Application of Growth Regulators and Saprophytic Fungi *Trichoderma viride* Pers ex Fr. to Improve the Sanitary Condition of the Soil under Pepper Crops

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Received: 11 March 2020 *Accepted: 10 May 2020*

Abstract

The article presents studies of the effect of the microbiological preparation "Trichodermine", bio-organic fertilizers and growth regulators based on humic acids "Nagro", "Stimix", "BioLife" and mineral fertilizers (NPK) on the biological activity of the soil, the degree of disease caused by Alternaria capsici-annui Savul & Sandu and the yield of pepper in the field. It was found that the differences in the indicator "Biological Activity of the Soil" depend on the number of microorganisms, the composition (species diversity) and the percentage of the main Micromycetes. Organic products help to optimize soil health and reduce the total content of fungal colonies, and in the composition of Micromycetes, the fungus Trichoderma sp. is more common. When mineral fertilizers are applied, the number of fungal pathogens and toxin producers increases. A higher total yield was obtained in the variants with biological treatment. The yield increase is from 9% to 29%.

Keywords: vegetable production, biological activity of the soil, resistance to diseases

Introduction

The development of fungal diseases in vegetable field crops is one of the limiting factors of yield. Black leaf spots (*Alternaria capsici-annui* Savul & Sandu) - one of the most important economic diseases on pepper. According to some authors (Chulkina, 1995; Bakker et al., 2014), the intensity of the disease depends on the amount of soil-seed infection and ecological and technological conditions of cultivation. The concept of biological protection of plants is determined by the amount of organic matter in the soil, the introduction of organic fertilizers and the use of biological agents (Rudakov, 2006).

Growth regulators and organic fertilizers based on natural materials have received considerable attention from both the scientific community and commercial enterprises, especially in the last two and a half decades (Apone et al., 2010; Craigie, 2011; Brown & Saa, 2015; Du Jardin, 2015; Yakhin et al., 2017). Biostimulants offer a potentially novel approach to regulating / modifying physiological processes in plants to stimulate growth, relieve stress and increase yields (Belakbir et al., 1998; Bhattacharyya & Jha, 2012; Ikrina & Kolbin, 2004; Linser et ai., 2006; Mora et al., 2010). The effect of organic fertilizers on the intensity of vegetable crop diseases has been studied by a number of researchers (Colla et al., 2015; Di Marco & Osti, 2009; Dixon &Walsh, 2004; Fuentes-Ramirez & Caballero-Mellado, 2006;

Hernandez et al., 2014; Hernandez-Herrera et al., 2015). It was found that the use of organic fertilizers on greenhouse tomatoes changes the nutrition balance and affects the phytosanitary status of this crop (Georgieva, 2013).

The aim of this work is to study the effect of new growth regulators on the following indicators in the production of pepper: the total microbiological activity and structural composition of soil Micromycetes, the index of damage by Alternaria black spots, the total yield and percentage of standard products.

Materials and Methods

Experimental work was carried out in 2009-2011 on the territory of Maritsa Vegetable Crops Research Institute on alluvial-meadow soil with pepper variety "Kurtovska kapia". Soil characteristics: humus content-2.1% (Turin), mineral nitrogen(NH4 + NO3-N), determined by the distillation method-1.8 mg/100 g soil, mobile P_2O_5-16.1 mg/100 g and mobile K_2O – 18.2 mg/100 g soil (Egner-Rhyme), soil reaction Ph/H_2O/- 6.9 (potentiometric). The experiment was carried out using the block method in four replications (100 replicated plants), with a test area of 12.8 m^2. The strategic direction of the experiment included the study of the influence of the Trichoderma viride Pers, the bio-fertilizers „Nagro", „Stimix", „BioLife" on the overall soil biogenicity, the composition and structure of soil Micromycetes and the development of black spots (*Alternaria capsici-annui* Savul & Sandu).

For this purpose, the pepper seeds were treated with "Nagro" and "Stimix" biofertilizers at a concentration of 1:100 for 4 h followed by treatment of plants with the same bio-fertilizers at a concentration of 1:100 during the flowering phase and after the first harvest. „BioLife" was introduced into the rhizosphere as a liquid solution at a concentration of 500ml/ 200L H_2O at 5L/m^2 immediately after planting, during the flowering period. The biological product "Trichodermine" (*Trichoderma viride* Pers ex Fr.) was introduced into the soil at a dose of 40 kg/Ha. Experimental variants were performed on a standard and twice-reduced mineral background. The standard mineral background contained N16P20K22: ammonium nitrate - 500 kg/Ha, super phosphate - 450 kg/Ha, potassium sulfate - 450 kg/Ha. Phosphorous and potassium fertilizers were applied during the autumn tillage, nitrogen fertilizers - during the growing season. The reduced mineral background contained N8P10K11, respectively.

Indicators: Total% Yield (kg/Ha), Attack Index of black spots on leaves affected by Alternaria, Percentage of Affected fruits (%), Total Biological Activity of the Soil (the number of CFU per 1 g of dry soil, the number of fungal colonies in 1 g of dry soil). The frequency of *Alternaria capsici-annui* attacks was calculated on a five-digit scale (Gannibal et al., 2011): 0 - no symptoms, 1- single spots, 2- up to 25%, 3- 26-50%, 4- more than 50 % of the leaf surface is covered with spots, the plant is strongly suppressed. The attack index was calculated using Mc. Kinnly formula, the effect of using organic fertilizers - according to the Abbott formula. During mass harvesting, the percentage of diseased fruits with signs of *Alternaria capsici-annui* was calculated (%). Microbiological analysis of soil samples was performed at the beginning, middle and end of the growing season - after harvesting. The biological activity of the soil was determined by standard microbiological methods of cultivation of microorganisms on elective nutrient media (Zvyagintsev, 1991).

Statistical processing of the obtained experimental data was performed using Excel (Microsoft Office 2002), Windows XP. The results of the study were analyzed using a standard statistical procedure and presented as average values from three replicas.

Characterization of bio-organic liquid fertilizers:

"Nagro" - 19.17% dry substance; 10.29 g/l organic substance; Ph-8.5; N(common)-0.77g/L; phosphorus (P2O5)-387 ml/L; potassium (K2O)-4624 ml/L; C:N (6:1); micro elements Cu-0.51 ml/L, MgO-541 ml/L, Mo-669 ml/l, S-1.8%, Zn-6.0 mg/Kg, Fe-154ml/L, B-1.02 ml/l, humic acids-1.63 g/L, polyacids-8.67 g/L;

„Stimix" - free amino acids-15-30 g / l, humic acids-30-50 g/l, organic silicon, live microbial cultures, biologically active substances.

„BioLife" - is a combined highly concentrated bacterial preparation. It contains enzymes and non-pathogenic microorganisms, as well as an active starter culture that accelerates and enhances the development of microorganisms and stimulates the direct action of soil bacteria.

„Trichodermine" (*Trichoderma viride* Pers ex Fr., strain No 12) is a biological product with a titer of 2.10^{10} c/g, obtained by solid-phase technology.

Results and Disscution

The results of our research confirm that the sanitary condition of the soil depends on the total effect of factors associated with the cultivation of agricultural crops. Microbiological analysis of soil samples taken from the pepper field at the beginning, middle and end of the growing season, shows that the biological activity of the soil in the experiment changes throughout the growing season (Table 1). The Total Biological Activity of the Soil in the experiment with pepper increases twice during the growing season. The increase in the biological activity of the soil in the control variant with mineral fertilizers (N16P20K22) during the flowering phase - the first harvest is 40%, at the end of the growing season – 26%. This shows that with standard pepper fertilization with a complex of mineral fertilizers (NPK), the mineralization process is most intense in the middle of the growing season, when the first harvest occurs. At the end of the growing season, the biological activity of the soil is still high, but with lower values than calculated in the middle of the growing season. The distribution of microbial colonies indicates an increase in the number of bacteria and Actinomycetes in the middle of the growing season, followed by an equalization of this indicator by the end of the period in almost all variants.

The number of fungal colonies is greatest at the beginning of the growing season and varies depending on the use of fertilizers and biologics (Table 1). The amount of imported fertilizers affects the biological activity of the soil. The introduction of a standard dose of mineral fertilizers (N16P20K22) in the control variant enhances the process of biological activity. The number of fungal colonies in 1 g of soil in the middle of the growing season is the highest in the variants of full mineral fertilizer-N16P20K22. Probably, the main factor regulating the number of microorganisms in the soil is the nitrogen content. It is well known that the study of the influence of the structural composition of the complex of Micromycetes in the soil is limited to four functional groups of fungi: saprophytes, parasites, antagonists, and fungi-producers of toxins. The variant with mineral fertilizers is dominated by fungi that produce toxins-*Penicillium sp., Aspergillus sp*. and *Fusarium sp.* (Table 2). The use of

"Trichodermine", "Nagro", "Stimix" and "BioLife" bioproducts leads to optimization of the sanitary state of the soil by reducing the content of fungal colonies in variants with bioproducts.

Table 1. *The content of germs in soil samples during the years of the experiment.*

	Season/ Variant	Number of microorganisms in 1 g of dry soil								
		Bacteria, mn/g			*Actinomycetes*, mn/g			*Micromycetes*, thsd/g		
		1	2	3	1	2	3	1	2	3
Start of the Vegetation Period										
Total sample		1,17	1,11	1,14	0,09	0,14	0,12	31,65	45,00	38,33
The Middle of the Vegetation Period										
1	$(N_{16}P_{20}K_{22})$	2,43	1,99	2,21	0,07	0,14	0,11	52,50	17,00	34,75
2	$(N_8P_{10}K_{11})$	0,87	1,87	1,34	0,23	0,18	0,21	26,50	27,00	26,75
3	$(N_8P_{10}K_{11})$ + "Trihodermine"	0,59	1,84	1,22	0,05	0,14	0,10	17,50	18,00	17,75
4	$(N_8P_{10}K_{11})$ + "BioLife"	1,44	2,34	1,89	0,07	0,27	0,17	20,00	28,00	24,00
5	$(N_8P_{10}K_{11})$ + "Nagro"	1,81	1,41	1,61	0,27	0,12	0,20	32,50	18,00	25,25
6	$(N_8P_{10}K_{11})$ + "Stimix"	0,66	2,10	1,38	0,33	0,17	0,25	25,00	16,00	15,50
The End of the Vegetation Period										
1	$(N_{16}P_{20}K_{22})$	1,71	1,62	1,67	0,05	0,13	0,09	24,75	30,00	27,38
2	$(N_8P_{10}K_{11})$	0,63	1,20	0,92	0,04	0,20	0,12	11,75	36,00	23,88
3	$(N_8P_{10}K_{11})$ + "Trihodermine"	0,52	0,51	0,52	0,18	0,10	0,14	20,00	27,00	23,50
4	$(N_8P_{10}K_{11})$ + "BioLife"	2,03	2,07	2,05	0,08	0,16	0,12	42,50	24,00	33,25
5	$(N_8P_{10}K_{11})$ Nagro	0,64	1,50	1,07	0,18	0,13	0,47	13,00	12,00	12,50
6	$(N_8P_{10}K_{11})$ + "Stimix"	2,45	1,04	1,75	0,10	0,06	0,08	6,25	13,00	9,63

The content of soil fungi taken from the variants with "Trichodermine" and "BioLife" is significantly lower than in the control variants, and Trichoderma colonies are more common. The effect of bio-products is better expressed on a reduced mineral background. Therefore, the introduction of "BioLife" into the rhizosphere when planting on a twice-reduced background increases the biological activity of the soil by 51% by the middle and by 43% by the end of the growing season. The study of the composition of microorganisms in the soil shows that the number of bacteria in this variant doubles - from 1.14 million/g at the beginning to 1.89 million/g by the middle and to 2.05 million/g of dry soil by the end of the growing season. Accordingly, in the same variant, the growth of Actinomycetes ranges from 0.14 million/g to 0.27 million/g in dry soil (Table 1).

Table 2. *The structural composition of Micromycetes in soil samples.*

№	Variant	Fungi colonies in petri dishes, per *sp.*cent							
		Alternaria sp.	*Fusarium sp.*	*Trichoderma sp.*	*Penicillium sp.*	*Aspergillus sp.*	*Mucor sp.*	*Cladosporium sp.*	*Mycelia sterilia*
1	($N_{16}P_{20}K_{22}$)	17,65	5,89	0,00	5,89	17,65	43,00	5,89	10,00
2	($N_8P_{10}K_{11}$)	5,50	0,00	5,50	2,25	21,75	6,25	5,25	23,50
3	"Nagro"	3,75	5,25	5,50	8,50	22,25	26,75	7,25	10,75
4	"Stimix"	0,00	0,00	6,66	6,66	23,33	30,00	2,00	12,25
5	"Trichodermine"	0,00	0,00	7,25	0,00	12,25	10,00	0,00	20,50
6	"BioLife"	0,00	3,50	6,50	6,95	23,75	5,00	7,15	37,15

The content of Micromycetes in the soil is maximum at the beginning of the growing season, after which the number of fungi decreases both in the variant with mineral nutrition and in the variant with bioproducts. The content of micromycetes in the soil is maximum at the beginning of the growing season, after which there is a decrease in the number of fungi both in variants with mineral nutrition and in variants with bioproducts. The introduction of bio-products on a low mineral background of bio-products creates favorable conditions for the development of microorganisms of all major groups. This guarantees the stability of functional relationships between the main ecological and trophic groups of microorganisms. The positive effect of biofertilizers on the development of fungal diseases of vegetables has been proven by many researchers (Egoshin, 2008; Yakovlev, 2006).

Monitoring of the incidence during harvesting showed differences in the rate of damage from Alternaria capsici-annui, depending on the rate of application of mineral (NPK) fertilizers and the applied biological product. In variants with mineral fertilizers, the development of black spots of Alternaria is 30-32%, with "BioLife" - 23-29% (Table 3).

The standard rate of basic mineral fertilizers (N16P20K22) is most effective for growing plants treated with "Nargo" and "Stimix" biologics. In variants with treatment of seeds with "Nagro" solution, the incidence reduction is 45% against the background (N8P10K11) and 55% against the background (N16P20K22). Seed treatment with "Stimix" solution reduces the development of black spots in pepper by 6% against the background (N8P10K11) and by 47% against the background (N16P20K22). Compared with the control variants, the effect of BioLife biofertilizer ranges from 11% on a reduced to 28% on a standard mineral background. A single treatment of the soil with the "Trichodermine" bioproduct gave good results for both fertilizer standards - standard (N16P20K22) and twice reduced (N8P10K11). The greatest effectiveness against black spots of Alternaria in seeding (56%) was registered when using the biological product "Trichodermine".

Table 3. *The influence of the organo-mineral system of fertilizers and bioproducts on the development of Alternaria black spots and the yield of sweet pepper.*

Variant	Alternaria black spots, per cent	Effect, per cent	Yield, kg/Ha	Effect, per cent	Standard production, per cent
Control	32,10	-	37730,01	-	66,76
($N_{16}P_{20}K_{22}$)	29,55	7,94	41490,20	9,79	87,82
($N_{16}P_{20}K_{22}$) + Nagro	14,5	54,82	46850,71	19,48	98,45
($N_8P_{10}K_{11}$) + Nagro	17,55	45,33	50570,14	25,39	98,96
($N_{16}P_{20}K_{22}$) + Stimix	20,55	35,98	48520,38	22,24	97,00
($N_8P_{10}K_{11}$) + Stimix	17,05	46,88	49870,57	24,35	92,54
($N_{16}P_{20}K_{22}$) + Trichodermine	14,05	56,23	49530,96	23,84	93,21
($N_8P_{10}K_{11}$) + Trichodermine	13,97	56,48	52110,15	27,60	97,72
($N_{16}P_{20}K_{22}$) + BioLife	28,65	10,75	53580,73	29,59	89,21
($N_8P_{10}K_{11}$) + BioLife	23,25	27,57	43880,89	14,033	94,81

Our results confirm that the use of microbial products in vegetable crops promotes growth, yield, and product quality. It was found that when growing field pepper, productivity by treating seeds with growth regulators and applying "Trichodermine" mineral fertilizer to the soil can be reduced by half without the risk of crop loss from Alternaria capsici-annui. As can be seen from table 3, the yield of pepper varieties "Kurtovskaya kapiya" in the version with a standard dose of mineral fertilizers was 41490 kg/Ha and 37730 kg/Ha on a reduced mineral background. Compared to a reduced mineral background, the yield increase on a standard mineral background is 10%. Treatment of pepper seeds with humic acids "Nagro" and "Stimix", treatment of the plant's rhizosphere with biological fertilizer "BioLife" and introduction of the biological product "Trichodermine" into the soil before planting enhance the effect of using complex mineral fertilizers. In the variants with "Nagro" and "Stimex", the yield increase is in the range of 19 – 24%, depending on the amount of mineral fertilizers in the experiment. On a reduced background of mineral fertilizers (N8P10K11), the effect of these drugs is higher than on the background created by the introduction of a full dose of mineral fertilizers (N16P20K22). The use of Trichodermin bio-product on a mineral background (NPK) increases the yield in field production of pepper by 27%. A significant increase in yield by 29% was observed with the introduction of “BioLife " on a mineral background (N16P20K22). This is probably due to the fact that BioLife helps the nitrification process and accelerates the absorption of mineral nitrogen by bacteria. An integrated system of organic and mineral fertilizers improves the quality of the products. The percentage of standard production is the lowest in the reduced mineral variant (N8P10K11) - 67%. In the variant using (N16P20K22), it is 88 percent. For comparison, 89-98% of standard products were obtained in variants using organic products "Nagro", "Stimix", "Trichodermine"and " BioLife".

Conclusion

The amount of imported mineral fertilizers affects the biological activity of the soil. The introduction of a standard dose of mineral fertilizers (N16P20K22) enhances the process of biological activity.

The use of "Trichodermine", "Stimix" and "BioLife" bio-products optimizes soil health by reducing the number of fungal colonies in the variants with bio-products.

The effect of bio-products is better expressed on a reduced mineral background.

The introduction of bio-products on a low mineral background creates favorable conditions for the development of microorganisms of all major groups. This ensures the stability of functional relationships between the main ecological and trophic groups of microorganisms.

The effectiveness of "Trichodermine" against black spots of Alternaria in pepper sowing is 56%. In variants with the use of "Nagro" for seed treatment, the incidence reduction is 45% against the background of N8P10K11 and 55% against the background of N16P20K22.

The use of an integrated system of fertilizer application (organic, mineral fertilizers and biological product "Trichodermine") increases the yield and yield percentage of standard pepper in the field. The increase in yield is 19-24% with the use of biofertilizers "Nagro" and "Stimix", 23% - " Trichodermine" and 29% - "BioLife".

Acknowledgments

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".

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