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INFLUENCE OF SEED-PROTECTANT PESTICIDES ON SOIL AZOTOBACTER AND CLOSTRIDIUM NITROGEN-FIXING BACTERIA

Andrei-Mihai GAFENCU, Andreea-Mihaela FLOREA, Eugen ULEA

"Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi, 3 Mihail Sadoveanu Alley, 700440, Iasi, Romania

Corresponding author email: eulea@uaiasi.ro

Abstract

This study was designed to evaluate the influence of five seed-protectant pesticide (fluxapiroxad, triticonazol, tetraconazol + tiofant metil, difenoconazol + fludioxonil and teflutrin + fludioxonil) at recommended concentration (for wheat seed treatment) on the numerical concentration in soil of Azotobacter spp. and Clostridium spp. nitrogen-fixing bacteria. The control variant was represend by soil sample where no seed treatment was applied. Soil samples were taken periodically and analyzed for the numerical determination of nitrogen-fixing bacteria of the genera Azotobacter and Clostridium.

Key words*: Azotobacter spp., Clostridium spp., nitrogen-fixing bacteria, soil, pesticides.*

INTRODUCTION

Pesticides are substances widely used in agriculture to control harmful organisms in crops (Johnsen et al., 2001). The most common use of pesticides is as plant protection products, which in general protect plants from damaging influence such as weeds, fungi, or insects (Adams, 2017). Pesticides can be classified by target organism (e.g., fungicides, insecticides, herbicides, rodenticides, bactericides etc.), chemical structure (e.g., organic, inorganic, synthetic, or biological), and physical state (Adams, 2017).

The use of plant protection products has been recognized and accepted as an essential ingredient in the modern agriculture for the control of pests, which damage crops and as result, they produce a severe loss in food production. However, the extended use of pesticides, together with the inadequate behaviours of prevention and use of basic protection requisites will increase the probability of accidental intoxication in a notorious manner.

Impact of pesticides on agronomic yield and profit margin makes them significant component of modern agricultural practices. However, the indiscriminate use of pesticides leads to the degradation of soil's microbial ecosystems.

Pesticides are often applied several times during one crop season and a part always reaches the soil. The wide use of pesticides has created numerous problems, including the pollution of the environment. The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides (Arora et al., 2016; Sethi et al., 2013).

The application of pesticides starts from the pre sowing stage. Different treatments include soil application, seed treatment, foliar spray, etc. Repeated applications of pesticides contaminate the soil. Soil is the most important site of biological interactions. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including microflora of soil, and also the physico-chemical properties of soil like pH, salinity, alkalinity leading to infertility of soil (Sarnaiket et al., 2006).

When pesticides are applied, the possibilities exist that these chemicals may exert certain effects on non-target organisms, including soil microorganisms (Simon-Sylvestre and Fournier, 1980).

The microbial biomass plays an important role in the soil ecosystem where they fulfil a crucial role in nutrient cycling and decomposition (De-Lorenzo et al., 2001).

Microorganisms are the primary soil decomposers driving key ecosystem processes such as organic matter decomposition, nutrient cycling and thereby, plant productivity (Devareet et al., 2007; Pandey and Singh, 2004).

In the last few decades, many soil microorganisms have been shown to have a positive effect on plant development. In addition to well-known symbiotic bacteria, free nitrogen-fixing organisms in the rhizosphere (*Azotobacter*) can stimulate plant development or limit damage caused by soil-borne plant pathogens, which is why it has been used as a possible nitrogen fertilizer to increase crop yields (Askar et al., 2013).

The aim of this study was to determine the effect of a number of five pesticides used for the treatment of wheat seeds on the numerical density of bacteria of the genera *Azotobacter* and *Clostridium* during the vegetation period of the winter wheat crop.

MATERIALS AND METHODS

Pesticides

Pesticides used for treatments in the research are commonly used for winter wheat seeds treatment, both by farmer's in Romania and by those in the European Union (ISSN, 2020). Pesticides used in the research were Fluxapyroxad (Systiva 333 FS), Triticonazol (Premis), Tifofant-metil + Tetraconazol (Biosild Top), Difenoconazol + Fludioxonil (Difend Extra), Teflutrin + Flodioxonil (Austral Plus) (Table 1). To compare the effect of pesticides on the numerical density of soil bacteria of the genera *Azotobacter* and *Clostridium*, the control of the experiment was represented by the variant where winter wheat seeds were not treated.

Table 1. Pesticide treatments under the study

S. No.	Pesticide used		Mode of application	Dosage
	Trade Name	Active ingredient (technical name)		
1	Systiva 333 FS	Fluxapyroxad 333 g/l	Seed application	150 ml/100 kg seeds
2	Premis	Triticonazol 25 g/l	Seed application	150 ml/100 kg seeds
3	Biosild Top	Tiofanat-metil 350 g/l Tetraconazol 20 g/l	Seed application	100 ml/100 kg seeds
4	Difend Extra	Difenoconazol 25 g/l Fludioxonil 25 g/l	Seed application	200 ml/100 kg seeds
5	Austral Plus	Teflutrin 40 g/l Fludioxonil 10 g/l	Seed application	500 ml/100 kg seeds

Field experience

Field plot experiment was carried out at Ezareni Farm - Iasi Didactic Station of "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine.

The experiment was monofactorial, studied factor was represented by seed-protectant pesticides. The design of the experiment was done according to the "randomized block method" in four replications.

Winter wheat crop technology was represented by classical technology. Wheat crop was sown after *Lupinus* sp. and was fertilized with moderate doses of fertilizer: 120 kg N/ha^{-1} and 45 kg P_2O_5/ha^{-1}.

It should be noted that no phytosanitary treatments were carried out during the growing season.

The sowing took place on October 22, 2019 and was done with the help of the Wintersteiger seed drill, and the harvesting took place on June 20, 2020 and was carried out with the Wintersteiger combine harvester.

Wheat variety

Wheat variety *Triticum aestivum* L. *cv. Glosa* was used in the experiment.

Total bacterial count

Soil sample were taken from each plot at 20 (11.XI.2019), 41 (02.XII.2019), 170 (08.IV.2020) and 184 (22.IV.2020) days after sowing (DAS). The number of *Azotobacter* and *Clostridium* population of wheat rhizosphere was determined by counting the colonies formed on solid Ashby medium.

Soil samples from experimental plots at 10 cm depth were collected randomly from five places from each plot and composited. 10% (w/v) suspensions of soil samples in sterile distilled water were serially diluted for determining total count of bacteria using plate technique.

For enumeration of nitrogen fixing bacteria solid Ashby medium containing: glucose 20 g,

KH_2PO_4 0.2 g, K_2SO_4 0.2 g, agar 15-18 g, $MgSO_4$ 0.2 g, NaCl 0.2 g, $CaCO_3$ 5 g, in one litre distilled water was used.

Soil dilutions

From the collected samples, successive dilutions were made in sterile water, using a dilution coefficient in the rate of 10 (dilutions 10^{-1}, 10^{-2}, …,10^{-6}). By this technique a series of dilutions are obtained in which the number of germs decreases in arithmetic progression. To prepare these dilutions, 9 ml of double-distilled water sterilized at 120°C for 30 minutes was distributed in sterile tubes of 30 ml capacity. Weighed 1 g of soil onto a sterile watch and placed it in the first dilution tube. After vigorous stirring for five minutes a 10^{-1} (1/10) dilution was obtained. From this dilution was taken with a sterile graduated pipette, 1 ml of suspension and transferred to another test tube with 9 ml of sterile water, obtaining the dilution 1/100 (10^{-2}). In the same way the other dilutions were obtained: 1/1000 (10^{-3}), 1/10000 (10^{-4}), 1/100,000 (10^{-5}) and 1 / 1,000,000 (10^{-6}). Under aseptic conditions, seedings were made from the obtained dilutions, introducing 1 ml of suspension from each dilution into a Petri plate. The plates were incubated at 28^0C up to 5 days.

Determination of the number of microorganisms in the soil

After incubation period at the thermostat, the colonies were counted with the help of Wolfhügel plates. With the help of the Wolfhügel plate, the colonies of bacteria from 15-20 surfaces of one cm^2 taken on the two diagonals of the Petri plate were counted. To calculate the number of colonies on the entire surface of the plate, the average number determined per cm^2 was multiplied by the surface area of the plate in cm^2. To determine the number of bacteria in a gram of soil, multiply the number of colonies in a Petri dish by the inverse value of that dilution. The result of the count is related to the dilution used, and the final result is expressed in colony forming units (CFU) per 1 g of soil.

Statistical analysis was performed using the SPSS program (IBM SPSS Statistics 20).

RESULTS AND DISCUSSIONS

Some studies on the action of pesticides on soil microorganisms have shown that some of the pesticides used to protect plants have a harmful effect on soil microbial growth, survival, and activity (Meena et al., 2020; Milenkovsky et al., 2010; Cycon et al., 2006; Chen et al., 2001).

Effect of seed protectant pesticides on *Azotobacter* spp.

Analyzing the results, it is observed that during the vegetation period, under the influence of applied pesticides on winter wheat seed, it was observed that the numerical density of bacteria of the genus *Azotobacter* decreased in the case of variants where the winter wheat seed was treated with pesticides. In the case of the control (no seed treatments) the number of bacterial colonies was higher. In the second part of the vegetation period, it is observed in the case of all the studied variants that the number of bacteria has increased, reaching close to the value registered by the control (Figure 1).

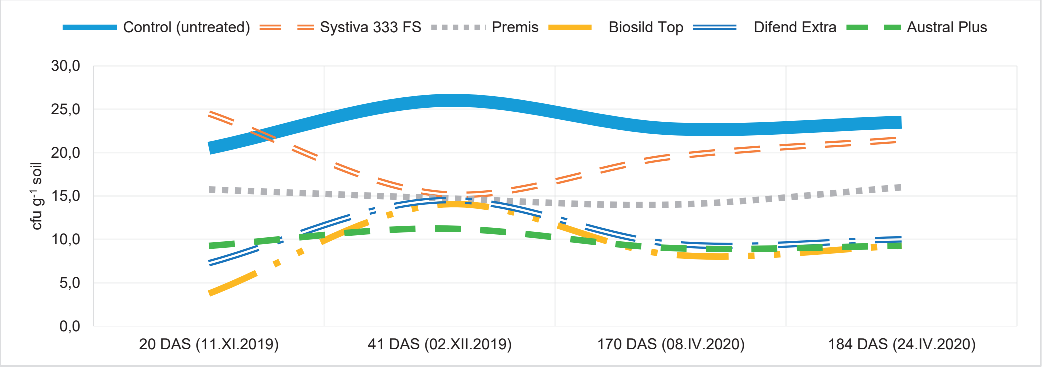

Figure 1. Numerical evolution of bacteria of the genus *Azotobacter* spp. during the vegetation period of winter wheat crop under the influence of seed-protectant pesticides (DAS = days after sowing)

Following the influence of seed-protectant pesticides on the numerical density of the genus *Azotobacter* (Figure 2) it is observed that at 20 days after sowing the lowest values were recorded when wheat seed was treated with Biosild Top, Difend Extra, and Austral Plus. The difference recorded compared to the control was statistically ensured. At the second observation, at 41 days after sowing, a balance of numerical density was observed between the researched variants, but compared to the control, in all cases the difference was statistically distinctly significant.

In the second part of the vegetation period, only the variant represented by Systiva 333 FS registered values of numerical density close to the control, in the other cases, the differences were major, being statistically ensured.

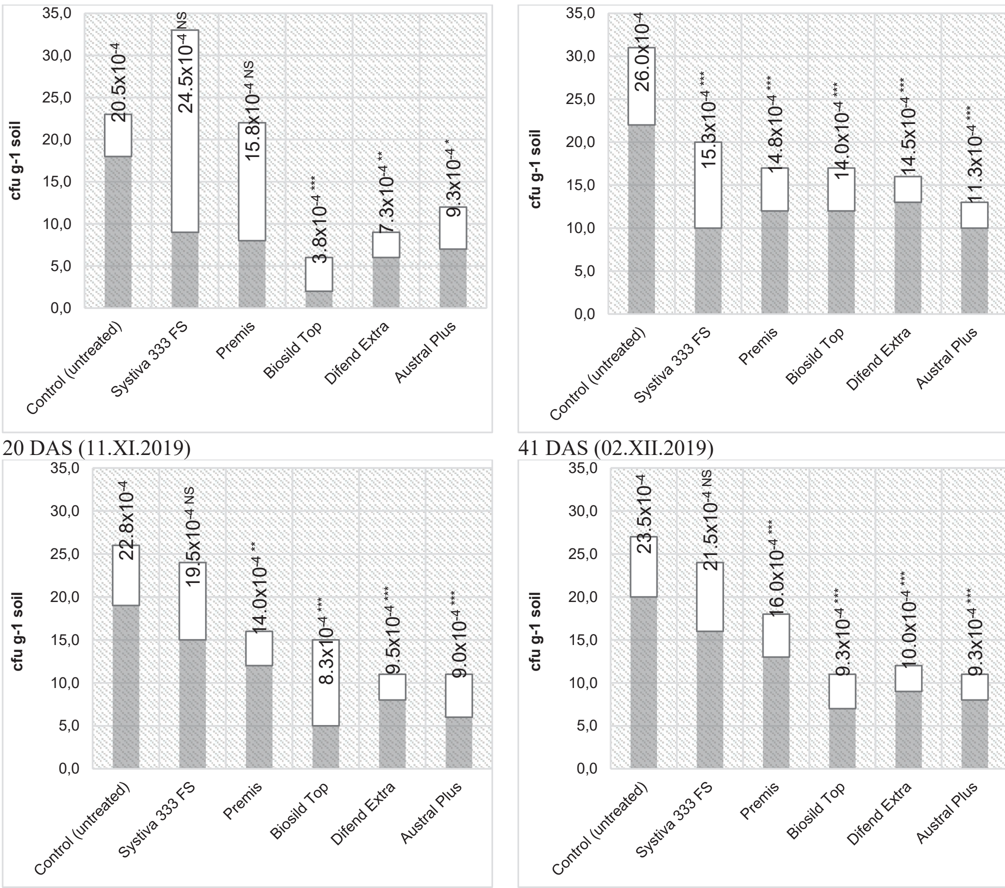

Figure 2. Effect of seed-protectant pesticides on *Azotobacter* spp. during winter wheat growing season (DAS = days after sowing) [NS = not significant; *not significant; **significant; ***distinctly significant]

Effect of seed protectant pesticides on *Clostridium* spp.

Analyzing the results, it is observed that during the vegetation period, under the influence of applied pesticides on winter wheat seed, it was observed that the numerical density of bacteria of the genus *Clostridium* decreased in the case of variants where the winter wheat seed was treated with pesticides. In the case of the control (no seed treatments) the number of bacterial colonies was higher and presented close values throughout the study period. In the second part of the vegetation period, it is observed in the case of all the studied variants that the number of bacteria has increased, reaching close to the value registered by the control (Figure 3). Following the influence of seed-protectant pesticides on the numerical density of the genus *Clostridium* (Figure 4) it is observed that at 20 days after sowing the lowest values were recorded when wheat seed was treated with Biosild Top and Austral Plus.

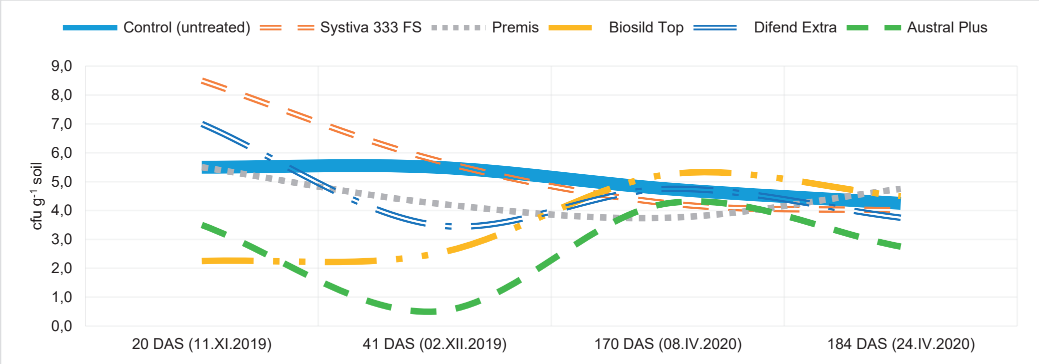

Figure 3. Numerical evolution of bacteria of the genus *Clostridium* spp. during the vegetation period of winter wheat crop under the influence of seed-protectant pesticides (DAS = days after sowing)

When wheat seed was treated with Systiva 333 FS and Difend Extra the values recorded were higher than the control variant. At the second observation, at 41 days after sowing, the largest differences between studied variants and control were registered. In the second part of the vegetation period, all the variants recorded a numerical density close to the control.

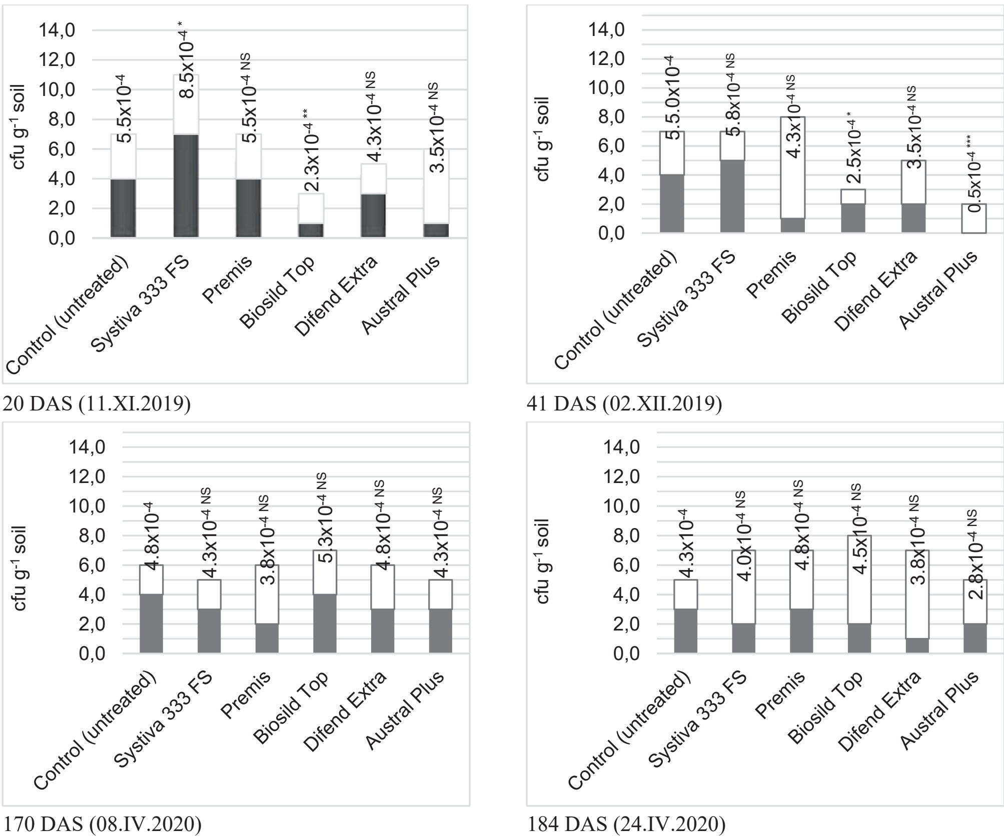

Figure 4. Effect of seed-protectant pesticides on *Clostridium* spp. during winter wheat growing season (DAS = days after sowing) [NS = not significant; *not significant; **significant; ***distinctly significant]

CONCLUSIONS

As a result of the study, it was observed that seed-protectant pesticides influence the numerical density of *Azotobacter* spp. and *Clostridium* spp. nitrogen-fixing bacteria in the soil.

Of the two genera, the genus *Azotobacter* is the one that is present with higher numerical values, being also the genus that is most strongly influenced by the action of pesticides.

It was observed that the numerical density of bacteria decreases in the first part of the vegetation period of the winter wheat crop, while in the second part of the vegetation period the numerical density of bacteria gradually increases reaching normal values, close to the control (no seed treatment).

It is well known that the use of pesticides for plant protection is an activity that cannot be replaced, but the application of the pesticides with the dose recommended by the manufacturer can avoid the occurrence of undesirable situations, like harmful effects on soil microbial growth, survival, and activity.

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