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USE OF GROWTH REGULATORS AND SAPROPHYTIC FUNGI *TRICHODERMA VIRIDE* PERS EX FR. TO IMPROVE THE HEALTH STATUS OF THE SOIL IN PEPPER FIELD

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Abstract

The article presents studies on the influence of the microbiological product “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 pepper yield in field production. It has been found that the differences in the "Soil Biological Activity" indicator depends on the number of micro-organisms, the composition (species diversity) and the percentage of major *Micromycetes*. Organic products help optimize soil health and reduce the total content of fungal colonies, and in the *Micromycetes* composition the fungus *Trichoderma sp.* is more common. When mineral fertilizers are introduced, the number of fungal pathogens and toxin producers increases. A higher total yield was obtained in variants with biological treatment. The increase in yield is between 9% and 29%.

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

INTRODUCTION

The development of fungal diseases in the vegetable field crops is one of the limiting factors of yield. Black leaf spots (*Alternaria capsici-annui* Savul & Sandu) is 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 and seed infection and the ecological and technological conditions of cultivation. The concept of biological protection of plants is determined by the amount of plant 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 physiological processes in plants to stimulate growth, relieve stress and increase yields (Belakbir et al., 1998; Bhattacharyya & Jha, 2012; Ikrina & Kolbin, 2004; Linser et al., 2006; Mora et al., 2010). The influence of organic fertilizers on the intensity of diseases in vegetable crops 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 balance of nutrition and affects the phytosanitary condition 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,



Alternaria damage index of black spots, the total yield and percentage of standard products.

MATERIALS AND METHODS

The experimental work was carried out in 2009-2011 on the territory of Maritsa Vegetable Crops Research Institute on alluvial-meadow soil with the 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 P₂O₅-16.1 mg/100 g and mobile K₂O – 18.2 mg/100 g soil (Egner-Rhyme), soil reaction Ph/H₂O/- 6.9 (potentiometric). The experiment was carried out by the block method in four replicates (100 replicated plants), with a test area of 12.8 m². 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 total biogenic of soil, composition and structure of the soil Micromycetes and the development of black spots (*Alternaria capsici-annui* Savul & Sandu). For this purpose, the pepper seeds were treated with the bio-fertilizers „Nagro” and „Stimix” in concentration 1:100 for 4 h followed by treatment of plants with the same bio-fertilizers in concentration 1:100 in the blooming phase and after the first harvest. The bioproduct „BioLife” was introduced into the rhizosphere of the plants as a liquid solution in concentration 500ml/ 200L H₂O at 5L/m² immediately after planting, in the flowering period. The bio-product “Trichodermine” (*Trichoderma viride* Pers ex Fr.) was introduced in the soil at 40 kg/Ha. Experimental variants were carried out on a standard and double reduced mineral background. The standard mineral background contained N₁₆P₂₀K₂₂: ammonium nitrate 500 kg/Ha, super phosphate - 450 kg/Ha, and potassium sulfate - 450 kg/Ha. Phosphorus and potassium fertilizers were introduced during

the autumn soil treatment, nitrogen - during the vegetation. Reduced mineral background contained N₈P₁₀K₁₁ respectively.

Indicators: Total% Yield (kg / Ha), *Alternaria* black spot Attack Index on the leaves, Percentage of Diseased fruits (%), Total Soil Biological Activity (CFU count per 1 g dry soil, amount of fungal colonies in 1 g of dry soil). The attack rate of *Alternaria capsici-annui* was counted on a five-digit scale (Gannibal et al., 2011): 0 - no symptoms, 1- single spots, 2- to 25%, 3- 26-50%, 4- over 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 - Abbott formula. The percentage of diseased fruits with signs of *Alternaria capsici-annui* was counted during mass harvesting (%). The microbiological analysis of the soil samples was made at the beginning, middle and end of the vegetation - after harvesting the produce. The biological activity of the soil was determined by the standard microbiological methods of cultivation of microorganisms on elective nutrient media (Zvyagintsev, 1991).

The statistical processing of the obtained experimental data was made with the program Excel (Microsoft Office 2002), Windows XP. The results of the study were analyzed by the standard statistical procedure and presented as means from three replicates.

Characterization of 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 crops, biologically active substances.



„BioLife” - is a combined highly concentrated bacterial preparation. It contains enzymes and non-pathogenic microorganisms and an active ferment that accelerates and increases the development of microorganisms and stimulates the direct action of soil bacteria.

„Trichodermine” (*Trichoderma viride* Pers ex Fr., strain N^o 12) – a bio-product with a titre $2 \cdot 10^{10}$ c/g, obtained by the solid phase technology.

RESULTS AND DISCUSSION

The results of our research confirm that the sanitary condition of the soil depends on the total action of factors associated with crop cultivation. The microbiological analysis of the soil samples taken from the pepper field at the beginning, middle and end of vegetation shows that the biological activity of the soil in the experiment changes during the whole growing season (Table 2). The Total Biological Activity of the soil in the experience with pepper increases twice during the vegetation. The increase of the soil biological activity in control variant with mineral fertilizers (N₁₆P₂₀K₂₂) during the flowering phase - the first harvest is 40 per cent, at the end of the vegetation – 26 per cent. This shows that with a standard fertilization of the pepper with the mineral fertilizer complex (NPK) the mineralization process is the highest in the middle of the vegetation when the first harvesting takes place. At the end of the vegetation the soil biological activity is still high, but with lower values than those calculated in the middle of the vegetation. The distribution of the colonies of microorganisms shows the increase in the number of bacteria and Actinomycetes in the middle of vegetation with the subsequent equalization of this indicator towards the end of the period in almost all variants. The number of fungi colonies is the highest at the beginning of the vegetation and varies, depending on the use of fertilizers and the bio-products. The amount

of imported fertilizers affects the biological activity of the soil. The introduction of the standard dose of mineral fertilizers (N₁₆P₂₀K₂₂) into the control variant enhances the process of biological activity. The amount of fungi colonies in 1 g of soil in the middle of vegetation is the highest in the full mineral fertilization options - N₁₆P₂₀K₂₂. Probably, a major 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 *Micromycetes* complex in the soil is limited to four functional groups of fungi: saprophytes, parasites, antagonists and fungi producers of toxins. In the variant with mineral fertilizers dominate fungi producing toxins - *Penicillium* sp., *Aspergillus* spp. and *Fusarium* spp. (Table 3). The use of the bioproducts “Trichodermine”, “Nagro”, “Stimix” and “BioLife” leads to the optimization of the sanitary state of the soil at the expense of the lower content of fungi colonies in the variants with bioproducts. The content of pathogenic fungi in soil samples with “Trichodermine” and “Bio Life” is significantly lower than in the control variants; except for *Trihoderma* spp. colonies are more common.

The effect of the bioproducts is better expressed on the reduced mineral background. Therefore, the introduction of „BioLife” into the rhizosphere during planting on the double-reduced background increases the Biological Activity of the Soil by 51 per cent to the middle and by 43 per cent towards the end of the vegetation. A study of the composition of the microorganisms in the soil shows that the amount of bacteria in this variant doubles - from 1.14 million/g in the beginning to 1.89 million/g - to the middle and up to 2.05 million/g of dry soil - at the end of the vegetation. Accordingly, in the same embodiment, the growth of Actinomycetes ranges from 0.14 million/g to 0.27 million/g in dry soil. The content of *Micromycetes* in the



soil is maximal at the beginning of the vegetation, after which there is a reduction of the fungi in the variant with the mineral nutrition as well as in the variant with bioproduct. The content of *Micromycetes* in the soil is maximal at the beginning of the growing season, after which there is a decrease in the fungi in the variants with mineral nutrition as well as in the variants with bioproducts. The introduction of bioproducts on the reduced mineral background of bio-products creates favorable conditions for the development of microorganisms from all major groups. This guarantees the stability of the functional links between the major ecological trophic groups of microorganisms. The positive influence of bio-fertilizers on the development of fungi diseases in vegetables has been proven by many researchers (Yegoshin, 2008; Yakovlev, 2006).

Monitoring of 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 bioproduct. In variants with mineral fertilizers, the development of black spots of *Alternaria* spp. is 30-32%, with "Biolife" - 23-29%. The standard rate of basic mineral fertilizers ($N_{16}P_{20}K_{22}$) is the most effective for growing plants treated with the bioproducts „Nargo” and „Stimix”. In variants with seed treatment in “Nagro” solution, the disease reduction is 45 per cent on background ($N_8P_{10}K_{11}$) and 55 per cent on background ($N_{16}P_{20}K_{22}$). The seed treatment with „Stimix” solution reduces the development of black spots in pepper at 6 per cent on background ($N_8P_{10}K_{11}$) and 47 per cent on background ($N_{16}P_{20}K_{22}$). Compared with the control variants, the effect of „BioLife” bio-fertilizer ranges from 11 per cent on reduced to 28 per cent on a standard mineral background. The single soil treatment with the bioproduct „Trichodermine” produced good results for both fertilizer norms - the standard ($N_{16}P_{20}K_{22}$) and the double reduced ($N_8P_{10}K_{11}$). The highest

efficacy against *Alternaria* black spots in the crop (56 per cent) was registered from the application of the bioproduct „Trichodermine”.

Our results confirm that the use of microbial products in the vegetable crops stimulates growth, yield and product quality. It was found that when growing pepper from treated seeds with growth regulators and applying Trichodermin to the soil, the dose of mineral fertilizers 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 the pepper variety “Kurtovska kapia” in the variant with a standard dose of mineral fertilizers was 41490 kg/Ha and 37730 kg/Ha on the reduced mineral background. Compared to the reduced mineral background, the increase in yield on a standard mineral background is 10 per cent. The processing of the pepper seeds with the humic acids „Nagro” and „Stimix”, the treatment of the rhizosphere of the plant with the biological fertilizer „BioLife” and the soil introduction of the bioproduct „Trichodermine” before planting enhance the effect of application of the complex mineral fertilizers. In the variants with „Nagro” and „Stimex”, the increase in the yield is in the range of 19 – 24 per cent depending on the amount of mineral fertilizers in the experiment.

On the reduced background of mineral fertilizers ($N_8P_{10}K_{11}$), the effect of these preparations is higher than on the background created by the introduction of a full dose of mineral fertilizers ($N_{16}P_{20}K_{22}$). The application of the bioproduct “Trichodermine” on a mineral background (NPK) increases the yield in the pepper field production by 27 per cent. A significant increase in yield of 29 per cent was observed with the introduction of “BioLife” on a mineral background ($N_{16}P_{20}K_{22}$). This probably due to the fact, that BioLife helps the process of nitrifying and accelerating the absorption of the mineral nitrogen by bacteria.

An integrated system of organic and



mineral fertilizers improves the quality of the product. The percentage of standard fruits is the lowest in the reduced mineral variant (N₈P₁₀K₁₁) - 67 per cent. In the variant using (N₁₆P₂₀K₂₂), it is 88 per cent. For comparison,

89-98 per cent of standard production was obtained in variants using the organic products "Nagro", "Stimix", "Trichodermine" and "Biolife".

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 ₈ P ₁₀ K ₁₁)	0,87	1,87	1,34	0,23	0,18	0,21	26,50	27,00	26,75
3	(N ₈ P ₁₀ K ₁₁) + "Trihodermine"	0,59	1,84	1,22	0,05	0,14	0,10	17,50	18,00	17,75
4	(N ₈ P ₁₀ K ₁₁) + "BioLife"	1,44	2,34	1,89	0,07	0,27	0,17	20,00	28,00	24,00
5	(N ₈ P ₁₀ K ₁₁) + "Nagro"	1,81	1,41	1,61	0,27	0,12	0,20	32,50	18,00	25,25
6	(N ₈ P 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 ₈ P ₁₀ K ₁₁)	0,63	1,20	0,92	0,04	0,20	0,12	11,75	36,00	23,88
3	(N ₈ P ₁₀ K ₁₁) + "Trihodermine"	0,52	0,51	0,52	0,18	0,10	0,14	20,00	27,00	23,50
4	(N ₈ P ₁₀ K ₁₁) + "BioLife"	2,03	2,07	2,05	0,08	0,16	0,12	42,50	24,00	33,25
5	(N ₈ P ₁₀ K ₁₁) Nagro	0,64	1,50	1,07	0,18	0,13	0,47	13,00	12,00	12,50
6	(N ₈ P ₁₀ K ₁₁) + "Stimix"	2,45	1,04	1,75	0,10	0,06	0,08	6,25	13,00	9,63



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

№	Variant	Fungi colonies in petri dishes, per <i>sp.</i> cent							
		<i>Alternaria sp.</i>	<i>Fusarium sp.</i>	<i>Trichoderma sp.</i>	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>	<i>Mucor sp.</i>	<i>Cladosporium sp.</i>	<i>Mycelia sterilia</i>
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 ₈ P 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

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	<i>Alternaria</i> 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 ₈ P 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 ₈ P 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 ₈ P 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 ₈ P ₁₀ K ₁₁) + BioLife	23,25	27,57	43880,89	14,033	94,81



CONCLUSIONS

The quantity of imported mineral fertilizers affects the biological activity of the soil. The introduction of the standard dose of mineral fertilizers (N₁₆P₂₀K₂₂) strengthens the process of biological activity.

The use of the bioproducts „Trihodermine”, „Stimix” and „BioLife” optimizes soil health at the expense of the less fungal colonies in variants with bio-products.

The effect of bioproducts is better expressed on the reduced mineral background.

The introduction of bioproducts on the reduced mineral background creates favourable conditions for the development of the microorganisms of all major groups. This ensures the stability of the functional links between the major ecological trophic groups of microorganisms.

The efficacy of „Trihodermine” against *Alternaria* black spots in the pepper crop is 56 per cent. In variants with the use of „Nagro” for seed treatment, the disease reduction is 45 per cent on background N₈P₁₀K₁₁ and 55 per cent on background N₁₆P₂₀K₂₂.

The application of the integration system for fertilization (organic, mineral fertilizes and the bio-product „Trihodermine” increases the yield and the percentage of the standard pepper production in field conditions. The increase of the yield is 19 – 24 per cent with the application of „Nagro” and „Stimix” bio-fertilizes, 23 per cent - „Trichodermine” and 29 per cent - „BioLife”.

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