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Agriculture and Environment

Effects of irrigation and fertilization on soil microorganisms

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Abstract. *The purpose of this study is to determine the influence of nitrogen fertilization and irrigation on the total count of aerobic mesophilic microorganisms, moulds and yeasts in the soil. The experiment was conducted in three variants and samples were taken as follows: from irrigated and fertilized furrow; from irrigated non-fertilized furrow and from furrow without irrigation and fertilization. For determination of total number of mesophilic aerobic microorganisms, moulds and yeasts the samples were diluted and cultivated on medium sheets coated with culture medium according to the requirements of the microorganisms. Data analysis shows that fertilization has the strongest multiplication effect on the number of aerobic mesophilic microorganisms in soil (their number increases from 10.522 x 10^6 to 12.8 x 10^6 cfu/g), whereas the irrigation does not have any statistically significant impact. The multiplication of moulds and yeasts in this trial is stimulated mainly by the increased humidity. When irrigation is applied the number of moulds and yeasts increases from 1.158 x 10^5 to 1.407 x 10^5 cfu/g, while fertilization does not affect their quantity in a statistically significant way.*

Keywords: aerobic mesophilic microorganisms, moulds, yeasts, fertilization, irrigation, soil

Introduction

Fertilization in modern agriculture not only maintains, but even gradually increases the yield of agricultural crops. Modern fertilization systems are not just an isolated element in crop production, but an essential link in the system of agricultural practices. The effective fertilizer provides plants with nutrients in appropriate proportions and quantities that stimulate the yield of crops with high biological and technological quality. Fertilization also gradually improves soil fertility (Li et al., 2005).

Years of research on the effects of agricultural practices clearly shows that mineral fertilizers, particularly nitrogen supplements have the strongest influence on the crop yield. The results of the research conducted by Jenkinson (1982) and Doran et al. (1996) show that mineral fertilization strongly influences a number of microorganisms and has pronounced effect on the qualitative selection of whole communities of soil microorganisms. According to many authors soil microbial biomass is closely related with soil fertility (Insam et al., 1991; Brookes, 1995; Yao et al., 2000). While soil bacteria are generally beneficial for soil fertility, because of their participation in the nutrients transformation, the fungi are very often toxigenic (Styla and Sawicka, 2010).

According to data from more than 20 years of studies submitted by Barabasz et al. (2002), high NPK fertilization rates significantly increase the number of the tested bacteria and fungi. It rose about 2-fold compared with the control. However, other studies indicate that fertilization sometimes decreases the number of many species of the selected microorganisms. High levels of nitrogen decrease the number of bacteria, but the change in the number of fungal species is negligible. It was discovered that fertilization with high levels of nitrogen fertilizer causes a reduction of the bacteria from genera *Arthrobacter* and *Streptomyces* by 50% on average and the total destruction of the bacteria from genera *Azotobacter*, *Rhizobium* and *Bradyrhizobium*. On the other hand, there was an increase in the number of microorganisms and biomass of the genera *Eubacterium*, *Pseudomonas* and *Bacillus* and in the number of moulds of the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Verticillium* (Barabasz et al., 2002). Styla and Sawicka (2010) believed that repeated mineral fertilization, especially with high doses of nitrogen, can cause strong acidification of the soils and increase the development of fungi. They reported that nitrogen fertilization increases the fungi number, but decreases the total count of aerobic microorganisms. According to Zong et al. (2010) nitrogen fertilization does not change the bacterial and fungal biomass in a statistically significant way.

The humidity is one of the most important parameters regulating the biological activity in the soil. Changes in humidity will affect soil microorganisms through complex interactions with nutrients, soil temperature, the arrangement of the soil pores and the soil atmosphere. Soil moisture and air temperature are some of the main factors regulating the activity of soil microorganisms. The study of Schnurer et al. (1986) showed that after heavy rains the number of bacteria doubles within three days, and in the subsequent dry period it decreases. The hyphae mass of the fungi also increased significantly during the experiment. Styla and Sawicka (2010) also found that after intensive irrigation the number of microorganisms

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increased, but the number of fungi decreased.

Because of the controversy in the literature data the purpose of the present experiment is to determine the impact of fertilization and irrigation on the development of aerobic mesophilic microorganisms, moulds and yeasts. This is one of the ways to determine the soil fertility after fertilization and irrigation as well as the possible presence of toxigenic fungi.

Material and methods

The field experiment was conducted at the experimental field of the Agricultural Institute, Stara Zagora. The predominant soil type is gleyic chromic luvisols. The humus layer has a thickness of 80 cm, the surface layer from 0 to 20 cm has loose structure, while the lower part is thick. The humus layer lies on a sandy carbonate layer. The irrigation norm is based on the established unit of field moisture capacity (FMC). For this type of soil it is found that the FMC is 27%, ie at the set depth of the active soil layer for watering to FMC watering at the amount of 95 m^3 is required.

The average temperature for 2015 in July and August was higher than the norm for the period 1930 – 2015, by 2.2 and 1.9°C, respectively. At the same time in June daily average temperatures were close to normal. This year was significantly warmer from March to August.

The study was conducted with corn grain. Irrigation was done by gravity. Irrigation water was supplied through a furrow. The size of the experimental plots was 70 m^2 (length of the furrows 10 m, 5 irrigated and 5 non-irrigated furrows). Nitrogen fertilizer was supplied three times in equal parts –before sowing, before furrowing and after first irrigation in total of 180 kg/ha of active ingredient. The whole fertilizer quantity was supplied during irrigation.

In the first variant samples for analysis were taken from the irrigated and fertilized furrow. In the second variant the irrigated non-fertilized furrow was sampled. In the third variant, which is a control, a sample was taken from a furrow without irrigation and fertilization. All samples were taken at the same time, 24 h after irrigation.

For determination of the total number of mesophilic aerobic microorganisms, mould and yeast samples were taken from the soil, they were decimally diluted to 10^{-4} for aerobic microorganisms and 10^{-3} for moulds and yeasts, and were cultivated on medium sheets coated with ready-to-use culture medium (R-Biopharm, Germany). 1 ml of the sample had been applied onto the fabric of the medium sheet. The sheets were incubated at 35°C for 24 – 48 h and the colonies were counted. The results are expressed in colony forming units per gram (cfu/g). The results were recalculated to 1 g of absolutely dry soil.

Statistical analysis of microbiological parameters was performed by a computer program "StatMost for Windows" (DataMost Co., USA, 1994), for statistical method was used Tukey's test, and the values were set as average, standard deviation and standard error.

Results and discussion

The study of the irrigation and fertilization impact on the total number of aerobic mesophilic microorganisms showed that during the period from June 3-rd to August 18-th, the total number of aerobic microorganisms was the lowest at irrigation without fertilization – an

Table 1. Total count of mesophilic aerobic microorganisms in different tillage practices

Irrigation and fertilization	Dates	Total aerobic count ($x10^6$ cfu/g)*	n	SD	SE
1. Irrigation+fertilization	03.06	16.5^a	3	2.18	1.27
1. Irrigation+fertilization	01.07	6.45^b	3	2.2	1.2
1. Irrigation+fertilization	16.07	11.71^c	3	1.8	1.03
1. Irrigation+fertilization	20.07	15.78^a	3	3.2	1.84
1. Irrigation+fertilization	18.08	13.55^c	3	1.8	1.04
Average		12.8^a	3	2.24	1.28
2. Irrigation+non-fertilization	03.06	8.73^b	3	1.82	2.43
2. Irrigation+non-fertilization	01.07	8.98^b	3	1.9	2.1
2. Irrigation+non-fertilization	16.07	7.64^b	3	0.9	0.51
2. Irrigation+non-fertilization	20.07	12.8^c	3	0.8	0.46
2. Irrigation+non-fertilization	18.08	13.06^c	3	2.89	1.61
Average		10.242^b	3	1.66	1.42
3. Non-irrigation+non-fertilization	03.06	16.5^a	3	2.18	1.27
3. Non-irrigation+non-fertilization	01.07	6.45^b	3	2.2	1.2
3. Non-irrigation+non-fertilization	16.07	9.82^b	3	1.7	0.98
3. Non-irrigation+non-fertilization	20.07	6.44^b	3	3.3	1.9
3. Non-irrigation+non-fertilization	18.08	13.45^c	3	1.3	0.75
Average		10.522^b	3	2.14	1.22
Average of all groups		11.188	3	2.01	1.31

*There is statistically significant difference ($P<0.05$), if the letters are not the same. The values are compared between the dates and between the average values

Table 2. Moulds and yeasts in different tillage practices

Irrigation and fertilization	Dates	Moulds and yeasts (x105 cfu/g)*	n	SD	SE
1. Irrigation+fertilization	03.06	2.18[a]	3	0.04	0.023
1. Irrigation+fertilization	01.07	1.91[a]	3	0.03	0.017
1. Irrigation+fertilization	16.07	0.95[b]	3	0.03	0.017
1. Irrigation+fertilization	20.07	0.9[b]	3	0.02	0.012
1. Irrigation+fertilization	18.08	0.75[b]	3	0.03	0.017
Average		1.338[a]	3	0.03	0.017
2. Irrigation+non-fertilization	03.06	1.46[c]	3	0.01	0.006
2. Irrigation+non-fertilization	01.07	3.4[d]	3	0.01	0.006
2. Irrigation+non-fertilization	16.07	1.53[c]	3	0.04	0.02
2. Irrigation+non-fertilization	20.07	0.95[b]	3	0.02	0.012
2. Irrigation+non-fertilization	18.08	0.72[b]	3	0.02	0.012
Average		1.407[a]	3	0.02	0.012
3. Non-irrigation+non-fertilization	03.06	2.18[a]	3	0.01	0.006
3. Non-irrigation+non-fertilization	01.07	1.91[a]	3	0.01	0.006
3. Non-irrigation+non-fertilization	16.07	0.55[e]	3	0.03	0.017
3. Non-irrigation+non-fertilization	20.07	0.472[e]	3	0.03	0.017
3. Non-irrigation+non-fertilization	18.08	0.676[e]	3	0.05	0.029
Average		1.158[b]	3	0.026	0.015
Average of all groups		1.301.01	3	0.0253	0.0146

*There is statistically significant difference ($P<0.05$), if the letters are not the same. The values are compared between the dates and between the average values

average of 10.242 x 10^6 cfu/g (Table 1). The amount of micro-organisms was the greatest when irrigation and fertilizing were applied – an average of 12.8 x 10^6 cfu/g. For the combination of non-fertilization with non-irrigation the number of microorganisms occupies middle position – 10.522 x 10^6 cfu/g. There is a statistically significant difference between the microorganisms that develop in irrigated and fertilized soils and the other two groups. On the other hand, between the microorganisms grown under irrigation+non-fertilization and non-irrigation+non-fertilization conditions there was not any statistically significant difference. This is an indication of the serious impact of fertilization on the increase of bacteria number. Such a trend of the nitrogen fertilization impact was described by Barabasz et al. (2002). On the other hand, according to the average values for different trial groups irrigation does not have serious impact on the number of aerobic mesophilic microorganisms. A possible reason for that is because soil humidity did not decrease below certain limit and in that way the multiplication of the aerobic mesophilic microorganisms was not affected. It is common belief that increasing the number of microorganisms has a favorable effect on soil fertility due to the many transformations of nutrients and synthesis of biologically active substances from the microorganisms (Kennedy and Pappendick, 1995).

According to Table 2 the number of moulds and yeasts is the greatest when irrigation without fertilization is applied – average of 1.407 x 10^5 cfu/g. The lowest average number is under non-irrigation+non-fertilization conditions – 1.158 x 10^5 cfu/g. Moulds and yeasts multiplied at moderate rate during irrigation and fertilization - their average values were 1.338 x 10^5 cfu/g. Between the average values during irrigation and fertilization and irrigation without fertilization there are not any statistically significant differences. These exist between the control values (without irrigation and fertilization) and the other two groups. Analysis of the data shows that humidity has the strongest impact on mould and yeast reproduction since water content increases the growth of moulds and yeasts. On the other hand, fertilization did not affect in a statistically significant way the number of moulds and yeasts.

According to Schnurer et al. (1986) and Barabasz et al. (2002) during irrigation and fertilization there is an increase in the number of moulds and yeasts. Our data confirm the general trend in respect to humidity, whereas after fertilization it is not well defined. However, other authors found that the number of moulds and yeasts decreases during intensive irrigation (Styla and Sawicka, 2010). Those findings may be due to the decreased oxygen content in the water saturated soil and the low supply of the moulds and yeasts with oxygen. In that way the intensity of irrigation probably has the greatest effect on mould and yeast reproduction.

Conclusion

The analysis of the data shows that fertilization has the strongest influence on the increase of the total number of aerobic mesophilic microorganisms, irrigation does not change these values in a statistically significant way. Reproduction of moulds and yeasts is stimulated mainly by the increased humidity while fertilization did not affect their values in a statistically significant way. On the basis of these findings we can recommend fertilization as a way to increase the number of microorganisms and therefore soil fertility. Moreover, we have to be careful with the application of irrigation because high levels of soil humidity can increase the number of moulds and yeasts and consequently the quantity of toxigenic fungi.

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