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Antifungal Activity of Inorganic Micro-and Nanoparticles Against Pathogenic Fungi Compared with Some Traditional Organic Drugs

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Abstract: Many organic and inorganic compounds are known to exhibit widespread anti-fungal activity with successful usage in agricultural and medicinal applications. The efficacy and safety of these compounds are crucial when used. In the present work, the effect of different chemicals characters, which are widely used in agriculture and medicine (sulfur and silver nanoparticles; micronized particles of tetramethylthiuramdisulfide, tebukanozol, carbendazim and inorganic peroxides (CaO_2, SrO_2, BaO_2); (water solvent of miramistin and flucytosine) against some fungal pathogens (*Alternaria alternata*, *Àspergillus niger*, *Candida àlbicans*, *Fusarium graminearum*, *Penicillium notatum*) were compared. The experimental data for the antifungal activities of various agents showed the following: 1) All tested compounds inhibit the growth of pathogens in varying degrees; the range of most active concentrations was from 0.3 mg/ml for tebukanozol to 200 mg/ml for micron sulfur particles. 2) The use of sulfur-based products revealed to suppression of tested fungal pathogens and becomes more active in form of nanoparticles with an average size 20 nm where their antifungal effectiveness increased by 10 times compared to micron particle average size of 70 microns. 3) Comparison of the effects of two types of elementary inorganic substances nanoparticles (sulfur and silver) the results revealed that the antifungal activity of 20 nm is almost equal to the activity of 20-25 mg/ml. 4) The data obtained from the mixture of sulfur and silver particles experiment indicate on their antagonistic effects which is possible due to the formation in mixtures as (Ag_2S) 5) Referring to the high antifungal properties of alkaline earth metal peroxides, the greatest interest is environmentally friendly calcium peroxide, it can be recommended as a seed dressing and antifungal agent for long-term storage of fruits and vegetables. The results allow evaluating the preparations "power of action" intended for use in a variety of areas. We recommend the mixture of sulfur and more toxic preparations (tetramethylthiuramdisulfide, tebukanozol and carbendazim) as alternative complex fungicide; it may be useful to reduce the pesticide load on the environment.

Key words: Nanoparticles · Fungal pathogens · Antifungal activities · Sulfur · Silver · CaO_2 · SrO_2 · BaO_2

INTRODUCTION

The history of world agriculture shows that unwillingness or inability to use chemicals in the form of fertilizers and means of plant protection from pests inevitably entails downswing of a sharp decline as overall production and quality of agricultural products. Nowadays over one hundred thousand species of fungi, hundreds species of pathogenic bacteria on the planet are found. Many of them have formed a stable food connection with the different groups of crops, adapted to wait a temporary absence of the main host plant and when it appears they accumulate their numbers very fast. Currently, in the developed countries the agricultural strategy of development involves the improvement and implementation of integrated farming systems, including

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widespread use of chemical agents in the form of fertilizers, pesticides, plant growth stimulants. All countries concerned about their own food self-sufficiency, as well as intergovernmental and non-governmental organizations, taking into account the demographic problems of the world and the problem of hunger in developing countries adhere to the strategy as a whole. The tense situation in the area of the food supply of the world's population has led to the fact that in most developed and developing countries, a huge amount of fertilizer and plant protection products are used, since the use of chemicals is the most economical way to obtain high yield. Chemical plant protection products (pesticides) are produced by the chemical industry and released to consumers at relatively low prices that causes a high return of their use and puts them in the first place in farming [1-4]. Pests reduce the cost of weed control and enhance crop. The sharp increase in the volume of use of herbicides, insecticides, desiccants has led to significant environmental pollutions. According to the need to ensure the safe use of chemicals to humans and the environment demands on the quality of these chemicals are increasing, there is a need to develop and implement new, more technologically advanced and the safest ways to use them [5, 6].

One of the promising directions of development of environmentally safe and efficient fertilizers, means of protection and stimulation of plant growth are nanotechnologies. The distinguishing characteristic of drugs based on nanotechnology is the ability to exert effective influence on plants in very low concentrations [7, 8]. The use of nanoformulatioms leads to increased resistance to adverse weather conditions and the increase of the harvest of almost all products (potatoes, cereals, vegetables, fruit) and technical crop (flax, cotton) [9]. In this work, the results of a study of anti-fungal properties of micro- and sulfur nanoparticles against the pathogenic fungi and compare their performance with traditional inorganic and organic preparations was presented. As inorganic micro- and nanoparticles were used well-known elemental silver and calcium peroxide were used. Also, the antifungal properties of the foregoing materials in micro and nano-forms with the corresponding characteristics of effective and well-known organic products such as tebukanozol, carbendazim and its derivative, having a negative impact on the environment were compared. Sulfur and its compounds play an important role in human life over the years. Bactericidal (sulfur ointment) and fungicides (in the processing of the vine finely divided powder) properties of sulfur already have been used at the dawn of civilization. The action mechanism of sulfur agents is that sulfur interacts with organic substances and forms sulfides and pentatonic acid having anti-microbial and anti-parasitic activities. Sulfur along with carbon, hydrogen, nitrogen, calcium is an essential element of the existence of living organisms. And so sulfur is essential mineral element nutrition of plants, animals and humans. But in this work only the antifungal properties of sulfur against phytopathogenic organisms was considered. Nowadays elemental sulfur is widely used in plant growing as a fungicide and acaricide. But in most cases sulfur was used as a micronized wettable powder in the USSR earlier and now in Russia, which were produced according to specifications [10-12]. Similar products are widely used in other countries, [13-15]. In general, micronized sulfur powder is one of the most widely used in plant growing chemicals which are used as well-proven and environmentally safe plant protection products [16-27].

In recent years great considerable attention is paid to the synthesis methods of antifungal properties of nanoscale sulfur due to the development of nanotechnology. A lot of work has achieved on the development of methods for producing nanoscale sulfur including chemical vapor deposition of sodium thiosulfate and polysulfide solution [28-36]. Biological properties of sulfur nanoparticles such as antifungal and growth-regulatory activities have been studied, with physical and chemical properties [37-52]. Sulfur nanoparticles have been synthesized from H_2S gas using new biodegradable iron chelates in w/o microemulsion system and it has been found that reducing the particle size increases the efficiency of the sulfur particles against all tested microorganisms (fungi, yeasts and bacteria) [37, 38]. Jagajjanani and Paria [39] found that, small sized sulfur particles (~35 nm) were very effective in inhibiting the fungal growth. Microscopic examination has confirmed the fungicidal effect due to the deposition of particles on the cell wall and its damage. Schneider *et al.* [40] mentioned the perspective use of sulfur nanoparticles in agriculture and medicine, as completely non-toxic compounds to human and plant cell structures, but at the same time have a selective toxicity against harmful lower organisms. Massalimov *et al.* [41-43] mentioned that the effectiveness of sulfur nanoparticles to accelerate the growth of wheat shoots and roots, it could be useful to produce of wheat seed dressing products. Interesting results concerning the effectiveness of antifungal effects of sulfur nanoparticles for a broad range of pathogenic organisms were obtained [44-47]. Antibacterial activity of sulfur nanoparticles (5.7 nm) was very high against Gram-positive *Staphylococcus aureus*, while this type of nanoparticles has not antibacterial activity against Gram-negative bacteria [48]. The obtained results of many experiments confirmed the use of sulfur nanoparticles as an antimicrobial agent in comparison with elemental sulfur [49]. Moreover, it has been revealed no toxicity of sulfur nanoparticles to plant and human cells. Bollig *et al.* [50] demonstrated that the existence of sulphur in the nutrition of tomato enhanced its resistance against *Verticillium dahliae* fungi by sulphur-containing defence compounds. Various aspects of the presence sulfur in plants of its toxicity to a wide range of pathogens, the problems of sulfur biogeneziz, sulfur localization and shape of its existence have been considered [51]. Antifungal effects of micronized sulfur and sulfur nanoparticles against two types of pathogenic fungi *Aspergillus niger* and *Fusarium* on sporulation, ultrastructural modifications and phospholipid contents of fungal strains have been studied and obtained results revealed to perspectives of using sulfur nanoparticles [52]. The broadest spectrum of antimicrobial activity of follow sulfur contained compounds-thiosulfinates; trisulfides and benzylsulfinic acid were observed [53].

Recently, one of the most actual problems related to the environment is to replace toxic pesticides by their safer analogs. The above described review shows a high potential of sulfur as an environmentally friendly fungicides especially in nanoform. The objective of this research was to evaluate the antifungal effects and properties of sulfur in two forms (powder of microparticles and nanoparticles). The efficacy of sulfur-based chemicals could be detected by examination of antifungal characteristics. Therefore, antifungal properties of sulfur have been studied in comparison to some of inorganic and organic substances. These tested agents have different mechanisms of suppression of pathogenic organisms and, therefore, it is interesting to compare the effect of such different antifungal drugs on the same pathogens. The most important characteristic of any drug is its critical concentration, leading to the complete destruction of the colony of pathogens. First of all, we have compared the efficiency of compounds as micro-and nanosulfur forms with tebukanozol and carbendazim, which are well-known and widely used in plant protection. We have also measured the anti-fungal characteristics of following inorganic matter silver in the form of nanoparticles and ions, as well as three representatives of the inorganic peroxides (CaO_2, SrO_2, BaO_2), which have same pronounced antifungal properties [54]. The results of measurements of the critical concentration of each of the above mentioned substances used five kinds of pathogenic fungi were presented in this work. Finally, was evaluated the efficacy of drugs widely used in medicine - miramistin and flucytosine. When comparing the properties of these antifungal agents it is necessary to mention that although all of them have antifungal activity, but their applications for various purposes. Elemental sulfur, tebukanozol and carbendazim were used in plant protection against fungi and mites. Calcium peroxide were used to purify water from pathogens, for decontamination of stored grains and vegetables, as well as the preparation for seed protection of wheat and other cereals [55-57].

MATERIALS AND METHODS

To study the following reagents qualification "chemically pure": elemental sulfur S, citric acid, silver nitrate, calcium, strontium and barium peroxides were used to explore antifungal properties of inorganic (sulfur, silver and peroxides CaO_2, SrO_2, BaO_2) and organic (tebukanozol, carbendazim, TMTD, miramistin, flucitozin), the tested compounds have been used against five pathogenic fungi cultured (*Alternaria alternats*, *Àspergillus niger, Candida àlbicans*, *Fusarium graminearum*, *Penicillium notatum*), purified and identified by scientific research institute of technology of herbicides and plant growth regulators of the Academy of Sciences Republic of Bashkortostan. The elemental sulfur were crushed in an industrial roller mill and used as microns sulfur. Calcium polysulfide solution was used to produce sulfur nanoparticles by gradually adding 10% citric acid, sulfur nanoparticles powder was obtained [35, 36]. Silver nitrate has been used to produce ions in the aqueous solution for prepare of silver nanoparticles by thermal decomposition at 500ºC. Inorganic peroxides, CaO_2, SrO_2 and BaO_2 were synthesized according to the method described by Kim *et al.* [53]. As organic products, tebukanozol, carbendazim, TMTD (tetramethylthiuram), miramistin, flutotsizin commercial preparations were used. Particle size distributions for sulfur and other materials-were determined by- a laser particle size analyzer of Shimadzu SÅLD 7101. Also the size and the shape of silver particles and sulfur with were determined by a scanning probe microscope Solver Pro M. Analysis of the structural characteristics of tested samples was carried out on X-ray diffractometer Rigaku Ultima IV. To study the antifungal effect of tested materials and their ability to inhibit growth of pathogenic fungi the growing medium Sabouraud agar was used, this media has been prepared in accordance with the technical specifications N9229-014-00419789-95.

RESULTS AND DISCUSSION

Study of Dispersed Agents: Due to the role of particle size in drugs antifungal activities, the particle size distribution were measured for all of synthesized materials: silver and elementary sulfur compounds, calcium peroxides, strontium and barium. Integral and differential particle size distributions of sulfur particles received after crushing in a roller mill are as shown in Fig. 1 as black color circles (•). The data revealed that distribution of particles in this case lies in the range from 40 micron to 200 micron and the average size is equal 70 micron. Integral and differential distributions of chemically precipitated sulfur particles were measured in aqueous medium and it was established that distribution of particles in this case lies in the range from 10 nm to 40 nm and the average size is equal 20 nm. Fig. 1 shows these particle size distributions by not painted over circles (o). Along with measuring particle size distribution using a laser article size analyzer, size and shape of sulfur particles were determined using Solver Pro M Scanning Probe Microscope, the data showed that sulfur nanoparticles are of spherical shape. Fig. 2 also the particle sizes ranged from 10 to 50 mm and the average size were equal 20 nm (Fig. 2a), the data obtained using the special program of images processing. Data in Fig. 2b presents the image of sulfur particles crushed in a roller mill, obtained with an optical microscope, which generally reflects the pattern shown in Fig. 1 with filled red circles. It is well known that the crystalline sulfur at room temperature has an orthorhombic structure. X-ray phase analysis of sulfur nanoparticles (Fig. 3) shows that they also have an orthorhombic structure this result is consistent with our previous findings [41] and other researches [30, 37]. Along with sulfur there are three alkaline earth metals (BaO_2, SrO_2, CaO_2) which have anti-fungal properties [54] were studied.

Distribution of integral and differential distributions of peroxide particle sizes, indicating that they all have a wide distribution in the range from 1 to 100 microns (Fig. 4). The curves of the integral and differential size distribution for silver nanoparticles were the same with case of sulfur nanoparticles, also the range was from 10 nm to 40 nm and the average particle size was 20 nm (Fig. 5).

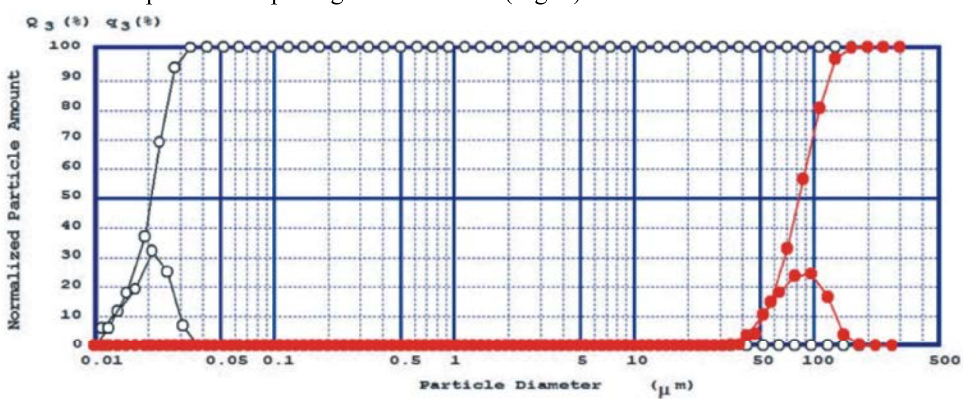

Fig. 1: Integral and differential particle size distributions of sulfur particles obtained: (o) - from calcium polysulfide, average particle size is 20 nm; • - after grinding in roller mill, average particle size is 70 micron.

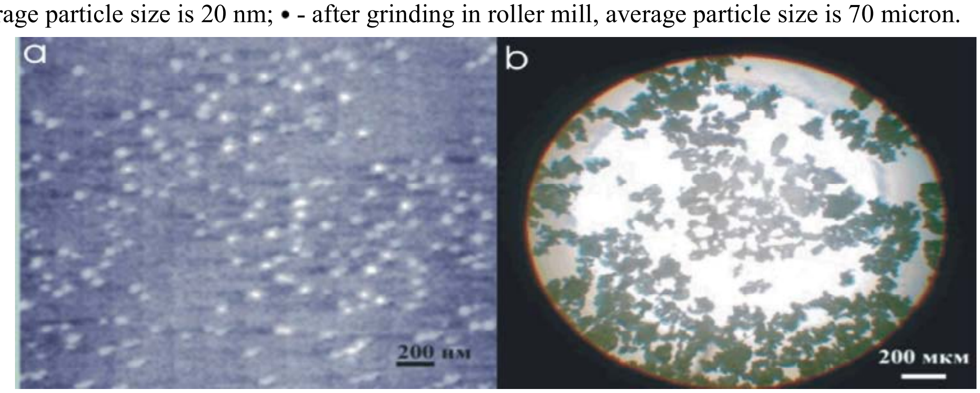

Fig. 2: Images of nanoparticles of sulfur (a) with an average size of 50 nm obtained with a scanning probe microscope and sulfur particles after grinding in roll mill (b) having an average size of 40 microns obtained by an optical microscope.

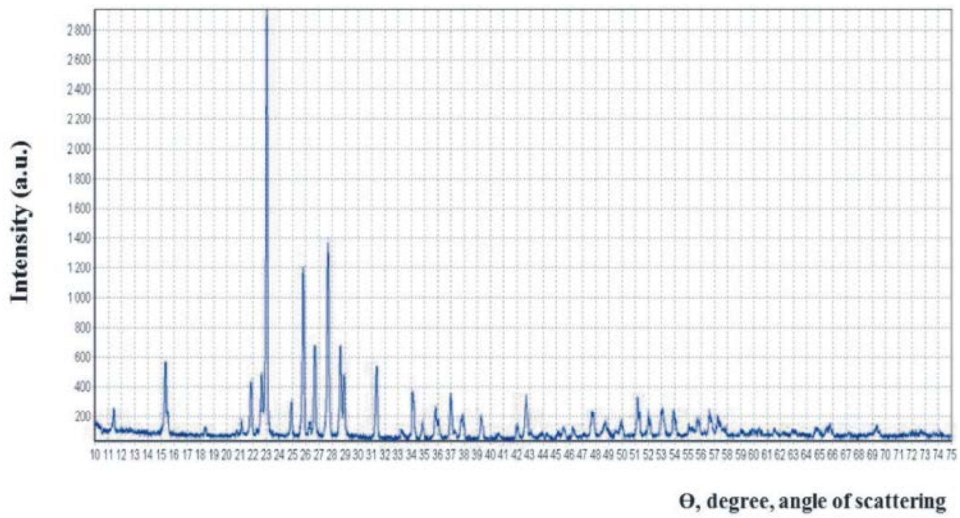

Fig. 3: XRD pattern of sulfur nanoparticles.

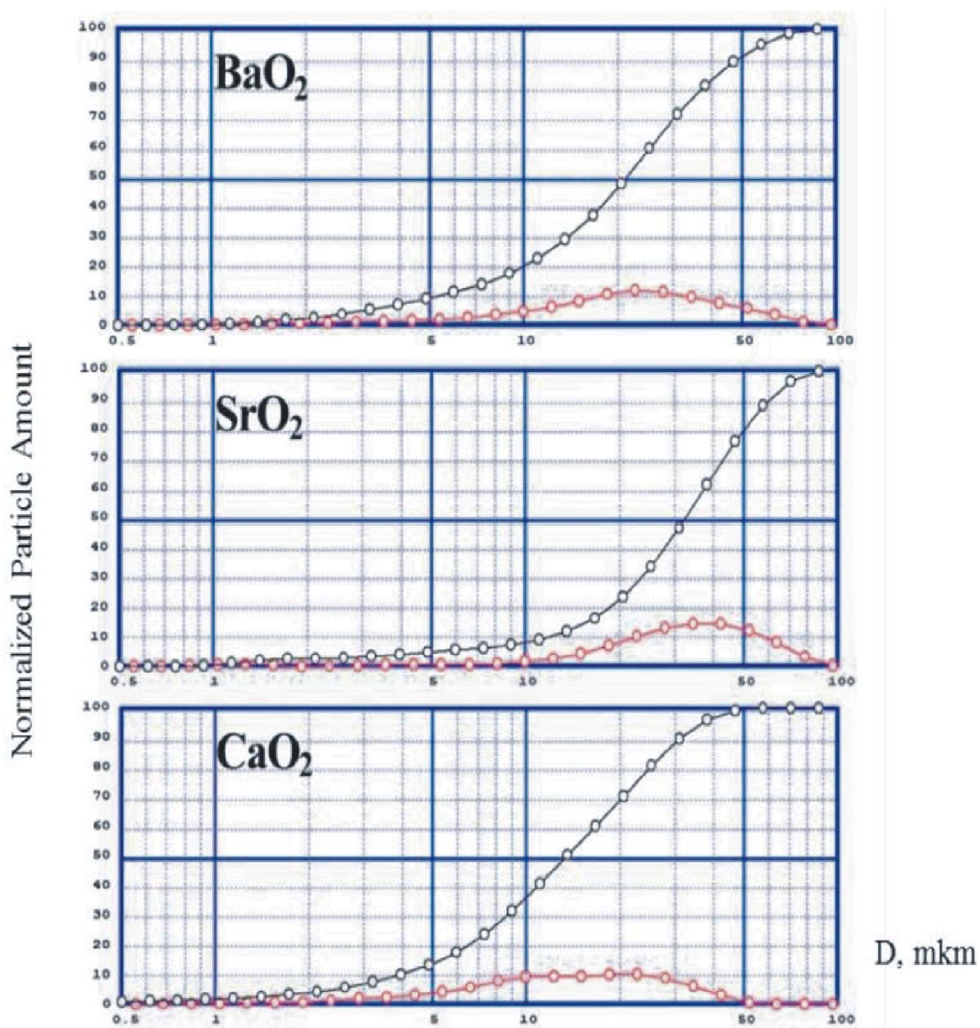

Fig. 4: The integral and differential distribution of peroxide particle size.

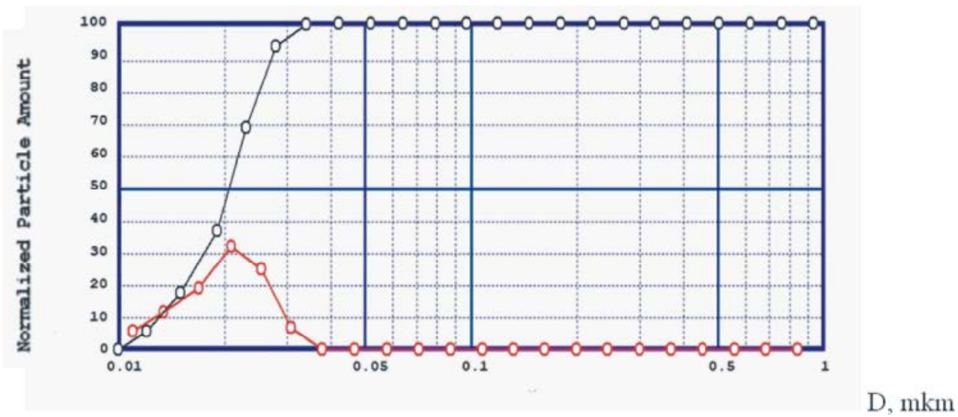

Fig. 5: The integral and differential distribution of silver particle size.

The fungicidal activity of the compounds and substances tested considering the full growth retardation- the minimum inhibitory concentration (MIC) against the tested fungal cultures on solid medium Sabouraud containing the tested compounds. Powdered preparations have been made into the molten Sabouraud medium (selective) in different concentrations then this mixture was poured into 20 ml of a Petri dish and evenly stirred until the medium polymerization. After polymerization (“pour point") on the medium with the preparations and the control medium (the medium without the addition of substances) test-culture of fungal was incubated at 25°C for 4 days. The results are visually recorded daily by the presence of typical colonies of fungal growth [58, 59]. As the tested microorganisms the following strains of fungal pathogens were used:

- *Candida albicans* is one of the simplest yeasts and belongs to a class of opportunistic fungi;
- *Aspergillus niger* is one of higher aerobic fungi, belongs to opportunistic fungi;
- *Penicillium notatum* - is one of fungi that form of food and causal agent of some post-harvest diseases.
- *Fusarium graminearum* is commonly found on cereal grains, most commonly on wheat, corn and barley.
- *Alternaria alternata* is common and widespread species this fungus has been recorded causing leaf spot and other diseases on over 380 host species of plant.

In this work, the anti-fungal effect of these inorganic materials and mixtures has been investigated as follow:

- Elemental sulfur, the average particle size is 8 microns.
- Nanoparticles of elemental sulfur, the average particle size of 20 nm.
- Calcium peroxide (CaO_2), average particle size 10 microns.
- Strontium peroxide (SrO_2), average particle size is 22 microns.
- Barium peroxide (BaO_2), the average particle size is 20 microns.
- Powder of elemental silver nanoparticles, average particle size is 30 nm.
- Solvent of silver nitrate.
- Mixture of nanoparticles of elemental sulfur and silver.
- Mixture of elemental sulfur nanoparticles and silver ions in an aqueous medium.
- Mixture of elemental sulfur particles of micron size and elemental silver nanoparticles.

The results presented in Table 1 revealed to measurement of anti-fungal activity of the above five tested inorganic substances; Inorganic substances are presented in two forms of sulfur (microns and nano particulate states), silver nanoparticle and silver ions. Also the data mentioned to micron particles of calcium, strontium and barium peroxides. Data in Table 2 revealed to sulfur nanoparticles concentration of 20 mg / ml leads to the completely growth inhibition of all tested pathogens. Also sulfur nanoparticles of instead of micron particles leads to the decrease of sulfur critical concentration factor of 10, this means that sulfur nanoparticles efficiently effective 10 times than micro particles. Comparison of the data obtained with silver nanoparticles against pathogenic strains showed that the effects were equally well as growth inhibiting of fungi and nanoparticle concentrations up to 25 mg/ml completely

Table 1: Critical concentration of inorganic compounds, leading to the complete inhibition of pathogenic fungal colonies.

	Critical concentration of inorganic drugs (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Fungal pathogens	S_m	S_n	CaO_2	SrO_2	BaO_2	Ag_n	Ag_k	$S_n + Ag_n$	$S_n + Ag_k$	$S_m + Ag_n$
Alternaria alternata	200	20	5	8	10	25	50	19+50	19+50	190+50
Àspergillus niger	200	20	5	8	10	25	10	19+80	19+80	190+50
Candida à1bicans	200	20	5	8	10	25	10	19+80	19+80	190+50
Fusarium graminearum	200	20	5	8	10	25	50	18+50	18+50	190+50
Penicillium notatum	200	20	5	8	10	25	20	19+80	19+80	190+50

Table 2: Critical nanoparticle concentration of sulfur and organic compounds, leading to the complete inhibition of pathogenic fungal colonies

	Critical concentration of inorganic drugs (mg/ml)						
	1	2	3	4	5	6	7
Strains variety of fungi	Tebukanozol	Carbendazim (BMA)	BMA● HCl● H_2O	BMA●SA	Flucytosine	Miramistin	S_n
Alternaria alternata	0.3	10	3	2	2	1	20
Àspergillus niger	0.4	6	5	2	2	1	20
Candida à1bicans	0.3	4	0.3	0.5	2	1	20
Fusarium graminearum	0.4	10	2	2	2	1	20
Penicillium notatum	0.4	6	0.4	2	2	1	20

inhibits the fungal growth. At the same, time the effect of silver ions on various pathogens is different. Strains *Candida a1bicans* and *Aspergillus niger* at a concentration of 10 mg/ml were completely suppressed, strain *Penicillium notatum* is inhibited at 20 mg/ml and for strains suppression of *Fusarium graminearum* and *Alternaria alternata* nanoparticle concentration was 50 mg/ml were required. A comparison of the data for sulfur nanoparticles with the obtained data for silver nanoparticles indicated that the effectiveness of sulfur nanoparticles slightly (to 25%) exceeded than the corresponding values for silver nanoparticles. Data in columns 8-10 shows data from a mixture of particulate silver and sulfur, which indicate antagonism of their action in the mixtures. Perhaps this is due to the formation of silver sulfide (Ag_2S) in mixtures which is less soluble in water, in addition to the antifungal effects of silver compound.

The most effective class of tested compounds is inorganic calcium peroxide (CaO_2), all pathogenic microorganisms were suppressed with concentration up to 5 mg/ml. Peroxides strontium and barium also effectively inhibit pathogens with higher critical concentration where caused less mass content of the corresponding peroxide. As organic substances have antifungal effects against pathogenic fungi with the following chemicals (Table 2). From organic preparations, the most effective one is tebukanozol which is widely used as a fungicide in plant cultivation where the concentration up to 0.3-0.4 mg/ml is sufficient to inhibit all the tested fungal pathogens. Carbendazim is 10-30 times less effective in comparison to preparation tebukanozol and to increase its efficiency are various derivatives of carbendazim, such as complexes with hydrochloric acid (HCl) and salicylic acids (SA) [60]. Also carbendazim has increasing efficiency for individual fungal species, also the data mentioned to high antifungal effects of miramistin and flucytosine against tested fungal pathogens (Table 2).

Efficiency of tebuconazole, carbendazim and its derivatives, higher than that of sulfur nanoparticles (Table 2), but we must consider that all of these substances have toxic higher than the toxicity of sulfur. To compare the prospects for practical application as fungicides of sulfur tebukanozol, carbendazim and also highly effective fungicide Tetramethylthiuramdisulphide (TMTD). For all tested compounds, we have chosen the same concentration which was equal to 1 g of fungicide per 1 kg of seeds. Before seed dressing formulation is diluted with water in a ratio of 1: 9. We incubated 7 days treated seeds in water at 20ºC and the fungicidal activity was then determined by measuring the percentage of particle sprouts do not contain spores of pathogenic organisms. Along with the evaluation of fungicidal activity of the preparations we have also measured the mass and number of seedlings germinated seeds [61, 62].

Antifungal effects of four types of tested compound TMTD, tebukanozol, carbendazim and sulfur nanoparticles as shown in Fig 6 where the TMTD is completely 100% germ suppresses the development of pathogens are no pathogenic fungi. Tebukanozol, carbendazim and sulfur nanoparticles inhibit the growth

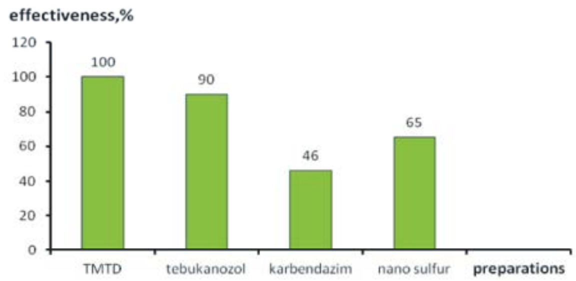

Fig. 6: The effectiveness of pathogenic organisms suppression using various drugs.

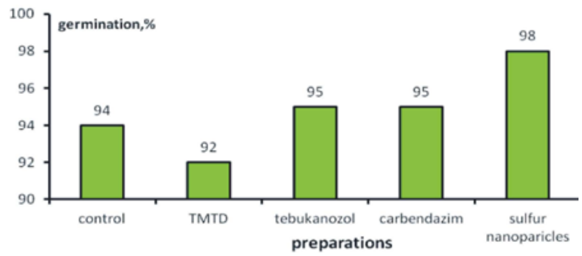

Fig. 7: Laboratory germination of seeds treated with various drugs.

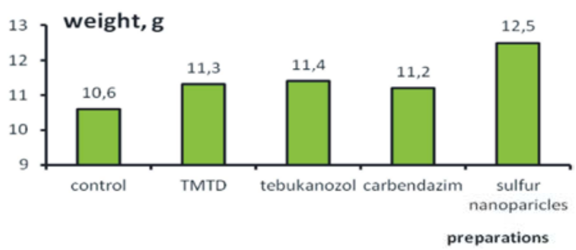

Fig. 8: Weight of 100 plant sprouts treated with various drugs.

by 90%, 46% and 65% respectively. The data indicated that the most effective agent is TMTD, tebuconazol acts on 10% less and carbendazim has the lowest impact.

In addition to determine the anti fungal effects of compounds on the plants the value of laboratory germination and germ weight were estimated (Figs 7 and 8). These parameters described the ability of tested particles to accelerate the growth of plants which considered very important indicators of the plant functioning. Fig. 7 shows a diagram of laboratory germination, which is calculated as a percentage of germinating plants as treatments compared to untreated seeds. These treatments leads to changes in seed germination, compared to the results obtained without drug treatment 94% from sown seeds were raising. The greatest negative impact on seed germination (reduction to 92%) obtained from TMTD processing while the greatest positive impact was obtained from treatments by sulfur nanoparticles where the seed germination increases to 97%. After storing seeds for 7 days, we measured the weight of the germ plants treated with different preparations as well. Fig. 8 shows the results of a mass of 100 shoots, as control results, the values of the untreated samples were used. From Fig. 8 shows that all tested drugs stimulate the growth of plants, but in varying degrees and nanoparticles of sulfur show the best results. Seed treatments by sulfur nanoparticles leads to an increase the weight by 19% compared with control, which is considerably higher than the corresponding values for the other compounds.

When considering the impact of drugs effectiveness on the plant is necessary to consider not only their anti-fungal and growth regulating properties, but also to evaluate their effect on the environment. In this respect, the sulfur-based drugs have no disadvantages because they are environmentally friendly. At the same time, all three tested compounds (TMTD, carbendazim and tebuconazole) present riskiness for the environment. For example, TMTD is a poison and medium lipotropic dangerous drug has a pronounced cumulative property. It is proved gonadotoxic and fetotoxic effect of TMTD on warm-blooded organisms [63, 64].

Although ñarbendazim belongs to the class of low-risk products in accordance with the World Health Organization, this drug is toxic to the liver, its effects on the reproductive system and it is considered to be potentially carcinogenic compounds. Carbendazim violates the human hormonal system and can cause cancer, because of the ability to penetrate the upper layer of the plants hence the presence of residues on the plant. Tebuconazol refers to the 2nd class of danger and is medium toxic to mammals; this drug does not affect the number of beneficial flora and fauna, but cannot prevent it from falling into the water. Taking into account the results of anti-fungal and growth-stimulating activity of the above drugs to reduce the pesticide load on the environment can be recommended to treat all plants and mixing the seeds with sulfur nanoparticles and one of the selected more toxic drugs (TMTD, carbendazim and tebukanozol) with a reduction in the dose of application of the latter.

On the basis of the obtained data for the antifungals for various purposes, it could conclude the following:

- The data in Tables 1 and 2 showed that all studied compounds inhibit the growth of pathogenic organisms in varying degrees; the range of critical concentrations varies from 0.3 mg/ml for tebukanozola to 200 mg/ml for micron sulfur particles.

- The use of sulfur-based products led to suppression of tested pathogenic organisms, especially in the nanoparticles form with an average size of 20 nm where anti-fungal effectiveness increased by 10 times compared with the data obtained with micron average particle size of 70 microns.
- Comparison of the effects of two nanoparticles types of elementary inorganic substances (sulfur and silver) in the same size of 20 nm indicated that they were approximately the same efficacy equal to 20-25 mg/ml.
- The data obtained from the mixture of sulfur and silver particles indicate on the antagonism of their action, which is possible due to the formation in mixtures of silver sulfide (Ag_2S) which is the least soluble in water, silver compound and has not anti-fungal effects.
- It should be noted the high anti-fungal properties of alkaline metal peroxides, the greatest interest is environmentally friendly calcium peroxide, it can be recommended as a seed dressing and antifungal agent for long-term storage of fruits and vegetables.
- The maximum impact of anti-fungal agents listed in Table 1 and 2 have tebukanozol which is widely used as a plant protection product.
- In the future to reduce the pesticide load on the environment to use mixtures in those toxic products must be replaced partially or completely safe and effective, for example, such as sulfur nanoparticles.
- Necessary to consider also that the sulfur nanoparticles also effect as plant growth promoter especially in seed dressing and help to increase the protein content [65].

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12. Technical specification N113-04-322-40 Sulfur Colloid - pasta.
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