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The Role of Organic and Mineral Fertilizers in the Regulation of Soil Fungi Stasis.

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ABSTRACT

One of the most active ways of regulating the processes in the soil aimed at increasing soil fertility and improving the phytosanitary condition of the soilis the application of fertilizers. The activity of soil microflora influences oil fungistasis. Joint application of organic and mineral fertilizers increases soil fungistasis. The research was conducted in the following crop rotation: first-year clover – spring wheat – vetch/oats for grain – winter wheat. While carrying out the research, the effect of organic (chopped straw of the previous crop and stubble of first-year clover) and mineral fertilizers ($N_{60}P_{60}K_{60}$). The structure of micromycete complex in the rhizosphere of spring wheat, vetch-oat mixture and winter wheat, biological activity in the soil, soil fungistasis, and infestation of winter wheat withroot rot in crop rotation were also studied. The greatest number of saprotrophs throughout the growth was after applying chopped straw, stubble of first-year clover and mineral fertilizers ata dose of $N_{60}P_{60}K_{60}$. The greatest number of pathogens was in the check. The growth in the number of microorganisms in the soil including antagonists boosted its fungistasis. The greatest number of ungerminated conidia of *F. gibbosum* throughout the growth was in the soil in which chopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$were added. The joint application of chopped straw, stubble of first-year clover and mineral fertilizers increased biological activity in the soil by 1.3 times. Winter wheat was least infested with pathogens of root rot after applying chopped straw, stubble of first-year clover without application of mineral fertilizers and at the beginning of the growth a degree of infestation was 11.3% and 8.2% after application of$N_{60}P_{60}K_{60}$; 17.9 % and 14.3 % –at the end of the growth, respectively. Mineral fertilization decreased infestation of winter wheat with root rot.

Keywords: organic fertilizers, mineral fertilizers, microorganisms, fungistasis, biological activity in the soil.

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INTRODUCTION

A large population of microorganisms in the soil exists in a state which is often characterized as an "unstable equilibrium", that is, when each microorganism at any given moment is in equilibrium with its neighbor, but the changes in external conditions lead to changes in balance. If the soil is not processed and remains in the same condition, the daily changes in the equilibrium arenot significant because there are no available sources of energy [1; 3].

Most generative cells of fungi are subject to external regulation in the form of fungi static action of the soil. Even under favorable soil conditions, a viable generative cells cannot grow, the growth of mycelium gets slow or stops not because of endogenous dormancy or temperature and water content in the soil, but due to other factors. Reaction to fungi static effects of the soil is possible for the life cycle of soil fungi that experience periods of minimum amount or absence of the substrate. If during this period the spores germinate spontaneously or hyphae start to grow, then it probably leads to their depletion and demise [2; 7]. Fungi static effect of the soil regulates such growth, and it is usually overcome in the rhizosphere by the factors which facilitate the development of the fungus. Spores germinate and mycelium grows around the roots. This phenomenon is of great importance to induce and maintain fungistasis and resolve it – these are processes through which the soil organism, pathogenic for plants, must pastor maintain inoculated potential to susceptible hosts. Any interference in these processes may lead to either a decrease or an increase in the number of the pathogens [5; 6; 15].

One of the most active ways of regulating the processes in the soil aimed at increasing soil fertility and improving the phytosanitary condition of the soilis the application of fertilizers. Fertilizers not only enrich the soil with nutrients, but also affect the development of micromycetesthat influence the growth and development of plants [4; 8].
The elements of mineral nutrition can have a specific impact on pathogens by altering the structure of mycocenosis, fungistasis and physical and chemical properties of the soil and reduce their number in the saprophytic, especially antagonistic microflora [11].

Combined application of organic and mineral fertilizers reduce the infectious potential of the soil. This is because the combined application of organic and mineral fertilizers stimulates the growth of microorganisms including antagonists.

A significant reduction in the level of infection in the soil is observed under the combined effect of crops and fertilizers. In this case, a significant phytosanitary effect is achieved only against the background of high biological activity in the soil. Organic fertilizers stimulate biological activity in the soil because microorganisms consume organic matter as a source of energy that contributes to the optimization of phytosanitary state of agro ecosystems, the conservation of soil fertility, since in the case of the lack of organic substances microorganisms use the humus of the soil [10; 14].

It was established that fungistasis of the soil is connected with the activity of soil microflora. Consequently, combined application of organic and mineral fertilizers increases soil fungistasis [9].

MATERIALS AND METHODS

The study was carried out in the field and in the laboratory. To do the research in the field of FBGNU "MariResearchInstitute of Agriculture", a two-factor experiment was conducted. The research was conducted in the following crop rotation: first-year clover – spring wheat – vetch/oats for grain – winter wheat.

The scheme of the experiment:

Factor A – the application of organic fertilizers
A_1 – check (without any fertilizers)
A_2 –chopped straw of the previous crop and stubble of first-year clover
Factor B – the application of mineral fertilizers
B_1 – check (without any fertilizers)
B_2 – $N_{60}P_{60}K_{60}$

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The location of the plots is systematic. The total area of the plot was 330 m^2 (30m x11m), experimental area–100m^2. Each of the trial plots was divided crosswise into two parts of 165 m^2 (15m × 11m) to study the effect of mineral fertilizers. Then each of the parts was divided into two parts of 82.5 m^2 (5.5m x 15m) to study the effect of organic fertilizers in the form of chopped straw of the previous crop and stubble of first-year clover. The sod-podzolic, medium loam soil of experimental plot had the following agrochemical parameters of the arable layer: humus content (by Tyurin) – 2.23%, hydrolyzable nitrogen – 6.0 mg/100 g of soil, mobile forms of phosphorus (by Kirsanov) – 27, exchangeable potassium (by Kirsanov) – 11 mg/100 g of soil, pH_{sal}–5.8.

At the stagesof tailoring, booting, earing and yellowing, samples of rhizosphere soil were selected. The roots of ten plants together with the soil were taken out from the soil with a soil knife and shovel. To determine the quantity of microorganisms, microbiological analysis of the soil samples was carried out in the laboratory. A sample of soil of 1g was placed in a sterile flask of 250 ml, poured with 100 ml of tap water and closed with sterile cotton plugs. The flasks with soil suspension was shaken up in a shaking bath for 30 minutes, then the suspension was settled for 30 seconds to let large mineral particles settle. Then the soil suspension was diluted to inoculate in the nutrient soil: 1 ml of this suspension was put into a test tube with 9 ml of sterile tap water with a sterile pipette; then 1 ml of the suspension from this test tube was put into a tube with 9 ml of sterile tap water with a new sterile pipette. Surface smears from the last dilution were performed in the Petri dishes four times: 1 ml of soil suspension was evenly distributed across the surface of the Petri dish with a glass spatula. Then the dishes were turned upside down and put into a thermostat. To identify and count fungi, Chapeckmedium was used. Fungi were kept at a temperature of 28 ºC. Identification and count of fungi were carried out after 7 days.

After calculating the number of colonies on all parallel dishes, the average number of colonies on each dish was counted. Then the number of microorganisms in 1g of air-dry or absolutely dry soil was calculated with the formula as follows:

$$a = \frac{b \times v}{d},$$

Where:

a – a number of microorganisms, CFU/g of soil;
b – The average number of colonies on the dish;
v – Dilution, from which surface smears were performed;
d – The weight of air-dry or absolutely dry soil taken for the analysis, g

MPA was used to isolate bacteria. Bacteria were incubated at a temperature 36ºC. They were counted on the 5th day.

To determine a specific set of microorganisms, typical and accidental species were identified. To establish whether a species is typical or accidental, the frequency index was used. The frequency index of a species is the percentage of frequency of the species in a sample from the total number of samples. This index allows us to find out if a species is typical of a certain agro-ecosystem or not. The frequency index of the species was calculated with the formula as follows:

$$FI = \frac{n \times 100}{N},$$

Where:

FI – frequency index, %;
n – A number of samples in which a species was found out;
N –the total number of samples.

Species are considered to be typical if frequency index is more than 30 %, accidental – less than 10 % [13].

To determine the biological activity in the soil, pre-weighed linen fabrics with the size of 5cm×15cm were sewed to the plastic film. Soil crossover (10 cm deep) was made in each sample with a shovel. The film with a linen fabric was applied to the smooth wall of the soil crossover; the opposite side of the plastic film was covered with soil, pressed tightly to the wall. The place with the linen fabric was marked with a stick. After 45 and 90 days the remainder of the linen fabric was taken out, washed with water to remove soil particles, dried and weighed. The mass loss showed the intensity of decomposition of linen fabric.

The scale of intensity of decomposition of linen fabric during the growth is as follows: very weak < 10%; weak – 10-30%; medium– 30-50%; strong – 50-80%; very strong > 80%.

The method of A. Dotsenko (1972) was used to determine soil fungistasis. The soil samples were placed in sterile Petri dishes and wetted up to 80% of the maximum water holding capacity. After that filter paper was put on the soil surface. Petri dishes were kept in the laboratory for 48 hours. Then cellophane disks, 10 mm in diameter, with conidia suspension of the tested fungus (5 per Petri dish) were put on the surface of the samples and left in a moist chamber for 24 hours. Then the discs were removed and micro scoped. The indicator of soil fungistasis is the percentage of ungerminated conidia of the fungus [12].

The intensity or the degree of infestation was determined on a 4-point scale:

0 – the signs of the disease are absent;
1 – single strokes, coleoptiles and underground inter node turned browns lightly;
2 –underground inter node or the base of the stalk and nodal roots turned brown greatly;
3 – Underground inter node turned black, the base of the stalk, nodal roots were infested, and they were rotten;
4 – The plant died out.

RESULTS AND DISCUSSION

As a result of the analyses, 16 species of microorganisms were identified. Such pathogenic fungi as Fusarium spp., Bipolaris spp. and Alternaria spp. were isolated. Of these, typical of the agro-ecosystem are the following species: F. culmorum (frequency index was 45.3%), F. avenaceum (49.7%), F. oxysporum (45.0%), F. gibbosum (70.3%), F. graminearum (55.7%) and B. sorokiniana (48.6%).

Such fungi as Alternariaalternata Fr. and CladosporiumgraminumCdaare accidental. The frequency index was about 20%. They mostly appear on weakened seedlings as secondary parasites.

Such saprotrophsas Penicillium freguentans West. (85.3 %), Penicillium virdicatum West. (76.7 %), Penicillium funiculosum Thom. (77.4 %), Penicillium lanosum West (45.7 %), Penicillium fumigatus Fres. (47.3 %), Aspergillus Niger van Tiegh (73.5 %), Aspergillus clavatureDesm. (54.3 %), RhizophusnigricansEhr. (61.3%) were isolated.Mucor piriformis Fisch. were also very frequent identified (82.4%). They are typical of the agro-ecosystem. From saprotrophic fungi, the fungus-antagonist Trichoderma lignorum (Tode) Haz.–a typical representative of the antagonistic microflora was isolated, the frequency index of which was 67.3%.

The structure of micromycete complex in the rhizosphere of spring wheat is presented in Table 1.

Table 1:The structure of micromycete complex in the rhizosphere of spring wheat in crop rotation

Variants		Saprotrophs, thousand/g of soil	Pathogens, thousand/g of soil
organic fertilizers – Factor A	mineral fertilizers – Factor B		
Tillering			
Check	Check	26.3	24.7
	$N_{60}P_{60}K_{60}$	30.2	20.5
Organic	Check	29.4	23.7
	$N_{60}P_{60}K_{60}$	35.7	20.1

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Booting			
Check	Check	36.4	31.4
	$N_{60}P_{60}K_{60}$	43.3	26.4
Organic	Check	38.6	28.6
	$N_{60}P_{60}K_{60}$	50.4	27.1
Earing			
Check	Check	44.3	36.7
	$N_{60}P_{60}K_{60}$	52.6	31.4
Organic	Check	48.1	33.2
	$N_{60}P_{60}K_{60}$	60.5	31.6
Yellowing			
Check	Check	55.4	37.4
	$N_{60}P_{60}K_{60}$	63.6	30.3
Organic	Check	60.3	34.8
	$N_{60}P_{60}K_{60}$	74.4	29.1

The greatest number of saprotrophs throughout the growth was after applying chopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$. At the beginning of the growth their number was 35.7 thousand/g of soil, and at the end of the growth– 74.4 thousand/g of soil. After applying mineral fertilizers the number of saprotrophs was higher. The greatest number of pathogens was in the check. Their number was 1.3 times more than in a variant where organic and mineral fertilizers were applied.

In rhizosphere soil of vetch-oat mixture during the growth the number of saprotrophs increased and the number of pathogens decreased in comparison with their number in rhizosphere soil of spring wheat. After applying organic and mineral fertilizers, the number of saprotrophs at the tailoring stage of oats was 35.7 thousand/g of soil, that is 1.4 times higher, and pathogens – 20.1 thousand /g of soil, that is 1.2 times lower in comparison with the check (Table2).

Table 2: The structure of micromycete complex in the rhizosphere of vetch-oat mixture in crop rotation

Variants		Saprotrophs, thousand/g of soil	Pathogens, thousand/g of soil
organic fertilizers – Factor A	mineral fertilizers – Factor B		
Tillering			
Check	Check	30.7	22.1
	$N_{60}P_{60}K_{60}$	35.6	19.2
Organic	Check	34.4	20.1
	$N_{60}P_{60}K_{60}$	42.8	18.7
Booting			
Check	Check	40.7	29.2
	$N_{60}P_{60}K_{60}$	59.4	25.3
Organic	Check	42.3	24.8
	$N_{60}P_{60}K_{60}$	55.1	23.5
Earing			
Check	Check	50.2	32.7
	$N_{60}P_{60}K_{60}$	57.6	29.2
Organic	Check	51.4	30.2
	$N_{60}P_{60}K_{60}$	67.8	28.8
Yellowing			
Check	Check	60.7	34.3
	$N_{60}P_{60}K_{60}$	69.3	28.1
Organic	Check	65.6	30.2
	$N_{60}P_{60}K_{60}$	78.9	28.7

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The structure of micromycete complex in the rhizosphere of winter wheat is presented in Table 3.

Table 3: The structure of micromycete complex in the rhizosphere of winter wheat in crop rotation

Variants		Saprotrophs, thousand/g of soil	Pathogens, thousand/g of soil
organic fertilizers – Factor A	mineral fertilizers – Factor B		
Tillering			
Check	Check	28.5	24.3
	$N_{60}P_{60}K_{60}$	34.4	21.6
Organic	Check	32.3	23.5
	$N_{60}P_{60}K_{60}$	39.7	22.8
Booting			
Check	Check	38.0	31.3
	$N_{60}P_{60}K_{60}$	47.5	27.3
Organic	Check	40.6	28.5
	$N_{60}P_{60}K_{60}$	53.4	26.2
Earing			
Check	Check	47.3	36.4
	$N_{60}P_{60}K_{60}$	55.7	31.3
Organic	Check	48.8	33.5
	$N_{60}P_{60}K_{60}$	65.3	31.7
Yellowing			
Check	Check	59.4	36.7
	$N_{60}P_{60}K_{60}$	67.8	30.1
Organic	Check	63.6	33.8
	$N_{60}P_{60}K_{60}$	78.3	29.3

The greatest number of saprotrophs throughout the growth was when chopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$were applied. At the tailoring stage, their number was 39.7 thousand/g of soil and at the yellowing stage it increased to 78.3 thousand/g of soil. In the check the number of saprotrophswas 1.4 times less. The greatest number of pathogenswas in the check. At the beginning of the growth it was 24.3 thousand/g of soil and at the end of the growth – 36.7 thousand/g of soil.

The ratios of saprotroph/pathogen in crop rotation depending on the studied variants can be represented in the following graphics (Fig.1):

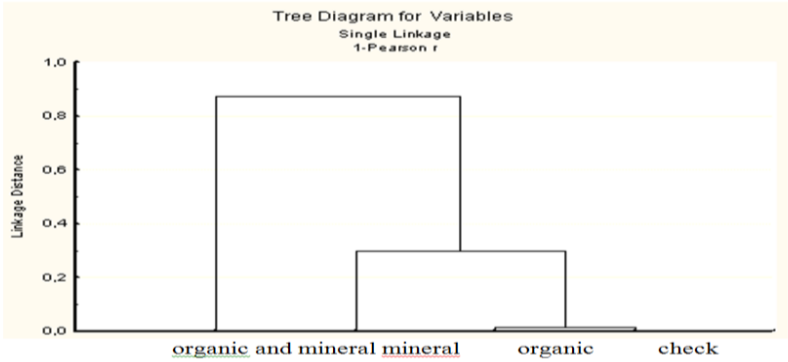

Fig 1: Dendrogram of the similarity of saprotroph/pathogen ratios in crop rotation depending on the fertilizers

When conducting a microbiological analysis of the rhizosphere soil of winter wheat, aerobic bacteria Pseudomonaswereidentified,the frequency index of which was about 40%, Bacillus (35.3%) and Myxococcus (30.1%). The greatest number of bacteria throughout the growth was when chopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$were applied. The highest number of them was at the tailoring stage – 80.5 thousand colonies. At the stage of yellowing, their number decreased to 74.7 thousand colonies. The smallest number of bacteria (55.3 thousand colonies) was in the check. At the beginning of the growth it was 1.5 times less in comparison with the variant where organic and mineral fertilizers were applied.

The application of organic and mineral fertilizers affected not only the growth of microorganisms in rhizosphere soil of winter wheat, but also fungistasis. The growth in the number of microorganisms in the soil, including antagonists, boosted its fungistasis. The greatest number of ungerminated conidia of F. gibbosum throughout the growth was in the variantwithchopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$. At the beginning of the growth the percentage of ungerminatedconidia was 73.8, and at the end of the growth – 73.5, which is 1.4 times more than in the check (Table4).

Table 4: The influence of fertilizers on the soil fungistasis (microfield experiment, winter wheat, F. gibbosumas a test-object)

Variants		Fungi stasis		
organic fertilizers – Factor A	mineral fertilizers – Factor B	tailoring	earing	yellowing
Check	Check	51.3	50.1	49.3
	$N_{60}P_{60}K_{60}$	49.5	49.3	48.5
Organic	Check	65.7	68.9	65.3
	$N_{60}P_{60}K_{60}$	73.8	75.3	73.5

The application of straw had a significant impact on fungistasis. Thus, the number of ungerminated conidia in the variant where fertilizers were not applied was 65.7 %, which is 1.3 times more than in the check. Along with the basic agrochemical parameters of soil fertility (the content of humus and mineral nutrients, soil reaction, physical and chemical properties) there is such parameter as biological activity in the soil, which mainly depends on the activity of soil microflora. The application of fertilizers, especially organic, improves biological activity in the soil, which contributes to the purification of soil from infection (Table5).

Table 5: Biological activity in the soil in crop rotation

Variants		Decomposition, %	
organic fertilizers – Factor A	mineral fertilizers – Factor B	45 days	90 days
Check	Check	32.0	97.6
	$N_{60}P_{60}K_{60}$	36.5	94.7
Organic	Check	33.9	98.2
	$N_{60}P_{60}K_{60}$	41.3	97.9

The highest percentage of decomposition of linen fabric was in the variant with applied chopped straw, stubble of first-year clover and mineral fertilizers. Other variants had slight differences from the check. After 90 days, the percentage of decomposition was slightly different in the variants. This is due to the fact that at the end of the growth the mineralization of organic matter slows down. The degree of decomposition on the 45thday is medium and on the 90th day it is very high.

The change infungistasisof rhizosphere soil of winter wheat influenced the infection of the crop withroot rot. In the check plants were infected more than in the variant with applied mineral fertilizers. In the variant where chopped straw was not applied, the degree of infestation of plants with root rot was very low.

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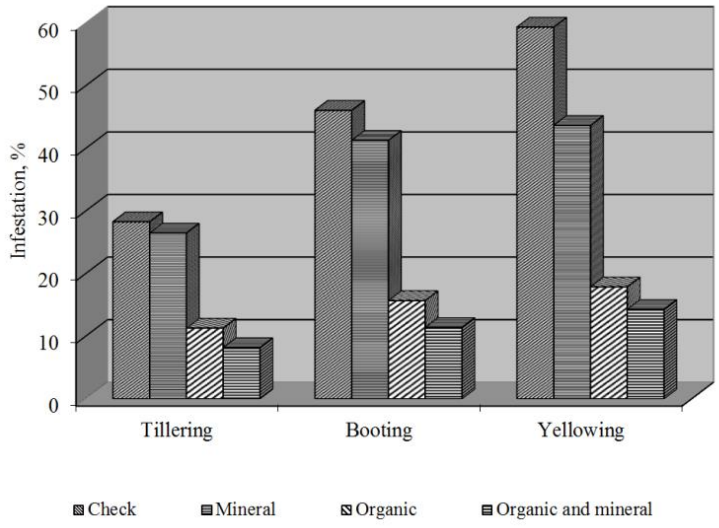

Fig 2: The influence of fertilizers on the infestation of winter wheat with root rot in crop rotation

With a high-level of soil fungistasisthe infestation of the crop with root rot was low (Fig.2). Thus, at the beginning of the growth the lowest level of infestation of winter wheat with root rot (11.3%)was in those variants where chopped straw, stubble of first-year clover were applied without application of mineral fertilizers and 8.2% when $N_{60}P_{60}K_{60}$was applied; at the end of the growth it was 17.9% and 14.3 %, respectively. In the check soil fungistasis was slight, consequently, the level of infestation of plants was higher. It should be noted that mineral fertilizers reduce the infestation of winter wheat with root rot.

CONCLUSIONS

1. The application of chopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$contributed to the increase in the number of saprotrophs in the soil and decrease in pathogens and a high level of soil fungistasis.
2. The combined application of organic and mineral fertilizers increased biological activity in the soil by 1.3 times in comparison with the check.
3. The least infestation of winter wheat with root rot during the growth was in the variant with applied chopped straw, stubble of first-year clover and mineral fertilizers at a dose of $N_{60}P_{60}K_{60}$.

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