

## eloidogyne (Goeldi, 1887)

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## Selenium – Nanoparticles as a Possible Method for Control to *Meloidogyne* (Goeldi, 1887)

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Original scientific paper

### SUMMARY

: (cv. Gamayun) –  
*Meloidogyne incognita* (cv. Red  
Cherry) – *Meloidogyne arenaria*.

P, S, K, Ca, Zn, Fe),

Influence of selenium nanoparticles on the systems: tomatoes (cv. Gamayun) – *M. incognita* and tomatoes (cv. Red Cherry) – *M. arenaria* was investigated. The changes of morphological and physiological characteristics and content and changes in the quantity of some elements which are of special importance for the normal development of the plants (Mg, P, S, K, Ca, Zn, Fe, and Se) were investigated. The stimulation effect of Se-nanoparticles on the growth and development of infected with root-knot nematodes was proved. Treatment of infected plants with Se-nanoparticles increases the activity of proteinase inhibition and in this way improves the innate plant immunity.



2016). (Wu et al., 2016). Now in the plant growing practice water nanoparticle solutions of trace elements are more and more in search. Selenium positively influences the structure of the cell membranes.

et al., 2001). (Xue et al., 2001). It also activates superoxide dismutase activity (Xue et al., 2001). Addition of Se strengthens the antioxidant capacity in senescing plants and also activates the plant growth by increasing of starch accumulation in chloroplasts.

(Udalova, 2018). After our experiments and analyses of the results obtained, we detected the stimulation effect on the plant development and inhibition on the morphological and physiological characteristics of the nematodes and on the degree of infestation of the roots (Udalova et al., 2018).

(Gupta and Gupta, 2017). Application of Se in low concentrations increases the plant stability to high levels of salinity, droughty, heavy metal pollution, high temperature, senescent and UV radiation (Gupta and Gupta, 2017).

UV (Gupta and Gupta, 2017). These characteristics of selenium determined our investigations of Se nanoparticle solutions as an abiogenic factor which increases the plant stability to stress factors including to *Meloidogyne* invasion.

The aim of our investigations was to study the effect of treatment with Se-nanoparticle solutions on the factors which influence the plant resistance of the host-parasite systems: (*Meloidogyne incognita*-tomato cv. Gamayn and *Meloidogyne arenaria*-tomato cv. Red Cherry).

(*Meloidogyne incognita*) – (cv. Gamayn) – (cv. Red Cherry) – (cv. Red Cherry).

## MATERIAL AND METHODS

: (cv. Gamayun) – *Meloidogyne incognita* (cv. Red Cherry-Bulgaria) – *Meloidogyne arenaria*.

(Niconov et al., 2009).

10 70 nm.

cv. Gamayun

2 h

2 h 3 h  
Cherry.

– cv. Red

ULTIMA-2 (Horiba Jobin-Yvon, France).

( Gamayun)

(2 ml/ ),  
Red Cherry –  
(2 ml/ ).

0.34 0.68 µg/ml.

( Gamayun - *M. inncognita* – 3000  
2- ( 2 )  
Red Cherry - *M. arenaria* – 2000 2  
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14

10

40

g/

(

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(IR)

(PIs).

Investigations have been carried out in the system tomatoes, cv. Gamayun – *Meloidogyne incognita* and tomatoes cv. Red Cherry-Bulgaria – *Meloidogyne arenaria*. Water colloidal solution of Se - nanoparticles has been used in the experiments. The aqueous colloidal solution of Se was obtained by laser ablation (Niconov et al., 2009). The size of Se-nanoparticle solution was between 10 and 70 nanometers (nm). Before planting the seeds of Gamayun were soaked in Se solution for 2h and for 2h and 3h for the seeds of Red Cherry. The Se concentration of the aqueous solution was measured by ULTIMA-2 spectrometer (Horiba Jobin-Yvon, France). During the vegetation the leaves of the plants (Gamayun) were sprayed with Se-nanoparticles solution (2 ml per plant). Tomatoes Red Cherry developed with no treatment of the leaves. The seeds of the control plants were soaked in water. On the bases of our previous investigations we selected the optimum selenium concentration equal to 0.34 and 0.68 mg/ml. Three weeks after germination the plants were infected with root-knot nematodes (Gamayun - *M. inncognita* – 3000 second stage juveniles (J<sub>2</sub>) per plant and Red Cherry - *M. arenaria* – 2000 J<sub>2</sub> per plant). Plant samples for analyses were collected every 14 days after infection and treatment with selenium nanoparticles.

Experiments were performed in ten replicates. The final results were analyzed 40 days after infection. The main criteria of the plant resistance were the number of root galls per 1 cm, as well as the morphological and physiological parameters of the nematodes (the status of the nematode population – number of mature females, their size and fecundity).

The biochemical marker of induction resistance (IR) was the proteinase inhibition (PIs). The activity of PIs was

et al. (1961), Erlanger - evaluated by the suppression of amidase activity of trypsin according to the method of (Erlanger et al., 1961, after Udalova, 2018). The activity of PIs is associated with changes in expression of PR6 gene which encodes PIs in tomato tissues. The product of this gene is pathogen induced protein (PP) of the plant protection system.

(PR6),

M4TORNADO ( ) S2Pico Fox - The analyses of chemical elements were made by means of M4TORNADO (quality) and S2Pico Fox (quantity). The producer of the two implements is Germany.

( ) (cv. Analyses of elements was carried out in the system Gamayun – *M. incognita*, treated with selenium at concentration of 0.5 and 1.0 µg/ml.

Gamayun) – *M. incognita* 0.5 1.0 µg/ml.

## RESULTS

### *Morphological and physiological characteristics*

On the base of the results obtained the stimulation effect on the treated plants with selenium nanoparticle solution was proved both for two experiments (Gamayun - *M. incognita* and (Red Cherry – *Meloidogyne arenaria*, Table 1). The length and weight of the treated plants significantly differed compared with the control plants (Table 1).

( cv. Gamayun – *M. incognita* -

( cv. Red Cherry – *Meloidogyne arenaria*, 1).

( 1).

**Table 1. (cv. Gamayun cv. Red Cherry) *M. incognita***

**and *M. arenaria***

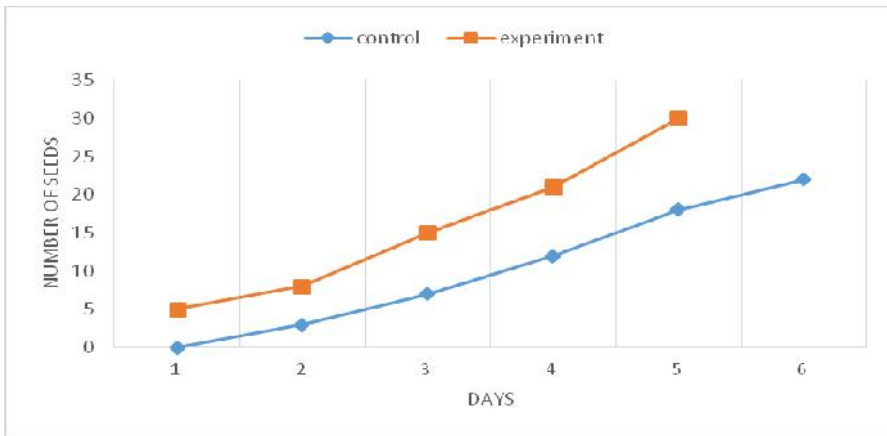
**Table 1. Effect of treatment with Se-nanoparticles on tomato plant development (cv. Gamayun cv. Red Cherry) and morphophysiological characteristics of the root-knot nematodes *M. incognita* and *M. arenaria***

Treatment of tomato plants	Number of galls (g/root)	Stem weight (g)	Stem length (cm)	Size of females (mm <sup>2</sup> )	Number of eggs in ootheca
<i>Gamayun - M. incognita</i>					
Se (0.34 g/ml)	246 *(80%)	22.7* (174%)	57.7* (120%)	0.34 (97%)	111* (60%)
Se (0.68 g/ml)	224 *(73%)	23.5* (179%)	68.4* (161%)	0.30* (86%)	100* (54%)
Control water	308 (100%)	13.1 (100%)	42.5 (100%)	0.35 (100%)	184 (100%)
LSD p 0.05	58	2,7	1,2	0,03	60
<i>Red Cherry - M. arenaria</i>					
Se (0.34 g/ml)	263 (91%)	17.2 (118%)	42.5 111.8%)	0.37* (120%)	190 (91%)
Se (0.68 g/ml)	230* (80.5%)	20, 1* (133%)	47.3 (123.1)	0.35* (113%)	178* (80%)
Control water	280 (100%)	15, 0 (100%)	38.2 (100%)	0.31 (100%)	210 (100%)
LSD p 0.05	42	4,2	7,6	0,05	28

\*Significant differences from control at p 0.05

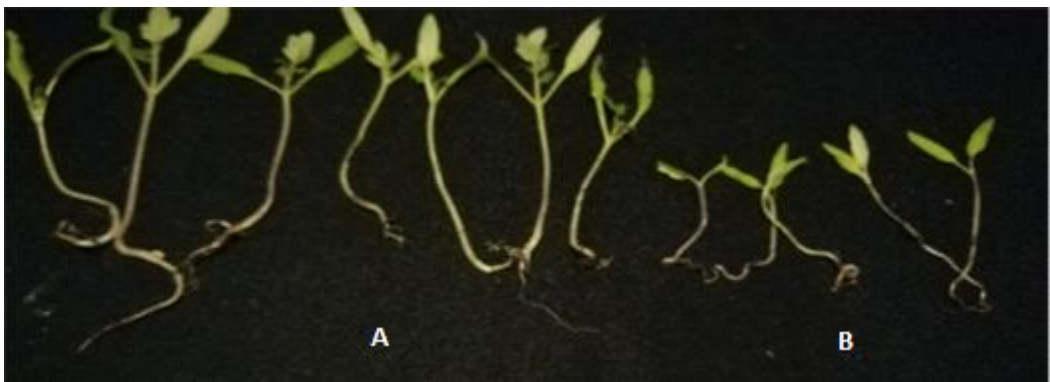
cv. Gamayun – *M. incognita*  
 Red Cherry – *M. arenaria*.  
 ( Red Cherry – *M. arenaria*)  
 3 h  
 ( Gamayun – *M. incognita*).  
 15  
 Red Cherry – *M. arenaria*, 1 2.)

The positive influence on the germination of the seeds was observed. The germination period was shortened (5 days for the system Gamayun – *M. incognita* and Red Cherry – *M. arenaria*. Effect of selenium nanoparticle (Red Cherry – *M. arenaria*, 3h treatment) was like the effect on (Gamayun – *M. incognita*). During the first 15 days after germination irregular development of the length of the plants was registered in experiment (Red Cherry – *M. arenaria*, Figures 1 and 2.)



1. cv. Red cherry (

Fig. 1. Germination of the tomato seeds cv. Red cherry (control-treated with water, experiment-treated with Se)



2. (A) (B – ) (cv. Red cherry)

Fig. 2. Fifteen days tomato plants (cv. Red cherry) from seeds treated with Se-nanoparticles (A) and with water (B)

15

(cv. Gamayun cv. Red Cherry)

( g/ 1).

( 1).

(Ahmad et al., 2016; Wu, 2016)

PIs.

PIs ( 2)

- During the next 15 days leveling of the length of the plants without statistically proved differences was obtained.

- The contemporary analyses showed that the preplant treating of the seeds (Gamayun and Red Cherry) reduced the infestation of the roots. The number of the galls was also reduced compared with the control plants (Table 1).

- Delay in the development of the nematodes was observed. The fecundity of the female nematodes was significantly lower (the number of eggs in ootheca, Table 1).

**Biochemical characteristics**

- It was previously shown (Wu et al., 2016, Ahmad, et al., 2016) that reduction in the fecundity of the nematodes and their delayed development are associated with expression of PIs. Our investigation showed significant increase in the activity of PIs in the tissues of the treated with selenium plants (Table 2).

cv. Gamayun

*Meloidogyne*

*incognita*

**Table 2. Effect of Se-nanoparticles on the activity of proteinase inhibitors in the tissues of tomato plants cv. Gamayun infected with *Meloidogyne incognita***

Treatments of plants	Juveniles second stage (J <sub>2</sub> )		Roots	
	Healthy plants	Infected plants	Healthy plants	Infected plants
Control	2.07	0.8	0.7	0.88
Se (0.68 µg/ml)	2.12	3.0*	1.2	1.92*
LSD p 0.05	1.13	1.8	1.06	0.87

\*Significant differences at p 0.05

et al., 1961 / Udalova, 2018/ (Erlanger

PIs PR6

(PIs) e PR6

- The biochemical markers of IR are proteinase inhibition (PIs). The activity of PIs was evaluated according to the method of (Erlanger, et al., 1961, after Udalova, 2018) Increase of PIs activity is closely connected with PR6 gene expression in the roots and leaves. The increase in the activity of PIs is due to an increased expression of PR6 gene and indicates the inducing properties of selenium nanoparticles.

3.  
(S, Mg, K, Ca,  
Fe, Zn)

(Gorbanov, 2010).

( 3).

*Meloidogyne*.

### Mineral content of the plants

The results obtained on the base of analyses of the mineral content of the plants are given in Table 3.

Selenium was not found in both of controls (Control – invaded and nontreated and Control – uninvaded and untreated, Table. 3).

Addition of Se changes the quantity in different way of the other elements investigated (S, Mg, K, Ca, Fe, Zn). These changes are into the framework for the normal development of the plants under *Meloidogyne* invasion.

3. (µg/ml)  
**Table 3. The mineral content of the elements in the experimental plants (µg/ml)**

	Se	P	S	K	Ca	Fe	Zn	Mg
<b>Variants</b>								
<b>Control – non invaded plants</b>								
Leaves	0	1.603±0.125	0.821±0.509	990±164.6	2456±72	454±43	52±3.27	0.019±0.0075
Stems	0	1.143±0.0737	0.54±0.055	2491±447	1629±42.5	589±33	63±5.26	0.03±0.002
Roots	0	2.25±0.156	0.8±0.303	2310±179	2390±84	669±37	81±5.43	0.038±0.012
<b>Control – invaded plants</b>								
Leaves	0	1.463±0.529	0.743±0.390	948±347	1041±206	348±39	34±5.63	0.019±0.0026
Stems	0	0.589±0.337	0.205±0.019	3169±215	878±259	484±14	47±6.79	0.033±0.0042
Roots	0	1.2±0.327	1.8±0.235	3230±534	1010±112	595±35	67±8.22	0.0404±0.0041
<b>Se - 0.5 µg/ml – non invaded plants</b>								
Leaves	2.87±0.112	1.03±0.094	0.752±0.267	2940±142	4277±216	457±11	55±5.4	0.0133±0.0021
Stems	1.84±0.222	1.5±0.913	0.517±0.098	3669±304	3778±668	532±13	58±6.7	0.043±0.0095
Roots	4.48±0.098	0.5±0.067	0.1±0.087	470±202	640±87	801±34	69±4.5	0.009±0.0013
<b>Se - 0.5 µg/ml – invaded plants</b>								
Leaves	2.05±0.066	1.56±0.89	1.19±1.020	1093±63.3	2947±1878	419±16	47±2.74	0.0363±0.0258
Stems	1.86±0.065	2.18±0.72	0.33±0.057	3185±285	2836±1355	525±5.5	52±2.62	0.0313±0.004
Roots	3.46±0.110	2.8±0.54	1.8±0.230	2820±180	3230±1212	690±21	68±5.31	0.061±0.012
<b>Se - 1.0 µg/ml – non invaded plants</b>								
Leaves	3.36±0.145	2.31±0.236	0.981±0.723	1215±203	3080±1349	508±28.5	50±5.10	0.038±0.0101
Stems	2.42±0.25	1.03±0.174	0.808±0.145	2977±111	2985±1080	508±14.5	56±3.68	0.047±0.0010
Roots	7.38±0.12	1.5±0.078	1±0.345	1360±245	2020±1112	742±25	61±7.8	0.045±0.0098
<b>Se - 1.0 µg/ml –invaded plants</b>								
Leaves	2.98±0.102	1.813±0.031	0.396±0.014	1845±210	2169±1029	394±6.5	47±2.88	0.019±0.0015
Stems	2.21±0.113	1.546±0.173	0.381±0.031	3750±332	2582±317	474±20	58±2.33	0.05±0.002
Roots	6.24±0.265	0.5±0.085	1±0.078	1740±450	4870±1032	721±34	74±4.23	0.015±0.002

N, P S - Calcium in combination with N, P, and S participate in composition of the proteins.



Fe

- Magnesium is a basic element of the chlorophyll molecule and has main role in the photosynthesis. Quantity of Iron in the plants is very low, but this element takes part in the plant nutrition and in innate resistance of the plants to infectious factors.

- Zinc is one of the elements which participates in many important processes in the plants – photosynthesis, chlorophyll syntheses, proteins, respiration, nitrogen and carbohydrate metabolism.

Phosphorous is a basic nutritive element for plants and participates in the processes of photosynthesis, breathing and cell division.

(Gorbanov, 2010).

- The treatment with Se-nanoparticles at the given concentrations increases the plant immunity and inhibits the normal development of the parasites (Tables 1, 2 and 3).

## DISCUSSION

- Addition of Se changes the quantity of other elements investigated. Treatment with Se in concentration investigated leads to increase of its amount in the plants (underground and above ground organs). Changes in quantity of other elements investigated at the experimental concentrations of Se influences in a positive way the development of the infected host and restricts the normal development of the nematodes. It was proved the role of Se in the processes connected with innate plant immunity. On the base of the results obtained of morphological and biochemical characteristics and the changes of the mineral content under Se influence, the aqueous colloidal solutions of Se-nanoparticles, can be regarded as a new abiogenic inducer of tomato resistance to infectious factors.

*Meloidogyne*

PIs

- The results obtained suggest that the content of PIs very probably may be one of the causes for the depression of the life

*Meloidogyne*.

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PIs  
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- activity of the nematodes. Addition of Se inhibits the synthesis of the proteinases of *Meloidogyne*. They are involved in different processes connected with the development of nematodes (nutrition, reproduction, and embryo genesis). Increase of the activity of PIs not only in the roots (site of localization of the nematodes) but also in the leaves indicates the systemic action of selenium nanoparticles. Changes in quantity of the elements are into the framework of the mineral balance for the normal plant metabolism.

- Treatment with Se-nanoparticles increases the plant immunity and the possibility of the plants to develop even under given infection.

## CONCLUSIONS

- Using of some microelements as abiotic elicitors to *Meloidogyne* invasion gives an opportunity to work-out new and ecological well-founded methods for control to infectious factors.

*Meloidogyne*

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- G. E. Folmanis (  
)

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## Impact of Wildlife Repellents Used on Corn and Potatoes Grown near Forests

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*Original scientific paper*

### SUMMARY

The everyday activities of useful game cause significant damage to the environment they inhabit.

- Especially relevant to agriculture is the damage caused by useful game on orchards, vegetable patches and vineyards, as well as corn, potato and cereal fields.

- The damage caused by useful game to forests and crops bordering woodland is currently a common problem for forest managers and farmers. They are found in all forest ranges, agricultural holdings and hunting reserves where game live.

- The most damage on agriculture is inflicted by the everyday activities of wild boars, rabbits and, to a lesser extent, deer and roes and other forest animals such as badgers and birds such as jackdaws, common wood pigeons and jays.

- This study looks at the use of the Porocol game repellent on corn and potatoes

- grown in places close to forests in two consecutive years. The results are optimistic, especially in the second year, after the first-year experiments were analysed and optimised. It has been shown that the repellent has a pungent, lasting odour, is not affected by weather, does not harm humans and other farm animals, and is easy to apply.

- Based on the results obtained in the two-year study and its conclusions, it is recommended to use the Porocol repellent in the organisation and implementation of crop protection measures, specifically in the production of corn and potatoes in Bulgaria's semi-mountainous and mountainous areas.

**Key words:** repellents, Porocol, wildlife, use in corn and potatoes

## INTRODUCTION

- Wild animals and their environment are interconnected. The environment provides shelter for the game and is a source of the food it needs. On the other hand, wild animals, by consuming that food, affect the environment and, more often than not, cause significant damage to crops and forest vegetation. Where forests have large numbers of wild animals, the cost of growing crops on adjacent land increases by 200-400%. Also, growing some tree species becomes unfeasible in certain places due to the constant damage inflicted by wild animals. Damage to forest ranges caused by red deer, fallow deer, roe deer and rabbits is especially significant.

- The issue of damage to forest vegetation by game has existed ever since humans began caring for forests to ensure their sustainable use, resulting in the creation of the concept of forest management.

- The causes of the damage are still unclear. However, a link has been

200-400%,

established between the change from natural forests composed of numerous species to coniferous monocultures and the increase in damage.

This change is believed to be a significant reason for the occurrence of damage, because changes in the composition and structure of forests in Northern Europe have reduced their grass and bush layer, which supplies most of the wild animals' food, forcing them to explore nearby farms.

The damage is gradually increasing as human activities expand throughout the forests and as a result of the desire to increase the number of wild animals. Wild animal damage to forest trees and crops was almost non-existent in the recent past.

This can be explained by the insignificant number of wild animals, on the one hand, and the poor exploitation of forests, on the other. Also important is the fact that no large-scale silvicultural activities have been carried out in our forests, especially in bare areas, with no restorations carried out either.

Despite the importance it has to forestry and farmers, the issue of damage is still unresolved. In addition, damage is now appearing in places where it previously did not occur. Moreover, the damage is increasing in all areas with intensively managed hunting reserves and forest ranges and nearby agricultural land in Bulgaria.

In order to obtain high yields from agricultural crops in places near large forests, it is essential to properly organise and conduct hunting, agricultural and plant protection activities.

Papers on the issue of damage and mitigating measures view the main task as the preservation of useful wild animals as forest dwellers and game.

- At the same time, effective measures are being developed to limit damage to forests and agricultural land to a tolerable minimum.

A solution to these issues is mainly sought in the following areas:

- Control the density of useful wild animal stock in order to create the right ratio between the number of animals and their natural food.
- Improve the food growing in the forest by measuring plant composition and form.
- Provide suitable (evidence-based) supplemental nutrition during the winter. In this regard, provide game with suitable mineral supplements.
- Ensure regular feeding to achieve so-called "chain feeding".
- Develop and test in practice effective protective mechanical, biotechnical and chemical means against damage.

#### **Damage caused by different types of wild animals**

*Damage by red deer.* Almost all species of game fauna are harmful to the forest, but the damage caused by red deer is especially significant.

In our conditions, there is a close link between the development of the stock of this species and the occurrence of damage. It was found in abundance in the mountains, foothills and plains. There is substantial written evidence of its former distribution in Bulgaria (Bubenov, 1959; Botev, 1967; Baychev, 1968; Stenin, 1969).

The damage observed includes bites on coniferous and deciduous saplings, bark peeling (Bubenik, 1960) and sapling plucking (Ruskov, 1963).

*Roe deer damage.* The roe deer is the most numerous member of big game in Bulgaria. Over the last few years, its

(Bubenov, 1959; Botev, 1967; Baichev, 1968; Stenin, 1969).

(Bubenik, A. 1960)  
(Ruskov and Petrov, 1963).

(Dragoev, 1964;  
 Botev, 1968).  
 1000 - 1200 m,  
 1500000  
 (Ruskov and Petrov, 1957; Dragoev,  
 1973; Petrov, 1975; Botev et al., 1985;  
 Milanov, 1993, 1995).  
 370 420  
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 0,68  
 1,  
 (Zhelev, 2015).  
 (Ruskov, 1967)  
 (Ruskov, 1957).

importance to hunting has grown in parallel with the expansion of its range to hunting reserves of small game.

- 
- Roe deer damage was unknown until recently. Damage by roe deer and red deer appears to result from an increase in their stock and intensified logging (Dragoev, 1964, Botev, 1968).

- The damage found in forests is similar to that by red deer, but in line with roe deer anatomy. It includes sapling biting, plucking and trampling, along with tree bark husking and peeling.

*Rabbit damage.* Rabbits are and will be the main wild animal species of our hunting reserves, because they find favourable living conditions in almost all places at an altitude of about 1,000-1,200 m, have good reproduction and relatively easily adapt to constantly changing environmental conditions.

- Based on the existing rating table and habitat rating instructions, the total normal rabbit stock in Bulgaria is 1,500,000 (Ruskov and Petrov, 1957; Dragoev, 1973; Petrov, 1975; Botev et al., 1985; Milanov, 1993, 1995). Official estimates indicate stocks in the range of 370 to 420 thousand individuals for the last 20 years.

- The population is under threat, with a growth of 0.68 (the normal value is around 1), which shows that maintaining stocks would be difficult (Zhelev, 2015). The damage observed includes sapling biting, and observations show that rabbits do not always eat the shoots they have bitten off (Ruskov, 1967) and the tree bark they have peeled off (Ruskov, 1957).

- *Wild boar damage.* The wild boar takes an important place among the members of big game. It brings valuable trophies and delicious meat, and hunting it requires courage. All this makes it an increasingly desirable target of hunters.



<p>20</p> <p>1984</p> <p>33500</p>	<p>About 20 years ago, the wild boar had a limited distribution across Bulgaria. It was only found in a few isolated places in Rila, Pirin, Ludogorie, Dobrudzha, Strandzha, the Rhodopes, etc. As a result of the protective measures taken by the authorities, as well as the resettlement of animals bred in purpose-built farms in the hunting reserves Sherba, Voden and Byala in the Ruse province, the stock of this species increased rapidly. According to official data, in 1984 there were about 33,500 wild boars in Bulgaria, with a trend for the numbers to increase.</p>
<p>(State Gazette (SG), 1974).</p>	<p>Along with the increase in wild boar stocks, damage started to appear. It is found wherever wild boars live and has great economic significance to agriculture.</p> <p>Data and scientific descriptions of wild boar damage to crops are currently limited or unavailable.</p> <p>The amount of damage can be assessed on the basis of reports by agricultural departments and private farmers, as prepared in accordance with the Law on protecting agricultural property from game (State Gazette (SG), 1974).</p> <p>Factors relevant to the amount of damage. Many factors affect the amount of damage useful game cause to the forest. They are important in the implementation of damage mitigation measures.</p>
<p>100 ha</p> <p>100 ha</p>	<p>The first factor in the study of hunting reserves and associated practices is game density. The term used most frequently is average density. It represents the average number of a species of game according to the summer stock per 100 ha of hunting area.</p> <p>The terms biotically acceptable density and economically acceptable density are also known. Biotically acceptable density means the number of a species of game per 100 ha that the game stock can reach</p>

(Wagenknecht, 1965).

(Koller, 1962).

Dragoev (1964)  
, Kolev (1966)  
Baichev (1968)

: 1) ; 2)

; 3)

(Petrov, 1964).

( )

” ”

( )

without causing any damage to its physical development. For the density to be economically acceptable, the damage game causes can be limited by available means (Wagenknecht, 1965).

The second factor – the insufficient amount of quality natural food in today's intensively managed forests – is one of the main reasons for damage caused by biting and peeling (Koller, 1962).

In Bulgaria, studies of the natural food supply in the forest were conducted by Dragoev (1964) for Ludogorie, Kolev (1966) for the Chepino part of the Rhodopes and Baichev (1968) for the region of Northeastern Bulgaria.

The measures to protect valuable forest tree vegetation from game damage are grouped as follows: 1) chemical means of damage mitigation; 2) mechanical means of damage mitigation; 3) biotechnical means.

There is a successful practice, especially in some Western European countries, to protect individual saplings of coniferous, deciduous and fruit trees using a perforated impregnated cardboard cover, which is placed on the top shoot of fruit trees and saplings needing protection (Petrov, 1964).

Polyethylene “socks” known by various names are used abroad (Austria) to protect the top shoots from being bitten.

These polyethylene cylinders are placed on the top shoot, with their lower end fastened with rubber rings.

They are designed to prevent coniferous saplings from biting. Beagles (dogs) tied to long ropes are used to drive game away from the green areas.

Elsewhere, lanterns are lit and suspended from trees next to the crops. The success of these methods is always short-lived

(Botev, 1968).

(Botev, 1968).

The most effective way to protect crops grown in small areas from game damage is to use solid fences. The fence is designed to prevent animals from entering the fenced areas. Although expensive, fences, as a means of protection from game damage, are widely used in all countries with intensive hunting and forestry. They should be used more widely in Bulgaria too (Petrov et al., 1968).

(Petrov et al., 1968).

In addition to the mechanical means of preventing damage caused by game in the forest set out above, so-called biotechnical or forest protection measures also exist. Although relatively new, these methods are cheap and sufficiently effective, so the fight to mitigate the damage caused by game has recently focused on them.

These measures to protect crops from game damage are numerous and varied.

From a biological point of view, it is important to improve the natural food supply for game in the forest to ensure it has the food it needs throughout the year.

Chemicals used to combat game damage are an important part of caring for forest and agricultural crops and supporting plant stock regeneration.

Special odorous substances are used to protect forest and agricultural vegetation from game damage.

At first, rags were soaked in them and placed near endangered areas.

Later, the composition of the mixtures was improved, enabling their application on plants.

In practice, these repellents are used as hand-made mixtures or in the form of

(Plant Protection Act), 1997).

, various manufactured agents known as  
- repellents (Plant Protection Act, 1997).

, Unlike agents used to control  
- insects and other pests, chemicals used  
- to control game damage are not intended  
- to poison the game, but only to repel it  
with their unpleasant odour or taste.

. In order to fulfil their purpose, the  
, chemicals used in practice must meet the  
- following important conditions:

- (a) they must have a pungent, lasting  
- odour that drives game away from  
- protected tree species and crops;

, b) they must not to be washed away by  
- precipitation, so they can serve their  
- purpose for a long time (5-7 months);

- (c) they must not to freeze at low  
- temperatures, because if they do, they will  
lose their properties and have no effect on  
game; d) they must be harmless to the  
people who handle them;

(e) they must not have a detrimental effect  
on saplings and crops; (g) they must not  
be flammable, because they would  
- otherwise increase the flammability of  
- saplings and crops and, in turn, increase  
; ) the risk of fires; h) their use should be  
economically justified – they must not be  
expensive and must be easy to handle.

- Chemicals used to mitigate game  
- damage can be made both at home or in  
chemical plants.

- Depending on the damage they are  
intended to prevent, they are anti-bite or  
- anti-peel agents.

: ) All known agents are mainly based on: a)  
- tar substances – hard and brown coal tar,  
- anthracite oil, wood tar of coniferous and  
- deciduous trees, carbolineum, asphalt, etc.;

- b) vegetable and animal oils and fats –  
palm oil, linseed oil, fish oil, bone oil, etc.;

<p>);</p>	<p>c) artificial resins – rosin; d) residues and waste substances from the preparation of Vaseline, paraffin, mineral oil, wax, etc.;</p>
<p>);</p> <p>(Wagenknecht, 1965).</p>	<p>e) waste substances from the processing of insecticides, paper, cellulose; f) organic and inorganic substances of various origin – blood, hair, sand, etc. (Wagenknecht, 1965).</p>
<p>;</p>	<p>In other countries, these raw materials are used to prepare various agents: - Tar agents. In raw form, they are intended mainly for spraying coniferous saplings in the period of vegetative dormancy. The most important of these are:</p>
<p>: Reuston Orcus ( ), Baunetcer Rutach ( ), Silvacol Hurbason ( ).</p>	<p>are: Reuston and Orcus (Czech Republic), Baunetcer and Rutach (Germany), and Silvacol and Hurbason (Austria). In addition, tar and anthracite oil are often used in a mixture with other fine tar sprays, such as Fursol (Germany) and Proherba (Austria).</p>
<p>( ) Proherba ( ).</p>	<p>- Fat agents. They are also designed to prevent coniferous and deciduous saplings from being bitten in winter. Wilderbisalbe, Jager, Coniferol, etc. are foreign examples of such agents.</p>
<p>Wilderbisalbe, Jager, Coniferol</p>	<p>- Resin-based agents.</p>
<p>;</p> <p>RZ ( ), „Heldebrand” ( ), Wipox Pinostris ( ).</p>	<p>Due to some of their properties – difficult to dilute with water, heavy vapours, etc. – they are applied in the period of vegetative dormancy and only by spraying. These include RZ (Czech Republic), Heldebrand agents (Germany) and Wipox and Pinostris (Austria). - More complex agents. This group includes emulsions, suspensions and fine powders. They are all made of substances that, due to their unsuitable physicochemical properties, cannot be used in raw form.</p>
<p>);</p> <p>Monacol ( );</p> <p>Spange V SRC ( );</p> <p>Carnofer ( ), Stahler ( ), Dendrocol, Forstan Marsuvin ( ),</p>	<p>Recent additions to the group of emulsions include Carnofer (Czech Republic), Stahler agents (Germany), Dendrocol, Forstan and Monacol (Austria), etc. Marsuvin (Czech Republic), Spange V and SRC (Germany), etc. are examples of suspensions. Substances such as kaolin, clay, lime, chalk are also</p>

Arboral, Arcotal (Austria), Apulin, Carnofer, Morsuvin (Czech Republic), Fekama W - 30M Fekama W-40M (Germany).

added to suspensions, which, in addition to improving their protective properties, help most of them adhere and dry.

In addition, they serve as emulsifiers of fatty and tar impurities. Fine powders are the most common type of winter bite repellents.

Being less phytotoxic, agents from the groups of suspensions and fine powders are most widely used in practice. In Bulgaria, hand-made mixtures of fish oil, animal blood, liquid soap, hexachlorane, creosote, formalin, caterpillar glue, phenol 40%, mercaptan, shoe polish, fusel oil and water in various combinations have been successfully tested against rabbit damage.

The use of chemicals is not always effective enough. In some places, repellents produce very good results, which is why their use is recommended. Arboral, Arcotal (Austria), Apulin, Carnofer, Morsuvin (Czech Republic), Fekama W-30M and Fekama W-40M (Germany) have been tested in Bulgaria.

These repellents have been very effective in protecting saplings from game. Saplings are smeared with various agents using flat brushes.

It is convenient and quick to use shoe shine brushes adapted by nailing 50-cm handles to them. It was found that the repellents' protective properties are not the same when used in different areas. This should not only be explained by whether the wild animals were hungry or not, but also by their individual qualities – habits, health, etc. – because their behaviour does not only consist of simple and consistent reactions to environmental requirements, but also of reactions provoked by a certain situation caused by environmental conditions.

The effect of the various agents against game damage also depends on the nature

50 cm.

2014). (Lecheva, Stoyanov, (Helianthus tuberosus L.). Ruskov (1957) (1997.) (01.10.1974., 05.07.1999 .) .19 8 2011 ).

of the habitat and on whether the game is provided with other sufficient forage areas in exchange for those taken away from it.

Several types of repellents based on different chemically active substances and proteins have been tested in Bulgaria (Lecheva, Stoyanov, 2014). The tested agents have been shown to be relatively good at protecting agricultural crops intended for game fields in TRICO CERTOSAN, REPELLOVIT and POROCOL in earth apple (*Helianthus tuberosus L.*).

Information and previous papers describing agricultural damage by forest animals are limited. Only Ruskov 1957 has studied and described the damage caused by rabbits on fruit crops.

Problems with crops and game damage have not even been fully addressed at the legislative level. At present, the Plant Protection Act (published in the SG, issue 91 of 10 October 1997) does not address this problem.

Historically, those problems have only been solved in the Law on protecting agricultural property (in force since 01/10/1974, with only the name changed in 05/07/1999), with any identical case of damage to agricultural crops only covered by the current Forest Act (published in the SG, issue 19 of 8 March 2011).

This study of the impact of the Porocol repellent on wild animals is an opportunity to expand the knowledge of how to counter the damage they cause to corn and potatoes grown near forests by comparing and proving the losses of agricultural production in two consecutive years in two areas of Bulgaria.

## MATERIAL AND METHODS

2018	2017	16 da	<ul style="list-style-type: none"> <li>- Experiments were conducted in the following two areas of Bulgaria: near Vasilovtsi Village, experiments were conducted in 2017 and 2018 to study game damage in a 1,66 ha cornfield; near Batak Town, an experiment was conducted in 2018 in a 0,24 ha potato field.</li> </ul>
2,4 da	2018	10-15	<ul style="list-style-type: none"> <li>- The sowing areas were tilled in the standard manner, including ploughing to 10-15 cm and milling after the area was cleared from the preceding crops.</li> </ul>
(NPK 2020)	( )		<ul style="list-style-type: none"> <li>- The purpose of the ploughing was to maintain soil moisture and hinder weed growth.</li> <li>- The soil was also fertilised, which is an important part of the cultivation of corn (NPK 2020) and potatoes (urea).</li> </ul>
Agriscience.	( )	8000	<ul style="list-style-type: none"> <li>- In our case, the soil surface was not profiled in advance. No herbicides were applied in the soil treatment. <i>Experimental crop seeds.</i></li> <li>- The corn was kindly provided by Pioneer Seeds®, Sofia, part of the Corteva Agriscience. Company. The seeds (FAO 360) were sown in May in both years of observation according to standard sowing rates of about 80,000 per ha, with the rows 70 cm apart and the seeds in each row about 17-20 cm apart.</li> </ul>
4500-5000 225-250 kg/da 70 m 28-31 cm.	17-20 m.	70	<ul style="list-style-type: none"> <li>- The potatoes, which were of the Picasso variety, were provided by a potato producer from Samokov. The tubers were planted in early May of the two-year observation period according to standard sowing rates of 45,000-50,000 tubers per ha or 2250-2500 kg/ha, with the rows 70 cm apart and the seeds in each row about 28-31 cm apart.</li> </ul>
(BBCH 12-16)		1 da	<ul style="list-style-type: none"> <li>- In the early growth stage (from the unfolding of the second leaf to the unfolding of the sixth leaf of the crop (BBCH 12-16), the areas were treated</li> </ul>



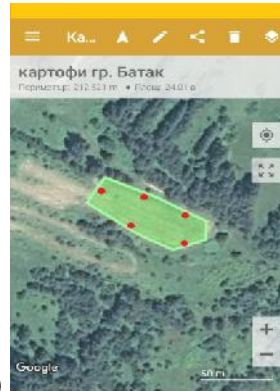
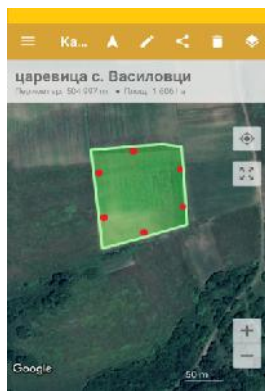


repellent installation. The POROKOL repellent was installed in accordance with the manufacturer's requirements.

All green columns were placed at the ends of the agricultural fields in late June, in the immediate vicinity of the forest.

The columns were arranged as shown in Fig. 1 a) and b). For the potatoes, which we only monitored in 2018, we placed the columns 55 metres apart (Figure 1b).

55 ( 1 ).



1. ● -

2018 (0,24ha) ● -

55 m

60 m

2017 ( 1 , 6 h a ) ;  
90 cm; b)

Fig. 1. Scheme of available columns ) in maize in 2017 (1,6ha); ● - columns POROKOL with a distance of 60 m from each other and a height of 90 cm; b) for potatoes in 2018 (0,24ha). ● - columns POROKOL with distance from each other 55m

*Morphological diagnostics.* The movements and composition of game species were monitored remotely using information received from the cameras installed in the forest, but near the agricultural fields. We programmed the cameras to monitor and film the animals in the evening (from 18:00 to 08:00) due to the frequent raids by wild animals in those hours.

The composition of species was determined on the basis of photos taken by the six cameras. The data were collected weekly. We identified the game by comparing them with references and

( 18:00 08:00),

other sources.

The sown agricultural areas were inspected every week after the first wild animal damage was established by route inspection. The purpose of these observations was to use specific techniques and methods to obtain as accurate information as possible to assess the extent of animal damage.

The damages inflicted were calculated as a percentage once, at the end of vegetation, just before the harvest. As a baseline for comparison, we used standard sowing rates for corn and potatoes per decare. The results of the damage inspections were averaged.

## RESULTS

The composition of game species visiting the cornfield near Vasilovtsi, Dragoman, were analysed in the first year of this study. Based on the 3,128 photos obtained from the cameras, we determined the species and monitored the dynamics of game appearing near the agricultural area. The observations started in the second half of June 2017 and lasted until October. The most common game was wild boar, badger, roe deer, common wood pigeons and other birds and rabbits, shown as the first attack on corn in Table 1 and Figure 2.

3128

2017

1

2.

1.

**Table 1. Species composition and dynamics of visits to cornfield during the first year**

	Wild animals	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Dynamics of attendance	Percentage of total (%)
		Camera	Camera	Camera		
1.	<b>/ Wild boar (<i>Sus scrofa</i>)</b>	81	82	49	212	<b>66,3</b>
2.	<b>/ Badger (<i>Meles meles</i>)</b>	20	14	9	43	<b>13,5</b>
3.	<b>/ Roe Deer (<i>Capreolus capreolus</i>)</b>	5	-	17	22	<b>6,8</b>
4.	<b>/ Common wood pigeon (<i>Columba palumbus</i>)</b>	-	5	30	35	<b>10,9</b>
5.	<b>/ wild rabbit (<i>Lepus europaeus</i>)</b>	2	1	5	8	<b>2,5</b>
Results of the total number of visits to the feeders near the cornfield:					320	100

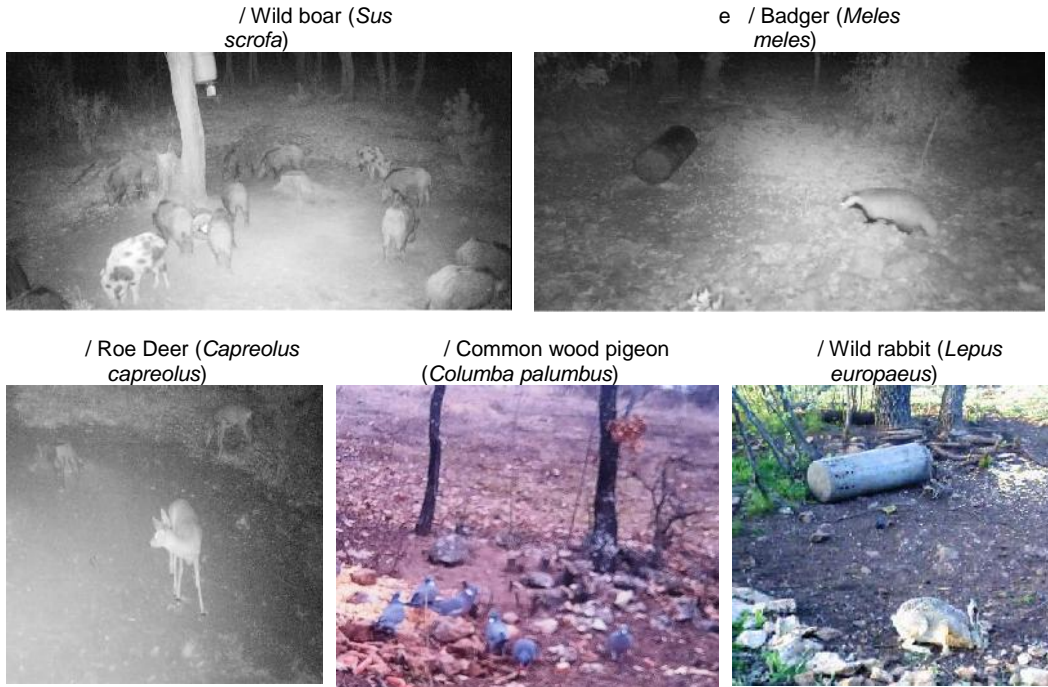


Fig. 2. The most common wild animals photographed on photo traps in 2017

2017. - Description of damage caused by game to corn in 2017. The first raids on game in the cornfield were discovered in early August 2017 or about 80 days after sowing. We had not detected digging out of sown corn seeds or gnawing of leaves.

2017 . 80

(BBCH 61-68) :

da 16

( 2000 ),

( 3).

5

**First symptoms:** In the phenophase of the tuber where the panicle flowers (BBCH 61-68), we observed plants that had fallen due to gnawing at the base or had been completely uprooted. Out of the total 16 decaire area under observation, we found damage in three places (approx. 2,000 plants) close to each other. The damage was limited to plant felling and gnawing of stems, leaves and developing cobs (Figure 3). We found that a column of repellent had been knocked down by high winds. The total number of columns at the end of August was 5.



3. 2017  
**Fig. 3. Fallen and gnawed corn plants in August 2017**

:  
 2700 m<sup>2</sup> ( 21600  
 ).  
 .  
 ( 71–79).  
 ( ).  
 .  
 ).  
 ,  
 ,  
 ,  
 .

**Second symptoms:** During the inspection carried out two weeks after the first raids, we found a huge number of plants had been felled and trampled in two patches of a total area of 2700 m<sup>2</sup> (approx. 21,600 plants). Most of the fallen plants had been gnawed, with the cobs eaten. The phenophase was determined as fruit development (BBCH 71-79).

We compared this increased presence of game with the data from the photos sent by the cameras and noticed a large herd (sounder) of wild boar. In all likelihood, this was a two-time visit by sows with piglets. The increased presence spurred us to quickly check the columns of Porocol repellent.

We saw that two columns near the forest were missing (probably stolen). We found that the animal tracks came and went from the cornfield exactly at the locations of the missing columns of Porocol repellent. The inspections carried out in September showed on-going wildlife raids on the corn, with the damage increasing progressively.

The patches had already merged, with the middle of the field completely trampled and no healthy plants left. The area of

8000 m<sup>2</sup>.  
 1300 m<sup>2</sup>  
 76%,  
 ( 4).

damage caused just in September was measured at 8,000 m<sup>2</sup>. Following outings for small wildfowl during hunting season, colleagues from the Vasilovtsi Hunting Club reported a large presence of wild animals and common wood pigeons in the corn. In late September, we reported new patches of damaged plants with a total area of around 1,300 m<sup>2</sup> or losses of approx. 76%, calculated on the basis of surviving plants (Figure 4).



4.  
**Fig. 4. Damage in the middle of the cornfield**

1000 m<sup>2</sup>  
 14,8 da  
 88%,

The inspections carried out in October showed an increase in damage by another 1,000 m<sup>2</sup>, with the total areas of damage of about 14.8 decare or 88%, as a result of which we decided not to harvest the area and leave it as a GAME FIELD.

**Results obtained in the second year of monitoring**

In the second year of this study, we also examined the impact of the Porocol repellent on game raids on potato fields near Batak, where the composition of game species was analysed.

We continued observing and analysing the dynamics of the visits to the cornfields near Vasilovtsi, Dragoman, taking into account the results of the previous year.

4014

3).

2.

The number of repellent columns installed there was at the upper limit recommended by the manufacturer, with another column added in excess of those recommendations. Based on the 4,014 photos obtained from the cameras, we determined the species and dynamics of the game appearing near the agricultural areas. We began the observations in the second half of May 2018 and ended the monitoring in October (Tables 2 and 3).

The results showed that the most common game found near the corn were the same as in 2017: wild boar, badger, roe deer, common wood pigeons and other birds and rabbits. The dynamics, however, were different, as shown in Table 2.

**Table 2. Species composition and dynamics of visits to cornfield during the second year**

	Wild animals	1 <sup>st</sup> Camera	2 <sup>nd</sup> Camera	3 <sup>rd</sup> Camera	Dynamics of attendance	Percentage of total (%)
1.	/ Wild boar ( <i>Sus scrofa</i> )	103	91	47	241	<b>64,78</b>
2.	/ Badger ( <i>Meles meles</i> )	15	21	11	47	<b>12,63</b>
3.	/ Roe Deer ( <i>Capreolus capreolus</i> )	9	10	11	30	<b>8,06</b>
4.	/ Common wood pigeon ( <i>Columba palumbus</i> )	-	2	38	40	<b>10,75</b>
5.	/ wild rabbit ( <i>Lepus europaeus</i> )	1	1	12	14	<b>3,76</b>
Results of the total number of visits to the feeders near the cornfield:					<b>372</b>	100

3.

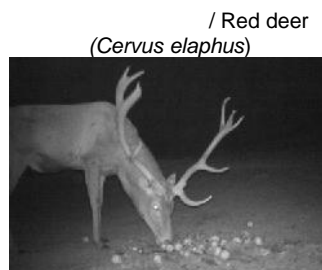
**Table 3. Species composition and dynamics of visits to potatofield**

	Wild animals	4 <sup>th</sup> Camera	5 <sup>th</sup> Camera	6 <sup>th</sup> Camera	Dynamics of attendance	Percentage of total (%)
1.	/ Wild boar ( <i>Sus scrofa</i> )	189	202	121	512	<b>87,37</b>
2.	/ Roe Deer ( <i>Capreolus capreolus</i> )	15	28	23	66	<b>11,26</b>
3.	/ Red deer ( <i>Cervus elaphus</i> )	5	1	2	8	<b>1,37</b>
Results of the total number of visits to the feeders near the potato field:					586	100

The results of the observations of the potato field in 2018 showed that the most common game in the vicinity were wild boar, red deer and roe deer (Table 2,

( 3,

5). Figure 5). The photos captured other animals too, mostly predators, probably tracking the movements of the wild boar as part of their transitional flow.



. 5. -

**Fig. 5. The most common wild animals photographed on photo traps near potato fields**

	Results of damage inflicted on corn by wildlife 2018
<p>( 16-18)</p> <p>6- – 8-</p>	<p>During the first inspection of the corn crop in the 6-8 leaf phase (BBCH 16-18), no damage by game was found.</p> <p>There was no digging out of the sown corn seeds or gnawing on the leaves. The visit was combined with the spraying of herbicide for cereal and deciduous weeds.</p>
<p>8</p>	<p>We inspected and restored all 8 columns of the Porocol repellent, placing an additional one in the middle, in response to the results of the previous year.</p>
<p>50 cm, 90 cm.</p> <p>12</p>	<p>The height of all columns was reduced from 90 cm to 50 cm. The results of 12 inspections carried out from June to mid-September were negative for damage to corn plants by game (Figure 6 A).</p>
<p>( 6 ).</p>	





Fig. 6. Surveys from June to mid-September (A) and at the end (B)

13- 1560  
 190 m<sup>2</sup> ( 6 ).  
 (15 ) -  
 350 m<sup>2</sup> 2900  
 (16 17 )  
 1000 m<sup>2</sup>  
 1,54 da  
 10% ( 7).

We noticed the first felling of isolated plants growing near the ends of the field during the 13th inspection in late September. A total of 1,560 felled and gnawed plants were identified, with cobs damaged in a section of about 190 m<sup>2</sup> (Figure 6 B).

The results of the second visit to cornfield in mid-October (15th inspection) showed new damage from pig raids on an area of about 350 m<sup>2</sup> and about 2,900 damaged plants.

The results of the inspections (16th and 17th) carried out in late October showed an increase in damage by another 1000 m<sup>2</sup>, with the total areas of damages of about 1.54 decares or 10% (Figure 7).



Fig. 7. Last examination of cornfield in the second year – general view

The corn crop was harvested in early November, with an average yield of

500 kg/da.

about 500 kg/decare.

2018

14

4

40 m<sup>2</sup>

5

( 8).

### Results of damage inflicted on potatoes by wildlife 2018

- In the total of 14 inspections of the potato field carried out during the year, one visit from a wild animal was found in September or 4 months after the installation of the Porocol columns.
- The damage covered about 40 m<sup>2</sup> of potatoes, but started from a meadow in the immediate vicinity and consisted of pieces of turf turned upside down in the meadow, as well as dug out and eaten potatoes, which indicated damage by wild boar.
- The results of all other inspections were negative. The crop was harvested in late September, 5 months after the columns of Porocol repellent were installed (Figure 8).



Variety Picasso



8. Fig. 8. Survey of potato field in the Batak region

### DISCUSSION

- 2017
- The results obtained in 2017 give us reason to confirm that, taking into account the damage we identified, the production of corn near forests is an extremely risky endeavour.

- The greatest damage to crops, in particular corn, is inflicted by wild boar, badgers and common wood pigeons.

2018

10%

1%

2017

12,5%

( ) 25%

( )

60 m

45 m).

18,75%

4-6

The use of Porocol is appropriate provided that the specialised repellent is applied in strict compliance with the manufacturer's requirements and in accordance with the weather and anthropogenic factors prevalent in the area.

The symptoms of damage to corn, as an agricultural crop, helped to expand the knowledge of local farmers and demonstrate ways to overcome similar problems encountered in agricultural activities conducted in areas bordering forests.

In 2018, the damage caused to the potatoes and the corn was respectively below 1% and around 10%, which is insignificant and reflects the effect of the Porocol repellent on wild animals. The recommended amount was at the lowest limits per unit area and linear arrangement of the Paracol columns. Taking into account the results of the previous 2017 for the corn, for the two experiments we increased the amount applied to the corn by 12.5% (per area) and to the potatoes by 25% (linear increase – instead of 60 m, we used columns placed 45 m apart).

The higher percentage of columns in both areas (18.75%) produced excellent results. The odour retains its effect in direct sunlight on the columns for a period of 4-6 months, as indicated by the manufacturer.

## CONCLUSIONS

1. (Porocol)
  - 2.
  - 3.
1. The Porocol repellent has an effect on wild animals.
  2. The use of Porocol is appropriate provided that the specialised repellent is applied in strict compliance with the manufacturer's requirements and in accordance with the weather and anthropogenic factors prevalent in the area.
  3. The repellent must be installed in accordance with the dynamics and species of wild animals, i.e. the height of

4.  
18,75%

5.

6. -  
(*Sus scrofa*),  
(*Capreolus capreolus*),  
(*Cervus elaphus*),  
(*Meles meles*), (Lepus euroraesus),  
(*Columba palumbus*)

7.

the columns and wicks has to be adjusted accordingly.

4. It is recommended to increase the density by 18.75% when installing the Porocol repellent because doing so produces very good results in corn and potatoes grown as crops.

5. The results of the study confirm that the damage identified means that growing corn near forests is an extremely risky endeavour.

6. The most common wild animals visiting the crops are wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), badger (*Meles meles*), rabbit (*Lepus euroraesus*), common wood pigeon (*Columba palumbus*) and other birds. This confirms the data generated in previous studies.

7. The symptoms of damage to corn, as an agricultural crop, helped to expand the knowledge of local farmers and demonstrate ways to overcome similar problems encountered in agricultural activities conducted in areas bordering forests.

Given the importance of the problem of game damage to agricultural crops grown near forests, this research should continue to enable the development recommendations for improving the hunting, agricultural and plant protection measures taken until now.

## ACKNOWLEDGEMENTS

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## Microbiological Properties of Soils from Mountainous Areas

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### SUMMARY

- The microbiological indicators of
- soils occupied by grass, deciduous and
- coniferous vegetation from regions in
- Lozenska, Rila and Maleshevska
- mountains were studied. The main groups
- of soil microorganisms, the enzymatic
- activity ( -glucosidase and phosphatase)
- and the total biological activity of the soil
- (by the released carbon dioxide) are
- determined. It was found that in each
- region the combination of specific soil
- characteristics, climatic conditions and
- vegetation type cause differences in
- microbiological properties. In general, in
- the Cinnamon and Brown Forest Soils
- under grass vegetation from the three
- regions a higher amount of actinomycetes
- is observed compared to those under
- coniferous vegetation. The soils of Lozen
- mountain are characterized by a large
- number of bacteria digesting mineral
- nitrogen, fungi and actinomycetes.
- 
- The amount of microorganisms in the
- surface horizon correlates with the bulk
- density and the C/N coefficient of the soil,

C/N

which is most strongly represented in the soils of Rila Mountain. Cinnamon forest soil under grass vegetation from Maleshevska Mountain stands out from the studied soils with the best microbiological indicators, where the highest amounts of heterotrophic and cellulose-decomposing microorganisms, the highest enzyme activity and the highest total biological activity were reported.

**Key words:** mountain soils, soil microorganisms, enzyme activity, general biological activity

## INTRODUCTION

Microorganisms and microbiological processes play a huge role in soil fertility and plant nutrition. The soil provides conditions for the development of the microflora, while it in its turn has a specific influence on it. With the specific physicochemical properties of a given soil, a certain amount and groups of microorganisms develop and a biological equilibrium is established, characteristic of the conditions and the season (Perfanova and Donkova, 2017).

The enzymatic activity of the soil is an indicator of the intensity of the biochemical transformation of organic residues, which releases nutrients available to plants. The enzymes that catalyze the mineralization of the main nutrients are important. The enzymatic activity is influenced by the properties of the soil, the vegetation cover, the way of land use, the interactions of the soil organisms, etc. (Nannipieri et al., 2011).

When the breathing of the micro-organisms was observed decomposition of various organic substances to simpler products and release of energy occurred (Vlahov, 1980). Quantitative characteristics of microorganisms and CO<sub>2</sub> production can be a biological indicator of soil condition (Hristeva, 2016).

(Perfanova et al., 2015 ).

- Cinnamon and Brown Forest soils are widespread in the mountainous regions of our country. Changes in the water, air and nutrient regime of soils under the influence of soil and climatic conditions, relief and vegetation cover significantly affect the microflora – changes the amount of individual groups of microorganisms, the dynamics and intensity of microbiological processes. (Perfanova et al., 2015).

- The aim of the present study is to make a comparative characterization of the microbiological properties of brown and cinnamon soils under different vegetation in three mountainous regions of the country.

## MATERIAL AND METHODS

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IUSS Working Group WRB (2015)  
Cambisols.

- In the present study, samples of Brown Forest soils (slightly eroded) from the lands of the village of Gabra (Lozenska mountain) and the village of Govedartsi (Rila mountain) and Cinnamon forest soils (underdeveloped, shallow, eroded) from the land of the village of Igralishte ( Maleshevaska mountain) were observed. According to the classification of the IUSS Working Group WRB (2015), these two types of soils are defined as Cambisols. In each mountainous area, soils under grass vegetation, deciduous forest and coniferous (or mixed) forest were studied. A description of the soil difference, vegetation cover, altitude and sampling depth, as well as the main soil characteristics are presented in Table 1. The physical and agrochemical properties of soil samples are presented in detail by Kercheva et al. (2019).

Kercheva et al. (2019).



## 1.

Table 1. Characteristics of the studied soils

sample	Altitude	/ Soil type (WRB (2015))	C %	C:N	pH (H <sub>2</sub> O)	Db g/cm <sup>3</sup>	Pt % Vol	W <sub>2.0</sub> % mass	W <sub>2.5</sub> % mass	W <sub>4.2</sub> % mass
<b>/ Gabra village - Lozenska mountain</b>										
1	916	Underdeveloped cinnamon forest soils, shallow, low and medium eroded ( <i>Eutric Leptic Cambisols Ochric</i> )	1,5	11	5,2	1,08	62	35	28	12
2										
3	937	/ Brown forest soils, not eroded and slightly eroded ( <i>Dystric Cambisols Ochric</i> )	2,7	13	3,9	1,02	61	29	24	8
4			0,9	10	3,8	1,38	49	17	15	7
5	920	Brown forest soils, shallow, low and medium eroded ( <i>Dystric Cambisols Ochric</i> )	1,5	14	4,0	1,26	53	18	15	7
<b>/ Govedartsi village - Rila mountain</b>										
6	1530	/ Brown forest soils, secondary grassed, not eroded, ( <i>Dystric Cambisols Humic</i> )	3,3	10	3,8	1,07	58	27	22	10
7	1503	/ Brown forest soils, not eroded and slightly eroded ( <i>Dystric Cambisols Humic</i> )	4,2	16	4,2	0,87	66	44	38	14
8	1579	/ Brown forest soils, not eroded and slightly eroded ( <i>Dystric Cambisols Humic</i> )	4,9	14	3,6	0,60	75	67	59	21
<b>/ Igralishte village - Maleshevska mountain</b>										
9	869		2,9	12	4,6	1,08	59	28	18	6
10	863	Underdeveloped cinnamon forest soils, shallow, low and medium eroded ( <i>Eutric Leptic Cambisols Ochric</i> )	0,4	10	4,2	1,44	51	15	7	3
11	-	Underdeveloped cinnamon forest soils, shallow, low and medium eroded ( <i>Eutric Leptic Cambisols Ochric</i> )	2,5	12	4,2	0,88	66	40	28	6
12	865	Underdeveloped cinnamon forest soils, shallow, low and medium eroded ( <i>Eutric Cambisols Ochric</i> )	1,7	10	4,2	0,83	68	30	22	7
13	855	Underdeveloped cinnamon forest soils, shallow, low and medium eroded ( <i>Eutric Cambisols Ochric</i> )	0,3	10	4,5	1,38	50	14	10	5
14	848	Underdeveloped cinnamon forest soils, shallow, low and medium eroded ( <i>Eutric Leptic Cambisols Ochric</i> )	1,6	21	4,0	1,04	61	19	16	7

(Grudeva et al., 2006).  
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 ( ) -  
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 Tabatabai-Bremner -  
 Tabatabai (Alef and Nannipieri, 1995).  
 CO<sub>2</sub>  
 (Alef, 1995).  
 ANOVA (LSD- <0.05).

The amount of the main groups of soil microorganisms was determined by the method of ten-fold dilutions by sowing soil suspensions on selective agar nutrient media (Grudeva et al., 2006). The following physiological and taxonomic groups of soil microorganisms were determined: heterotrophic microorganisms – on meat-peptone agar (MPA) after three days of incubation; microscopic fungi – on acidified Chapek’s medium – after seven days of incubation; actinomycetes and bacteria absorbing mineral nitrogen – starch-ammonia agar (SAA) – after seven days of incubation and cellulose-decomposing microorganisms – on Hutchinson’s medium – after fourteen days of incubation. The activity of the enzymes acid phosphatase and -glucosidase was determined. The analyses were performed by introducing an appropriate substrate and reading the product of the enzymatic reaction by the following methods: phosphatase activity – by Tabatabai-Bremner and -glucosidase – by Tabatabai (Alef and Nannipieri, 1995). CO<sub>2</sub> production was reported by titrimetric method (Alef, 1995).

Data were statistically processed by ANOVA (LSD test at p<0.05). Correlation analysis was used to study the relationships between microbiological parameters and soil properties.

## RESULTS AND DISCUSSION

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In the soils of Lozenska mountain (Gabra village) the largest amount of heterotrophic microorganisms is observed under grass vegetation. This indicates that with this vegetation in the soil the processes of decomposition of the difficult to mineralize organic substances such as humus, take place more strongly, compared to the deciduous and mixed forest. A similar trend is observed in the cinnamon forest soils of Maleshevska Mountain (Igralishte village), where the largest number of heterotrophic

15 cm)

0-8 cm 2,9 %, 10-15 cm 0,4 %.

Mergel et al. (2001)

0-5 cm,

( . ) -

- 1579 m,

- 3,7.

10-20 cm

g/ - 26-27 10<sup>3</sup>

- microorganisms is reported in the surface layer under grass vegetation, but with increasing depth (10-15 cm) their number decreases significantly.

Probably the substantial difference of this group of microorganisms in the two depths is due to the carbon content in the soil, which at a depth of 0-8 cm is 2.9%, and at 10-15 cm – 0.4%. Similar results were obtained by Mergel et al. (2001) for nitrogen-fixing microorganisms in acidic forest soil, with the largest number found in the 0-5 cm layer, and the amount of these bacteria decreases with increasing depth.

In the Brown Forest soils of Rila Mountain (Govedartsi village) most heterotrophic microorganisms are reported under grassy vegetation and under pine, while in the soil under spruce these microorganisms are absent. The reason for this may be altitude – 1579 m, which is the highest compared to all other options, as well as the lowest pH of this soil – 3.7.

In all types of vegetation in both types of soils from Lozenska mountain the greatest amount of bacteria absorbing mineral nitrogen is observed, in comparison with the soils from Rila and Maleshevska mountains. Substantially high values were reported in the surface layer under the mixed forest and in the layer 10-20 cm below the deciduous forest in the Brown Forest soils of Lozenska mountain.

The distribution of actinomycetes is similar to that of bacteria that absorb mineral nitrogen. The largest number was reported in the Cinnamon forest soil from Lozenska mountain, with grass vegetation – 26-27 CFU 10<sup>3</sup> g/soil.

It is noteworthy that in all three mountainous regions the amount of actinomycetes is highest in soils under grass vegetation, compared to those under forest vegetation. This is most likely due to the higher root density and the higher amount of plant biomass in the soil,

			which is mineralized by microorganisms.
			The highest number of microscopic fungi was reported in the soils of Lozenska mountain, while in the soils of the other two mountain regions these microorganisms are not widespread. The higher number of bacteria absorbing mineral nitrogen, microscopic fungi and actinomycetes in the soils of Lozenska mountain compared to the other two regions may be associated with more intensive processes of mineralization of organic matter in these soils.
Brant et al. (2006)			Brant et al. (2006) found that in soils occupied by forest vegetation, root-produced carbon compounds play a decisive role in the structure of microbial communities.
			The lowest number of cellulose-decomposing microorganisms has been reported in the Brown Forest Soil of the Rila Mountains, which may be due to the high altitude. The number of these microorganisms is the largest in the soil occupied by grass vegetation from Maleshevska mountain – 53 CFU 10 <sup>3</sup> g/soil, where the highest carbon content in the soil is 2.9%. It has been established that plant residues contain 20-30% cellulose, in the decomposition of which microorganisms play a dominant role and that the decomposition of cellulose in the soil and in the soil litter is carried out by different types of fungi and bacteria depending on soil conditions, species and the amount of plant residues (Štursová et al., 2012) (Table 2).
		– 53 10 <sup>3</sup>	
g/		– 2,9 %.	
		20-30 %	
(Štursová et al., 2012) (		2).	

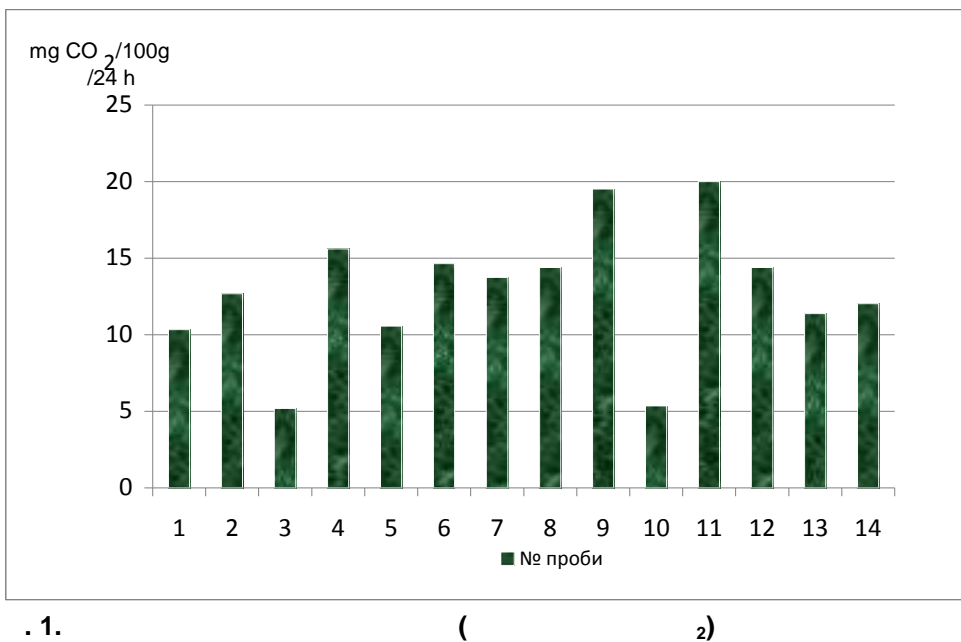
## 2.

**Table 2. Distribution of major groups of soil microorganisms in Brown forest and Cinnamon forest soils under different vegetation**

sample	cm / Plant cover, the depth of the soil sample in cm	Ammonifying microorganisms	/ Bacteria absorbing mineral nitrogen	Actino-mycetes	Microscopic fungi	Cellulose-decomposing microorganisms
/ Gabra village - Lozenska mountain						
1	Grass vegetation, 0-10	50	61	26	31	17
2	Grass vegetation, 10-20	48	61	27	21	25
3	Deciduous forest, 10-20	41	275	3	13	9
4	Deciduous forest, 0-5	38	80	9	57	18
5	Mixed forest, 0-5	30	126	17	30	25
/ Govedartsı village - Rila mountain						
6	Grass vegetation, 4-9	52	24	13	2	7
7	Solid white pine, 0-10	62	25	2	2	15
8	0-15 / Spruce, 0-15	0	44	2	1	5
/ Igralishte village - Maleshevka mountain						
9	Grass vegetation, 0-8	67	30	12	2	53
10	Grass vegetation, 10-15	7	51	8	8	10
11	Grass vegetation, 0-10	94	31	8	1	50
12	0-10 / Oak, 0-10	65	17	6	1	12
13	0-10 / Pine, 0-10	25	10	6	1	15
14	0-5 / Pine, 0-5	23	5	2	3	34

( 1).  
C/N  
(r=0,72)  
(r=0,61).  
C/N (r= 0,68).

The data from the correlation analysis show the presence of correlations between the amount of microorganisms in the surface horizons and the soil properties in the different mountain areas (Figure 1). In the region of Lozenska mountain a positive correlation was found between the cellulose-decomposing microorganisms with the C/N coefficient (r=0.72) and with the volume density of the soil (r=0.61). Bacteria using mineral nitrogen have a negative relationship with the C/N ratio (r= 0.68).



**Fig. 1. General biological activity (CO<sub>2</sub> production) in brown forest and cinnamon forest soils under different vegetation**

<p>C/N</p> <p>(r= 0,97)</p> <p>(r= 0,90),</p> <p>(r= 0,80),</p> <p>(r= 0,57).</p>	<p>(r= 0,98),</p> <p>(r= 0,57),</p> <p>(r=0,72)</p> <p>(r= 0,82)</p> <p>(r= 0,90).</p> <p>C/N</p> <p>(r=0,94)</p> <p>(r=0,74),</p> <p>(r= 0,65).</p>	<p>- In the soils of Rila Mountain almost all groups of microorganisms correlate with the C/N coefficient and with the bulk density of the soil. High negative relationships between the C/N ratio and fungi (r= 0.98), actinomycetes (r= 0.97) and heterotrophic microorganisms (r= 0.57) predominate, and the relationship with bacteria using mineral nitrogen (r=0.72) is positive. The relationships with soil bulk density are opposite – high positive correlation with fungi (r=0.90), actinomycetes (r=0.82) and heterotrophic microorganisms (r=0.80), and negative with bacteria using mineral nitrogen (r= 0.90).</p> <p>- In the soils of Maleshevska Mountain only actinomycetes correlate negatively with the C/N coefficient (r= 0.57). The relationships with bulk density were positive for fungi (r= 0.94) and bacteria using mineral nitrogen (r= 0.74), and negative for heterotrophic micro-organisms (r= 0.65).</p> <p>The enzymatic activity of the soils is</p>
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0-8 cm.

( 3).

### 3.

**Table 3. Enzyme activity of Brown forest and Cinnamon forest soils under different vegetation**

sample	cm / Plant cover, the depth of the soil sample in cm	-glucosidase	Acid phosphatase
		μ-mol NP/g /h	
/ Gabra village - Lozenska mountain			
1	Grass vegetation, 0-10	1,22	2,05
2	Grass vegetation, 10-20	0,59	2,02
3	Deciduous forest, 10-20	-	-
4	Deciduous forest, 0-5	0,90	2,54
5	, 0-5 / Mixed forest, 0-5	0,13	2,57
/ Govedartsi village - Rila mountain			
6	Grass vegetation, 4-9	0,69	4,92
7	, 0-10 / Solid white pine, 0-10	0,67	6,41
8	, 0-15 / Spruce, 0-15	0,96	8,15
/ Igralishte village - Maleshevska mountain			
9	Grass vegetation, 0-8	2,42	13,14
10	Grass vegetation, 10-15	0,22	1,12
11	Grass vegetation, 0-10	1,44	10,01
12	, 0-10 / Oak, 0-10	1,72	8,69
13	, 0-10 / Pine, 0-10	0,27	0,54
14	, 0-5 / Pine, 0-5	-	2,12

Soil enzymes play a significant role in the mineralization of organic matter and their activity is an important sensor for predicting the capacity to supply plants with nutrients. Sardans and Peñuelas (2005) found a decrease in soil enzyme activity under oak forest with a 21% decrease in soil moisture, a 35-83%

Sardans and Peñuelas (2005)

<p>21%, 35-83 %, - 31-40%.</p>	<p>- decrease in <math>\alpha</math>-glucosidase and a 31-40% decrease in acid phosphatase.</p>
<p>-</p>	<p>- The activity of all studied enzymes strongly decreases with the depth of the soil and is greater in spring than in autumn.</p>
<p>10 cm – 20,0 mg CO<sub>2</sub>/100 g /24 h.</p>	<p>- The highest values of the total biological activity (CO<sub>2</sub> – production) were reported in the Cinnamon Forest soils from Maleshevska Mountain under grass vegetation at a depth of 0-10 cm – 20.0 mg CO<sub>2</sub>/100 g soil/24 h. These samples showed the highest values of the activity of the two enzymes, as well as the highest amount of heterotrophic and cellulose-decomposing microorganisms.</p>
<p>0-8 cm - 2,9 %,   10 – -15 cm - 0,4 % 2 – 5,36 mg CO<sub>2</sub>/100 g /24 h.</p>	<p>- The data show that in the soil-climatic conditions of the Cinnamon Forest Soil in Maleshevska Mountain, occupied with grass vegetation, at a depth of 0-8 cm and carbon content in the soil - 2.9%, the most intensive microbiological processes take place. While in the same soil, occupied with grass vegetation, but at a depth of 10 – -15 cm and carbon content – 0.4%, the lowest CO<sub>2</sub> production was reported – 5.36 mg CO<sub>2</sub>/100 g soil/24 h. Our data are similar to the results of the study by Davidson et al (2006), which found that in the surface horizon of forest soils 40-48% of the CO<sub>2</sub> production of the entire soil profile is obtained.</p>
<p>Davidson et al (2006),  40-48 %</p>	<p>-</p>
<p>Chen et al. (2004)  0-10 cm</p>	<p>- Chen et al. (2004) found differences in the number of bacteria, actinomycetes and fungi, as well as in microbial respiration and enzyme activity in the soil layer 0-10 cm depending on the type of forest vegetation and the season. The highest number of bacteria and fungi was reported in natural forest, while in the soil under Chinese fir the highest number of actinomycetes.</p>

## CONCLUSIONS

- In each of the studied mountain areas the combination of the specific soil



properties, climatic conditions and the type of vegetation determine the differences in the microbiological indicators. In general, in the Cinnamon and Brown Forest soils under grass vegetation from Lozenska, Rila and Maleshevska mountains a larger amount of actinomycetes is observed in comparison with those under coniferous vegetation. In the soils of Lozenska Mountain a higher number of bacteria assimilating mineral nitrogen, microscopic fungi and actinomycetes was found in comparison with the other two regions. The amount of microorganisms in the surface horizon correlates with the bulk density of the soil and the C/N coefficient, which is most strongly represented in the soils of Rila Mountain.

The activity of the studied enzymes varies with different types of vegetation in each region. The highest values are reported in the surface layer of the Cinnamon forest soil under grass vegetation in Maleshevska Mountain, the activity is lower in the Brown forest soils from Rila mountain, and the lowest values are in the Cinnamon soils from Lozenska mountain.

Of all the studied soils with best microbiological indicators, Cinnamon Forest soil under grass vegetation from Maleshevska mountain stands out, where the highest amounts of heterotrophic and cellulose-decomposing microorganisms, the highest enzyme activity and the highest total biological activity (CO<sub>2</sub> production) are reported.

( CO<sub>2</sub>).

“ ( 16/11 20.12.2017),

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