EFFECT OF TRIFLURALINE HERBICIDE ON SOIL MICROFLORA IN TOMATOSEEDLINGSINOUTDOOR CONDITIONS IN KARAORMAN, STIP AREA

Full professor Snezana Stavreva Veselinovska
Full professor Jordan Zivanovik
MA Aleksandra Todorovska
Faculty of Educational Sciences, University "Goce Delcev", Stip
R. Macedonia

Aim: Microorganisms play an important role in herbicide degradation as they are able to utilize the biogenic elements from those compounds, as well as energy for their physiological processes. On the other hand, herbicides are more or less toxic substances that can have adverse effect on populations of microorganisms and prevent their development, reduce their abundance, deplete their taxonomic complexity and create communities with a lower level of diversity and reduced physiological activity.

Material and methods: The experiment was set according to the method of random block system in three iterations, with size of experimental plots from 10 m². In examination was included genus pink tomato (*Lycopersicon Lycopersicum*). In the examination were included two variants: 1. Control (without applied herbicide) and 2. Variant with trifluralin. The application of herbicide trifluralin was performed 1 day before tomato seedling (Tab. 2). The treatment was carried out with dorsal nozzle according to the recommended field dose of 480 g/L, with direct application to the soil surface. The soil samples for analysis were taken on 10, 20, 40, 60 and 90 days after the application of herbicides, and two depths 0-10 cm and 10-20 cm. For number determination of examined groups of microorganisms is used indirect (cultural) method dilution plating, appropriate dilution soil suspension was made on selective nutrient substrates (Govedarica Jarak, 1997; Kongulovski, 2001). The Petrie-dish, in which the seeding was performed examined soil microorganisms worn thermostat for incubation on temperature of 30° C for 24 to 48 hours for the total counts of microorganisms at a temperature of 28° C for a period of 7 days, aktinomycetes and fungi 5-7 days.

Results: In this article are presented complex interactions between herbicides and microorganisms in soil immediately after application and over the ensuing period, data of changes in the abundance of some systematic and physiological groups of microorganisms. In the article is also presented the effect of trifluralin on soil microorganisms. In this article it's shown the impact from the tested herbicide is on the number of *Actinomycetes* and yeasts.

Conclusions: NMO directly depends on the initial contamination of milk with microorganisms from the milk gland and microorganisms (contaminants) from the surroundings (afterbirth, urine, feces) that can populate the surfaces of the milking equipment and surfaces that milk comes into contact with in a considerable number. Minimizing the milk contamination level from these sources would significantly prevent the level of psychrophilic microorganisms.

Keywords: herbicides, microorganisms, trifluralin, soil

Introduction

Having in consideration the use of herbicides it's very important to know about their influence on soil microorganisms. When herbicide is applied in soil is exposed to numerous chemical, physical and biological processes, which in turn, depend on the actual compound, physical, chemical and biological characteristics of the soil (Nešković, N., Budimir, M. 1981;

Koskinen and Harper, 1990). Because herbicides have a special pathways in metabolism of soil microorganisms (Van Eerd et al., 2003), they are one of the main participants in transformation of herbicides in the soil. Influence of herbicides on microbial communities is different and depends from chemical composition of the herbicides applied, concentrations, preparations and more. For some microbial communities, herbicides can have stimulus effect on number and diversity of some groups of microorganisms (Nannipieri, 2003, Anderson, 1978). Some herbicides are susceptible to microbial degradation, and some do not. Trifluralin as subject of our research, can be subject for degradation by soil microorganisms (Worthing, C.R., ed. 1987). The available data reveal that the degradation, persistence and mobility of this herbicide in soil depend on the present organic matter content, moisture, pH and clay minerals (Miller et.al., 1975; Banks et. al., 1986; Renner et. al., 1988; Andrew et. al., 1990; Stougard et.al., 1990; Petkova and Donkovak, 1993). The trifluralin influence in soil is active for a period of 45-60 days (Tomlin, C. et al. 1997).

Materials and methods

The field experiment was carried out during 2010 and 201, on agricultural areas in the village Karaorman-Stip (Fig. 1 and 2). The experiment was set according to the method of random block system in three iterations, with size of experimental plots from 10 m². Tomatoes seedling was made on 17.05.2010 and 14.05.2011. In examination was included genus pink tomato (*Lycopersicon Lycopersicum*). In the examination were included two variants: 1. Control (without applied herbicide) and 2. Variant with trifluralin. The application of herbicide trifluralin was performed 1 day before tomato seedling, on 16.05.2010 and 13.05.2011 (Tab. 2). The treatment was carried out with dorsal nozzle according to the recommended field dose of 480 g/L, with direct application to the soil surface. The type of soil where the experiment took place is garden soil (hortisol). The soil samples for analysis were taken on 10, 20, 40, 60 and 90 days after the application of herbicides, and two depths 0-10 cm and 10-20 cm. For comparison with the herbicide varieties, soil samples were taken from the same depths as the control variant.

For number determination of examined groups of microorganisms is used indirect (cultural) method dilution plating, appropriate dilution soil suspension was made on selective nutrient substrates (Govedarica, M and all, 1997; Kongulovski 2001). The Petrie-dish, in which the seeding was performed examined soil microorganisms worn thermostat for incubation on temperature of 30°C for 24 to 48 hours for the total counts of microorganisms at a temperature of 28°C for a period of 7 days, aktinomycetes and fungi 5-7 days. Samples are placed in 3 iterations, 1 g/fresh soil. Heterotrophic soil microorganisms were examined: Total number of microorganisms nutritious substrate MPA (mesopepton-agar) (10^{-6}); for counts of actinomycetes was used nutritious substrate Waksman agar (10^{-5}) and for the counts of fungi was used the nutrient agar Sabourald, (10^{-4});

Result and discussion

Effect of trifluralin on total counts of microorganisms per gram of fresh soil, for soil depth of 0-10 cm and 10-20 cm, is given in Table 1 and Chart 1 and 2. According to the results presented in this table, at a depth of 0-10 cm, it can be seen that the total counts of microorganisms, is the lowest in the period from the 10th to the 40th day after the application of herbicides (DPA), for variant with trifluralin (Table1 and chart 1). During the 60th and 90th day their counts starts to increase. Different is the dynamics of the total counts of microorganisms at a depth of 10-20 cm. This depth in 10th DPA there are not large differences in the total counts of microorganisms softer control variant and the variant with trifluralin. This situation with the counts of microorganisms is due to the slow migration or movement of herbicides in the lower layers of the soil. Lowest value in the counts of microorganisms had appeared in the variant with trifluralin DPA is in the 20th day (Table 1 and Chart 2).

Table. 1 - Total count of microorganisms (x10^{-6} g/ fresh soil); 2010

Variants	Days									
	Soil depth from 0-10 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	47,0	100,0	46,0	100,0	39,7	100,0	43,7	100,0	45,5	100,0
Trifluralin	22,0	46,80	28,0	60,86	30,5	76,82	37,0	84,66	39,0	85,71
	Days									
Variants	Soil depth from 10-20 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	46,5	100,0	45,5	100,0	43,5	100,0	43,5	100,0	44,0	100,0
Trifluralin	40,0	86,02	39,0	85,71	37,5	86,20	42,0	96,55	43,5	98,86

As can be seen from Table 1 and the chart 2 at 90th DPA the total counts of microorganisms it's similar with control.

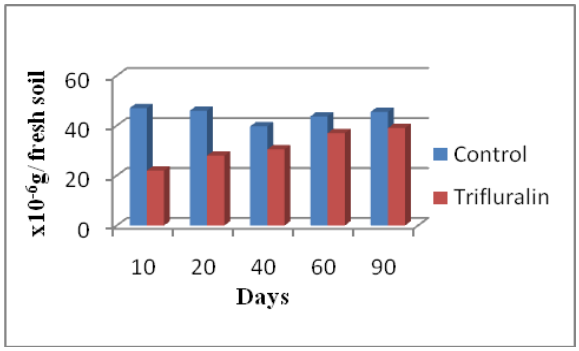

Chart 1. Total count of microorganisms for soil depth from 0-10 cm; 2010

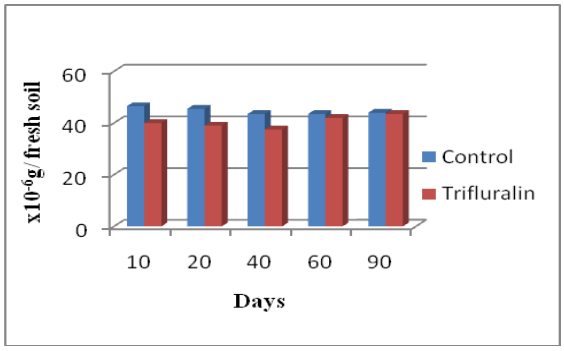

Chart 2. Total count of microorganisms for soil depth from 10-20 cm; 2010

From the results presented in Table 2, in 2011, for depth of 0-10 cm the lowest values in the total count of microorganisms, from 10th to 40th DPA was noticed at variant with trifluralin. After the 60th and 90th DPA, their counts gradually increases (Table 2, Chart 3).

Table. 2 - Total count of microorganisms (x10^{-6} g/ fresh soil); 2011

Variants	Days									
	Soil depth from 0-10 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	44,5	100,0	43,5	100,0	40,0	100,0	40,5	100,0	43,0	100,0
Trifluralin	19,5	43,82	21,0	48,27	28,0	70,00	36,0	88,88	40,5	94,18
	Days									
Variants	Soil depth from 10-20 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	45,5	100,0	46,0	100,0	42,0	100,0	46,0	100,0	44,0	100,0
Trifluralin	40,0	87,91	36,0	78,26	38,0	90,47	40,0	86,95	43,5	98,86

Similar to the results obtained from the previous year 2010, in 2011, there was less influence of trifluralin on microorganism's counts at depth of 10-20 cm during all days of examination (Chart. 4). The total counts of microorganisms is lowest at 20th DPA, whereas in variant with trifluralin 36,0 x10^{-6} g/fresh soil, while the total counts of microorganisms in the control is 46, 0 x10^{-6} g/fresh soil. After 60th DPA their counts is gradually increased. In examinations conducted by Breazeale and Camper (1970), decrease in the total counts of microorganisms from 46-50% was noticed 7 days after application of trifluralin and 2,4-D.

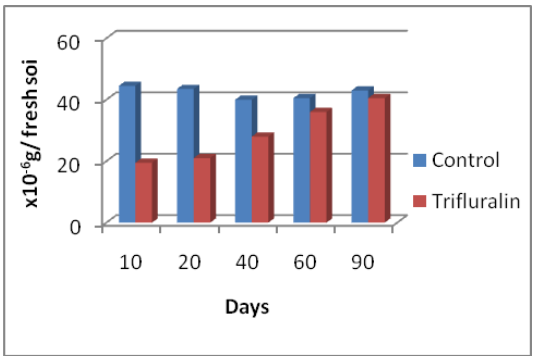

Chart 3. Total count of microorganisms for soil depth from 0-10 cm; 2011

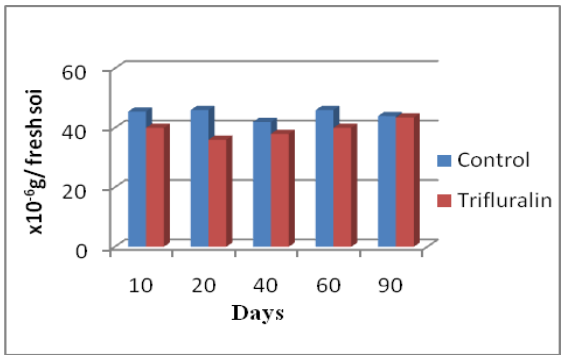

Chart 4. Total count of microorganisms for soil depth from 10-20 cm; 2011

❖ **Table 3-** Actinomycetes counts (x10^{-5} g/ fresh soil); 2010

Variants	Days									
	Soil depth from 0-10 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	105,0	100,0	95,0	100,0	90,0	100,0	85,0	100,0	75,0	100,0
Trifluralin	86,0	81,9	84,0	88,4	85,0	94,0	78,0	91,7	71,0	94,6
	Days									
Variants	Soil depth from 10-20 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	115,0	100,0	110,0	100,0	99,0	100,0	85,0	100,0	75,0	100,0
Trifluralin	105,5	91,7	100,1	91,0	89,5	90,4	75,0	88,2	65,5	87,3

According to the results presented in Table 3, the counts aktinomycetes in variant with trifluralin, gradually reduced from 10th to 40th DPA. Lowest value aktinomycetes have at 20th DPA, for variant with trifluralin. Their counts after 60th DPA gradually increases at 90-DPA where both variants have similar values (Table 3 and graph. 5). At a depth of 10-20 cm, during the whole period of examination the counts of aktinomycetes gradually reduced in the variant with trifluralin. Aktinomycetes lowest value had at 40th DPA (Table 3 and graph. 6).

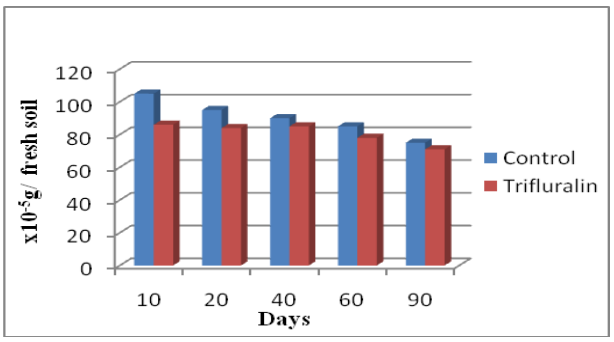

Chart 5. Total count of actinomycetes for soil depth from 0-10 cm; 2010

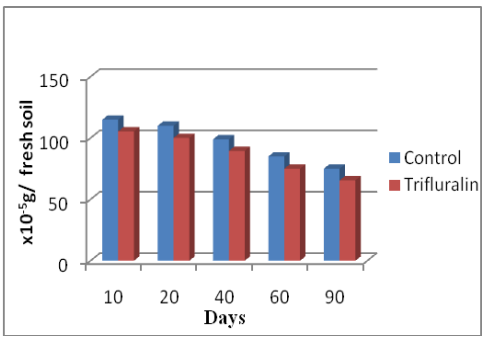

Chart 6. Total count of actinomycetes for soil depth from 10-20 cm; 2010

❖ **Table 4-** Actinomycetes counts (x10^{-5} g/ fresh soil); 2011

Variants	Days									
	Soil depth from 0-10 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	90,0	100,0	91,0	100,0	80,0	100,0	71,0	100,0	65,0	100,0
Trifluralin	85,0	94,4	86,5	95,0	75,0	93,7	70,5	99,2	75,5	116,1
	Days									
Variants	Soil depth from 10-20 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	100,0	100,0	98,0	100,0	95,0	100,0	81,0	100,0	78,0	100,0
Trifluralin	99,5	99,5	95,5	97,4	85,5	90,0	72,5	89,5	70,5	90,3

Similar to the previous year 2010, in 2011 resulted in changes in the total counts of actinomycetes at a depth of 0-10 cm caused by the herbicide. Due to the gradual migration of the studied herbicide in the lower layers of the soil, for depth of 10-20 cm, have a tendency to reduce the counts of aktinomycetes throughout the research period from 10th to 90th DPA (table 5 and graph. 8). According to the reported results for the effect of trifluralin from the counts of aktinomycetes we can say that trifluralin have an inhibitory effect on both investigated depths (0-10 cm and 10-20 cm), over the whole period of research.

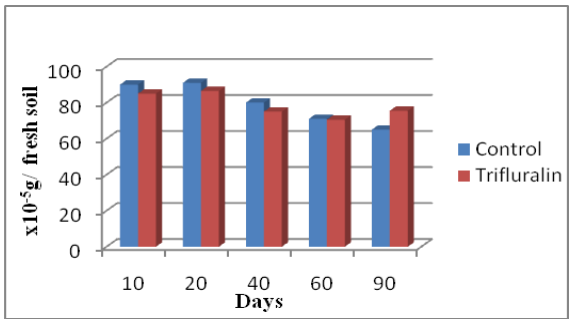

Chart 7. Total count of actinomycetes for soil depth from 0-10 cm; 2011

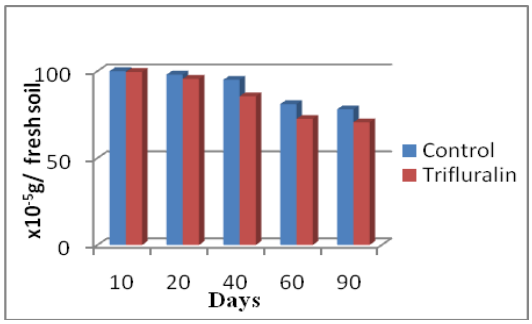

Chart 8. Total count of actinomycetes for soil depth from 10-20 cm; 2011

From the results presented in Table 5 and Chart 9, in 2010, it's shown that there is a tendency of increasing the total number of fungi at a depth of 0-10 cm, from the 10th day to the 60th day after application (DPA) triflurlin in comparison with control variant. In addition, the lowest values were at 10th and 40th DPA in variant with trifluralin, compared with control. At a depth of 10-20 cm, during the entire period of study, the counts of fungi have steady growth trajectory and the control variant with variant trifluralin (chart 10).

❖ **Table 5 –** Fungi counts (x10^{-4} g/ fresh soil); 2010

Variants	Days									
	Soil depth from 0-10 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	13,5	100,0	17,0	100,0	16,5	100,0	30,5	100,0	29,0	100,0
Trifluralin	11,0	81,4	16,5	97,0	15,5	93,9	27,5	90,1	16,5	56,8
	Days									
Variants	Soil depth from 10-20 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	14,5	100,0	21,0	100,0	23,0	100,0	30,0	100,0	29,5	100,0
Trifluralin	13,0	89,6	20,0	95,2	22,5	97,8	27,5	91,6	28,5	96,6

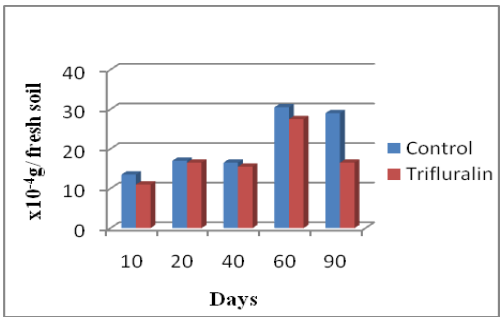

Chart 9. Total count of fungi for soil depth from 0-10 cm; 2010

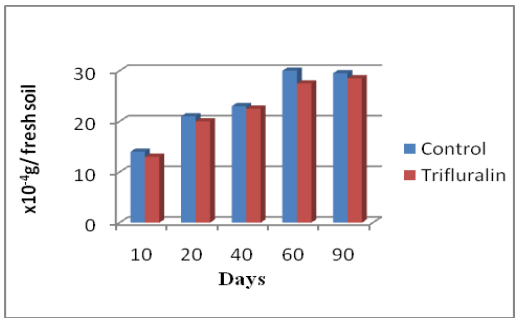

Chart 10. Total count of fungi for soil depth from 10-20 cm; 2010

Much like in 2010, and 2011 resulted with changes in the total counts of fungi at a depth of 0-10 cm impact caused trifluralin, throughout the survey period with a tendency to increase their number from 10th day to the 90-th day after application of herbicides (DPA). In addition, these changes are most pronounced 40th, 60th and 90th DPA (Table 6, Chart 11).

❖ **Table 6–** Fungi counts (x10^{-4} g/ fresh soil); 2011

Variants	Days									
	Soil depth from 0-10 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	13,5	100,0	17,8	100,0	20,0	100,0	25,0	100,0	28,0	100,0
Trifluralin	12,0	88,8	17,5	98,3	19,0	95,0	22,5	90,0	27,0	96,42

Variants	Days									
	Soil depth from 10-20 cm									
	10		20		40		60		90	
	No.	%	No.	%	No.	%	No.	%	No.	%
Control	13,9	100,0	16,9	100,0	23,5	100,0	29,9	100,0	30,0	100,0
Trifluralin	12,0	86,3	15,5	91,7	22,0	93,6	28,0	93,6	27,5	91,6

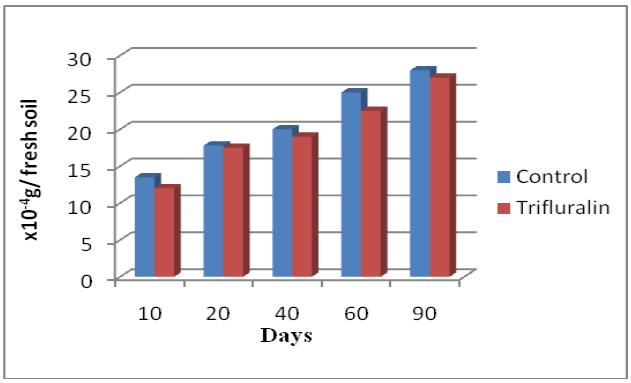

Chart 11. Total count of fungi for soil depth from 0-10 cm; 2011

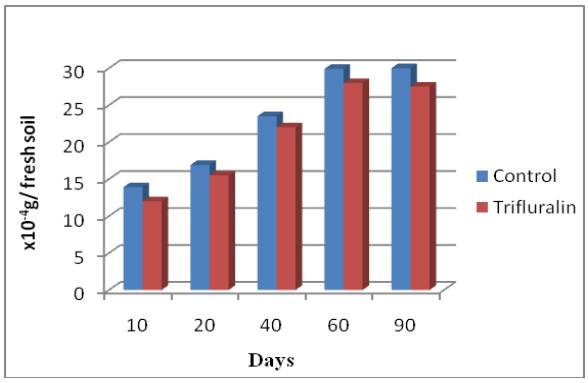

Chart 12. Total count of fungi for soil depth from 10-20 cm; 2011

The dynamics of fungi growth population, in both varieties, is observed at a depth of 10-20 cm. Especially this growth is expressed in the period from 40th and 90th DPA (Table 6, Chart 11). In the research of Breazeale and Camper, 1970, at the variant with trifluralin was noticed a reduction in the fungi counts for 81% less than control.

Conclusion

From the presented results it can be concluded that trifluralin has an inhibitory effect on the total counts of microorganisms, aktinomycetes and fungi in the soil. In addition, trifluralin had the most expressed effect during the entire period of the examination on total counts of aktinomycetes for both investigated depths of 0-10 cm and 10-20 cm, from 10th till 90th DPA. Slightly weaker inhibitory effect was found in fungi counts for both depths of 0-10 cm and 10-20 cm, during the whole period of research.

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