrogen (N) fertilizer- is significantly increased day after day. The biological N_2-fixation is the main source of N input in agricultural soil. However, international emphasis on environmentally sustainable development with the use of renewable resources is likely to focus attention on the potential role of biological N_2-fixation in supplying N for agriculture. Several environmental conditions are limiting factors to the growth and activity of the N_2-fixing root nodule rhizobia. One of the most important and potentially limiting factors to biological N_2 fixation is the use of pesticides including the insecticides. The objective of the study is to investigate the adverse effect of insecticides on N_2-fixing rhizobia. Experiments were conducted to study the impact of seven insecticides [Anthio 33 EC (Formotion (33%), Sevin 85 WP (Carbaryl (85%), Rogor L-40 (Dimethoate (40%), Thiodan 50 WP (Endosulfan (50%), Phosphotion (Malathion (50%), Lannate 20 L (Methomyl (200 g/l), Pirimor 50 DP (Pirimicarb (50%)] on the growth of seven strains of N_2-fixing root-nodule rhizobia (four strains belonging to Rhizobium leguminosarum, one strain belonging to R. phaseoli, one strain belonging to R. trifolii and one strain belonging to R. loti) in vitro by measuring optical density. The rhizobial strains were grown in yeast extract mannitol broth medium treated each with different concentrations (0, 0.1, 1, 10, and 100 mg/litre of active ingredient) of each insecticide and incubated for 48 hours in microfermentor at 28°C. The results illustrated that Phosphotion and Sevin had an adverse effect on the growth of the investigated rhizobial strains, whereas Thiodan was safe to these rhizobial strains. Whereas the strain Lóbab Z of R. leguminosarum was the most tolerant and R. trifolii Ló133/64 was the most sensitive one among the tested strains. The adverse effects of insecticides on rhizobia were observed at concentrations not normally expected to occur under field conditions. For further task, it is required to investigate the adverse effects of these insecticides on nodulation and N_2-fixation capacity of specific host plants with their rhizobia in pot and field experiments as well as other vital microbial processes in a wide range of soils.*

Keywords: *Insecticides, N_2-fixation, root-nodule bacteria, rhizobium, relative growth*

INTRODUCTION

A tremendous amount of anthropogenic nitrogen (N) fertilizer has been applied to agricultural lands to sustain crop production all over the world. However, inappropriate N management applications have caused numerous ecological and environmental problems. Rather than anthropogenic nitrogen fertilizer, biological nitrogen fixation is more emphasized on supplying N for agriculture.
The main problem of using pesticides including insecticides is that the amount of applied pesticides which

actually reaches the target organism is about 0.1 % and the rest usually ends up contaminating the soil ecosystem. [1] Invention of pesticides and insecticides has also promoted crop production by controlling the pests effectively which has also had negative impacts on environment due to its xenobiotics characteristics. The negative impact of these chemicals on the soil environment and their processes has received more attention and has been described by many researchers. [2] Recent studies show that the applied pesticides may harm the natural microorganisms, disrupt soil ecosystem, and which eventually may affect human health by entering in the food chain.

Soil has its beneficial microorganisms and their associated biotransformations. Soil microorganisms have the ability to perform biochemical transformations of different elements like nitrogen (N), phosphorus (P), sulfur (S), and carbon (C). Pesticides and insecticides may directly or indirectly has an effect on the important biochemical reactions such as mineralization of organic matter, nitrogen fixation, nitrification, denitrification, and ammonification by activating/deactivating specific soil microorganisms and/or enzymes. [3] The fact that information on possible effects of pesticides on all biochemical processes is sparse proves that it deserves more emphasis from the researchers. The legume–rhizobia symbiosis has distinctive role in agriculture and it results in huge quantities of nitrogen fixation throughout the world and any adverse effect on rhizobia results in decreased rates of biological nitrogen fixation. The activity of the N_2-fixing root nodule rhizobia is limited by many factors such as increased use of pesticides and insecticides. [4] Some pesticides can be detrimental to N_2-fixing bacteria. It slows down the nitrogen fixation process in bacteria and reduces the bacterium's respiration rate which later prevents its positive effects. By infecting the roots of legumes, rhizobia bacteria can form nodules where N_2 fixation occurs. [5]

This research focused on finding out how these seven insecticides may have effect on growth of root nodule rhizobia strains namely *R. leguminosarum* strains, *R. phaseoli, R. trifolii*, and *R. loti* in a field condition.

MATERIALS AND METHODS

An *in vitro* experiment was carried out to evaluate the response of four *Rhizobium leguminosarum* strains and each strains of *R. phaseoli*, *R.trifolii*, *R.loti* mentioned in Table (1) originated from different soil types to seven insecticides mentioned in Table (2) using microfermentor technique.

The microfermentor technique was carried out as following: Insecticides were applied at four concentrations (0.0, 0.1, 1.0, 10, and 100 mg/l). These range of concentrations was tested in 5 ml yeast extract mannitol (YEM) broth medium inoculated with 125 µl bacterial suspension of cell density (10^6 CFU/ml).

Sterilized, defined yeast mannitol broth medium [6] contains the following ingredients in 1 litre of distilled water: yeast extract 1 g, mannitol 10 g. The growth was measured at 550 nm from a shaken culture using a Sanyo-Gallenkamp SP50 spectrophotometer U.K after 48 hours incubation in rotary shaker (150 rpm) at 28°C.

Table (1) Detailed data on tested Rhizobium strains

Rhizobium strain	Laboratory code	Host plant	Origin
R.Leguminosarum	HB-3841^{str+}	Vicia faba	Libya
	E 1012		England
	Lóbab Z		Hungary
	Bükköny 75/4		
R.phaseoli	Feryol 1/4	Phaseolus vulgaris	
R.trifolii	Ló 133/64	Trifolium pratense	
R.loti	Baltacim-3	Onobrychis viciifolia	

Table (2) Detailed data on inoculated insecticides [7]

Commercial names	Active Ingredients	Active Ingredients	Molecular Formula
Anthio 33 EC	Formothion	33%	$C_6H_{12}NO_4PS_2$
Sevin 85 WP	Carbaryl	85%	$C_{12}H_{11}NO_2$
Rogor L-40	Dimethoate	40%	$C_5H_{12}NO_3PS_2$
Thiodan 50WP	Endosulfan	50%	$C_9H_6C_{l6}O_3S$
Phosphotion	Malathion	50%	$C_{10}H_{19}O_6PS_2$
Lannate 20L	Methomyl	200g/L	$C_5H_{10}N_2O_2S$
Pirimor 50DP	Pirimicarb	50%	$C_{11}H_{18}N_4O_2$

RESULTS AND DISCUSSION

The results were presented as relative growth rate of control tubes.The results from this study provide critical baseline information for further examinations to optimize the growth of these root nodule N_2-fixing bacteria. Figure (1) summarizes the average growth of all strains by each insecticide effect, Phosphotion and Sevin 85 WP had an adverse effect on all strains. To contrast, Thiodon 50 WP, Piriimor 50 DP were safer to use.

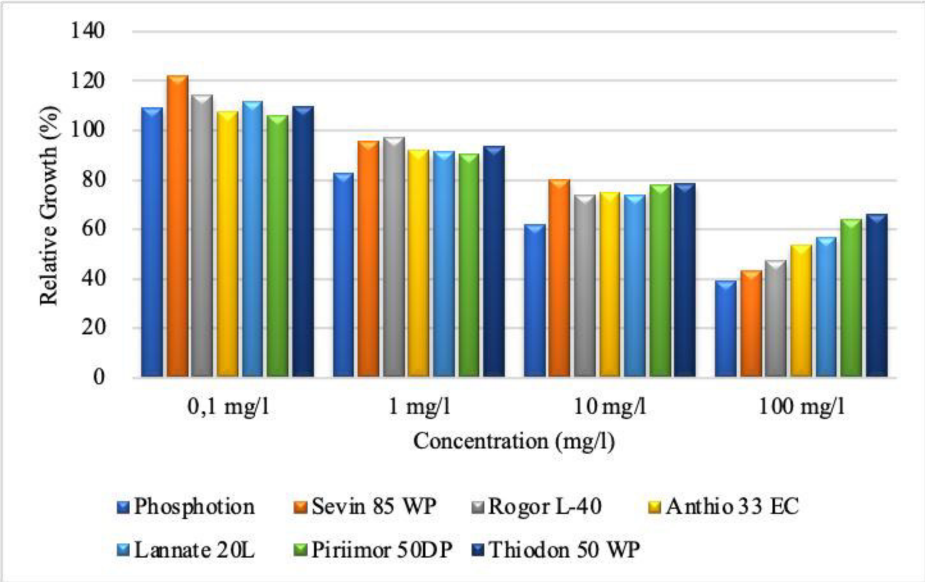

Figure (1) Average relative growth of all strains for each insecticide

Figure (2) shows that Lóbab Z has showed tolerance against Sewin 85 WP insecticide, Ló 133/64 was the sensitive one. Overall, there are huge differences between the growths of each strain at each concentrations, it indicates that sewin 85 WP had *the most* adverse effect on root-nodule bacteria.

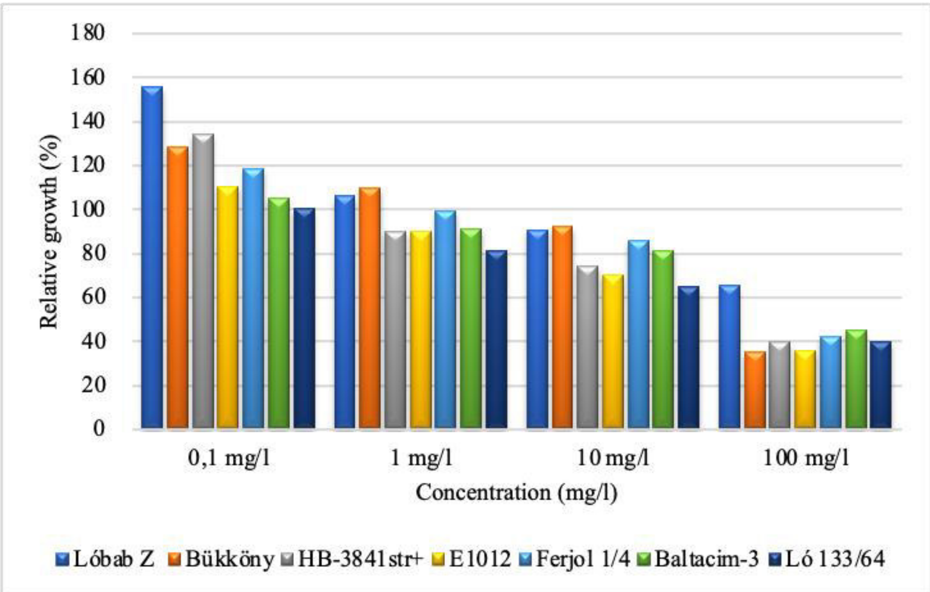

Figure (2) Relative growth of each strain for Sewin 85 WP insecticide

Figure (3) shows the effect of Thiodon 50 WP insecticide on the different strains. Bükköny and Ferjol 1/4 showed bigger growth while this insecticide was harmful for strains Ló 133/64, E1012, Baltacim 3.

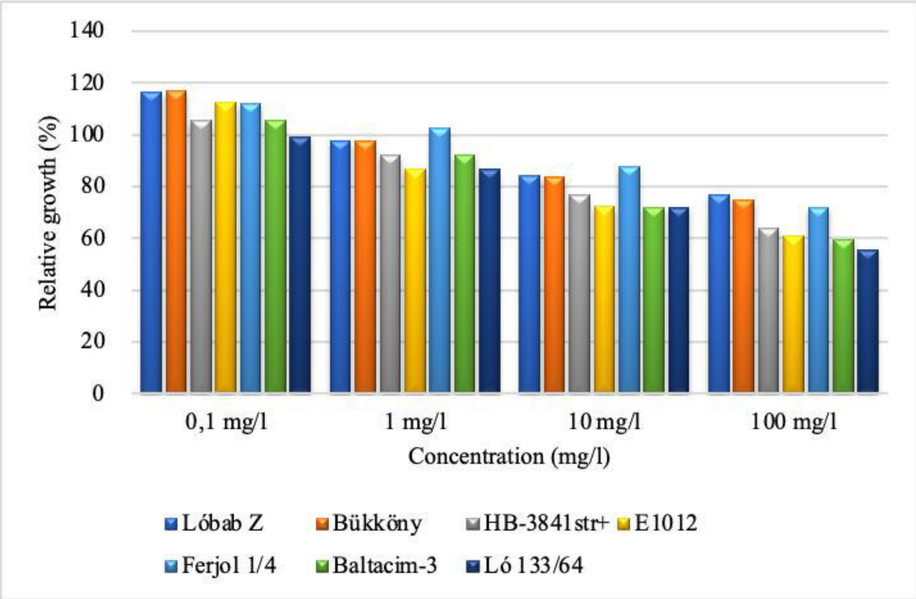

Figure (3)10 Relative growth of each strain for Thiodon 50 WP

Nevertheless, the insecticides used for aphid control may cause adverse effects on non-target organisms. [8] It is concluded that *Rhizobium* strains vary in their sensitivity to the insecticides. The Lóbab Z strain (isolated from Hungarian soil) proved to be broad-tolerant (the most tolerant) to most of the insecticides investigated, Bükköny 75/4 (Hungarian) and HB 3841 (Libyan) strains were the following tolerant strains. Strain E1012 (of English origin) was relative sensitive strain which depended on the toxicity of insecticides. Thus, it can be said that insecticides react differently with the strains depending on their toxicity and concentration. It was found that Thiodon 50 WP, Piriimor 50 DP were the least toxic, compared with Phosphotion, Sevin 85 WP being the most toxic. Rogor L-40, Anthio 33 E, Lannate 20 L have moderately influenced strain survival and reproduction.

These results provided a partial explanation of the compatibility of *Rhizobium* strains and insecticides under laboratory conditions. As mentioned earlier, any adverse effect on legume-rhizobia symbiosis results in reduced rates of biological N_2-fixation which counts and contributes more than artificial nitrogen supply. Therefore, the research with definite results and comparison to show how insecticides and pesticides affect this bacteria and its N_2-fixation process by decreasing the bacterium's respiration rate should be carried out in the future.

There is a significant impact of pesticide contamination in soil ecosystem. The applied insecticides affect the growth, survival, and working capacity of symbiotic rhizobial associationwith roots of legume plants resulting in dwindled atmospheric N_2-fixation [9].

The antagonistic interaction between the applied insecticides and symbiotic N-fixers differ with the specific chemical group of insecticide and the specificN_2-fixer group. However, the field recommended doses of these chemicals had little effect on symbiotic N-fixing bacteria [10]. Consequently, the soil receives the bulk of complex agrochemical compounds, several of which are poisonous to the activity of non-target beneficial soil micro-organisms [11].

More than 95% of the applied herbicides and 98% of insecticides reach non-target soil micro-organisms than their target pest, as they are sprayed proportionately across the entire field, irrespective of the affected areas [12].

The application of insecticides chlorpyrifos, imidacloprid, cypermethrin, endosulfan and carbofuran under field conditions causes considerable variation in soil bacterial populations [13]. Among the applied insecticides, chlorpyrifos has the most destructive effect on soil bacterial diversity. The residues of insecticides, when applied at field-recommended rates, do not cause any harmful influence on the nitrification [9].

However, it is the prolonged use and the amounts of such insecticides that cause concerns. Nevertheless, at higher rates, it inhibits the process of nitrification and microbes involved in it [11].

Some insecticides have a neutral effect on ammonification (e.g., superacids (25 and 500 g/ha) and nuvacron (100 and 600 g/ha) did not affect the ammonification process but is significantly reduced at higher concentrations of 1000 g/ha and 750 g/ha, respectively under controlled laboratory conditions [14]. Similarly, insecticides belonging to the chemical group of organophosphates (i.e., dimethoate, diazinon, chlorpyrifos, quinalphos, and malathion) inhibit the growth and population of soil bacteria [99].

Hence, it was reviewed that (1) the impact of various agrochemicals on the soil microbial diversity and environment; (2) the importance of smallholder farmers for sustainable crop protection and enhancement solutions, and (3) management strategies that serve the scientific community, policymakers, and land managers in integrating soil enhancement and sustainability practices in smallholder farming households. The current review provides an improved understanding of agricultural soilmanagement for food and nutritional security. [15]

CONCLUSIONS AND RECOMMENDATIONS

This study was designed to evaluate the effects of seven insecticides in vitro on the growth rate of seven rhizobial strains of N_2-fixing root-nodule. The adverse effects of insecticides on rhizobia strains were observed at concentrations not normally expected to occur under field conditions. From this study, it can be concluded that the results obtained under in vitro experiment show the sensitivity of the studied *Rhizobium* strains to chosen insecticides. It is evident that accumulations of high insecticide concentration are more destructive to rhizobia bacteria as compared to less concentration. Scientific studies on strain tolerance of *Rhizobium* and their reproduction is not widespread. Therefore, more investigations should be carried out using different techniques and other species of symbiotic N_2-fixing bacteria. What other factors affect the strain tolerance towards any kind of pesticides and how insecticides specially affect N_2-fixing bacteria should be studied as well in order to understand the relationship between the genes and symbiotic genes.

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