ATLANTIS PRESS

Advances in Social Science, Education and Humanities Research, volume 393

The Fifth Technological Order: Prospects for the Development and Modernization of the Russian Agro-Industrial Sector (TFTS 2019)

Assessment of the Effectiveness of the Use of Biological Products of Associative Nitrogen Fixation in Potato Cultivation

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Abstract* – At present time, in the current economic and environmental conditions in Russia, with limited use of mineral fertilizers in agricultural production, the use of biological cropping techniques is relevant. One of the directions is application of microbial preparations safe for the person and environment, as an element of resource-saving technologies as their production is less power-intensive, than mineral fertilizers, and application is economically favorable. Purpose of research: with the help of microbiological indicators sensitive to agrogenic loads to evaluate and compare the effect of various biological products on the number of microorganisms and the intensity of the transformation processes of organic compounds in the potato rhizosphere, the formation of crops and the development of potato diseases. In the field experiments of 2013-2018, agents Rizoagrin, Azorizin, Mizorin and agents with fungicidal, insecticidal and stimulating properties – Mival-agro, potassium humate, Prestige were used. The quantity of microorganisms in the rhizosphere of potatoes was the highest in the variant with the treatment of tubers with the Mival-agro agent (126% to control). All groups of determined microorganisms in this variant reacted positively to the treatment. The Rizoagrin and Mizorin agents stimulated the microbial abundance by 61 and 17%, respectively, the agent showed fungicidal properties by 38.6% in comparison with the control variant. The coefficients of mineralization, immobilization of nitrogen and transformation of organic matter in potato rhizosphere were calculated from the ratio of groups of microorganisms. The predominance of immobilization processes in the soil was established. Nitrogen of non-symbiotic fixation, the amount of which increases as a result of the action of microorganisms of biological products, remains in the rhizosphere of the crop. Agents of complex action, including microbial, increased potato yield by 1.3-7.9 t/ha, reduced disease damage: rhizoctonoise (*Rhizoctonia solani*), scab (*Streptomyces scabies*), late blight (*Phytophthora infestans*). An average positive correlation was established between the total number of microorganisms in the rhizosphere, the number of saprophyte bacteria on meat-and-peptone agar and yield.

Keywords—potato, rhizosphere, microorganisms, yield, potato diseases.

I. INTRODUCTION

Among the elements of plant nutrition, nitrogen occupies one of the most important places. Without the presence of this element, protein substances cannot be synthesized, and without protein substances, the protoplasm of a living cell cannot be built. "Nitrogen is more precious from general biological point of view than the rarest of noble metals" - wrote academician V.L. Omelyanskiy [1].

It is well known that the low yield of many agricultural crops, most often, is due to the lack of nitrogen nutrition of plants, although the total nitrogen reserves in the soil are very significant [2,3].

One of the main sources of increasing the amount of available nitrogen in the soil and plant productivity is the use of mineral fertilizers. Along with mineral fertilizers, biological products with different directions of action are used in agricultural production, including those based on associative nitrogen-fixing bacteria living in the rhizosphere of plants. The use of biological products enhances nitrogen fixation of the atmosphere, contributing to increased productivity of crops, improves quality indicators of products, increases plant resistance to stress, in particular, to drought, reduces the development of phytopathogens [4-7].

Researches of Potato Department of the Siberian Agricultural Research Institute (now Omsk Agrarian Scientific Center) in field experiments of 1997-2015 established positive influence of biopreparations of complex action on productivity and marketability of potato tubers [8].

Purpose of research: with the help of microbiological indicators sensitive to agrogenic loads to evaluate and compare the effect of various biological products on the number of microorganisms and the intensity of the transformation processes of organic compounds in the crop rhizosphere, the formation of crops and the development of potato diseases.

II. RESEARCH METHODOLOGY

In field experiments with potatoes (2013-2018) were used biological preparations of associative nitrogen fixators produced by the All-Russian Research and Development Center of Agricultural Meteorology (city of St. Petersburg, Pushkin) - Rizoagrin, Azorizin, Mizorin, as well as agents that favorably affect the structure and fertility of the soil, containing salts of humic acids, plant growth stimulants (potassium humate, Mival-agro) and Prestige agent, which has a fungicidal and insecticidal effect.

Potato cultivation technology is common in the southern forest steppe zone of Omsk region: the main processing - moldboard plowing, secondary tillage with rotary cultivator;

The article was prepared as part of a subprogram for the development of selection and seed production of potatoes in Russian Federation.

planting - with a clone seed planter СН-4БК in the second decade of May; cutting ridges, weed and pest control, mowing the tops, desiccation with a Raglon, harvesting - in the first decade of September with a 2-row digger with manual harvesting of tubers. The area of the plot is 30 m^2, accounting area is 15 m^2, 4-times replication. Tubers after inoculation with biopreparations were planted on backgrounds with the introduction of mineral fertilizers: 30-40 kg r.a./ha of ammonium nitrate and ammophos, 100 kg/ha of potash fertilizers in accordance with the All-Russian Research and Development Center of Agricultural Meteorology guidelines [5,7].

The soil is meadow-chernozem medium-thick medium-humus heavy-loamy, the reaction of the medium is neutral, the humus content is 6.5% by method of I.V. Tyurin, provision with mobile phosphorus-average (100-130 mg/kg), mobile potassium - high 300-350 mg/kg (according to F.V. Chirikov) [9]. Toxicity of soil extract was determined by bio test on radish seeds [10].

In the experiments, an early-maturing variety of potatoes Alyona was used. Accounting of diseases was carried out by the method of the All-Russian Potato Research Institute [11].

The microbial abundance in the potato rhizosphere was determined on solid nutrient media: MPA (meat-and-peptone agar) for bacteria that utilize organic nitrogen compounds, including ammonifiers; SAA (starch-and-ammonia agar) for microorganisms that consume nitrogen in mineral form; Mishustina's medium – for oligonitrophils; Muromtsev-Gerretsen medium - for bacteria that mobilize mineral phosphates. Cellulose-destroying microorganisms were taken into account on Getchinson medium, nitrate bacteria - on aqueous leached agar with the addition of double ammonium-magnesium salt of phosphoric acid, fungi - on Chapek medium with the addition of lactic acid [12]. The coefficients of mineralization (SAA/MPA), immobilization of nitrogen (MPA/SAA) and transformation of organic matter – Ct = МПА/SAA x (МПА+SAA) were calculated by the method of V.D. Mukha [13] as presented by L.N. Korobova et al. [14]. Statistical processing of the obtained data was carried out according to manual of B.A. Dospekhov's [15] using PC program Microsoft Excel.

Weather conditions during the potato growing season varied: dry 2014 and 2017 (hydrothermal coefficients 0.68 and 0.70, respectively), humid - 2018 (HTC 1.31). Relatively favorable for the growth and development of crops in terms of precipitation and air temperature were in 2013, 2015, 2016 years (HTI 1.08-1.11).

III. Results

Associative nitrogen fixation in the rhizosphere of non-leguminous plants was discovered in the middle of the last century. Nitrogen, fixed by associative microorganisms, in local conditions for cereals is from 3 to 30 kg/ha and serves as an additional source of nitrogen nutrition of plants. Biological preparations based on rhizosphere nitrogen-fixing microorganisms were used to enhance nitrogen-fixing activity of microorganisms in agricultural crops. Toxicity of the soil of the rhizosphere of plants when using biological agents was not detected [16].

The microbial abundance in the potato rhizosphere was the highest (126% to control) in the variant with Mival-agro, which is an organosilicon complex biostimulator. All groups of isolated microorganisms reacted positively to the use of this preparations, which is consistent with the studies of A.Kh. Kulikova et al. [17].

Among the studied microbial preparations, the number of microorganisms of the rhizosphere was most stimulated by Rizoagrin by 61% to control, respectively. At the same level there was the total (total) quantity of determined microorganisms when using prestige agent, although it has fungicidal properties. Mival-agro, Mizorin agents contributed to an increase in the number of fungi in the rhizosphere by 28-52% relative to the control (Fig. 1). According to the methods [13,14], the coefficient of transformation of organic matter – Ct, was calculated, which shows the direction of microbiological transformation of organic residues in the rhizosphere towards mineralization or towards the synthesis of humus substances, that is, the predominance of immobilization processes in the rhizosphere of potato plants.

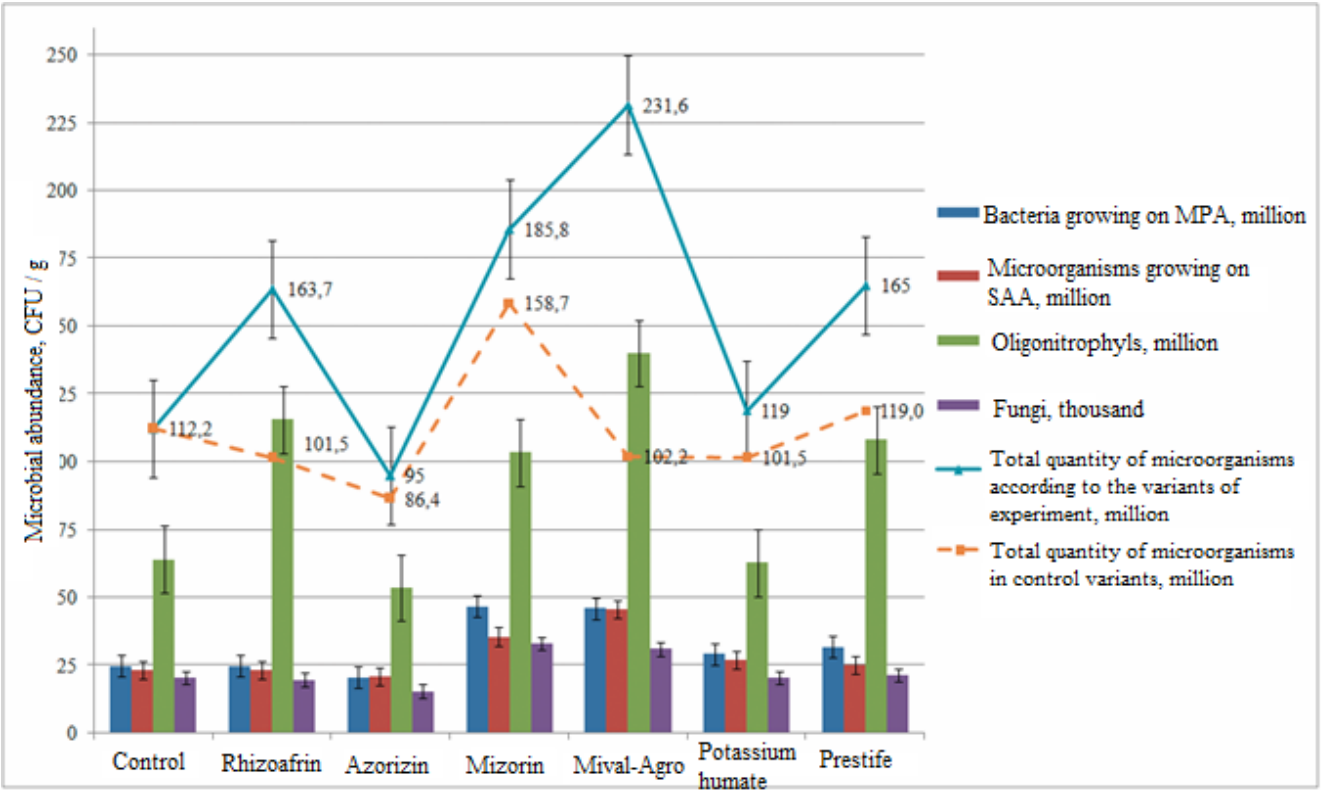

Fig. 1. Microbial abundance in potato rhizosphere depending on tubers treatment with agents, 2013-2018, CFU/g.

The highest values of Ct were obtained in the processing of potato tubers with Mizorin, Mival-agro, potassium humate, Prestige. Consequently, in the potato rhizosphere, using these preparations, immobilization processes predominate.

Nitrogen of non-symbiotic nitrogen fixation remains in the rhizosphere, improving the nutrition of microorganisms and fixing in the organic matter. The use of microbial preparations - Rhizoagrin, Azorizin for inoculation of potato tubers practically did not affect the value of Ct (table 1).

Studies have shown that the incidence of potato tubers with scab after presowing inoculation with biopreparations decreased by 2 or more times. To the greatest extent, compared with the control without treatment, the quantity of potato tubers affected by scab decreased when they were treated with the Prestige agent, as well as with the biological preparation Rizoagrin.

The use of the biological preparations Rizogrin, Azorizin allowed to reduce or eliminate completely the incidence of late blight that appeared to be comparable in chemical effectiveness of the Prestige agent..

TABLE I. INFLUENCE OF SECONDARY TILLAGE OF POTATO TUBERS WITH COMPLEX AGENTS ON THE INTENSITY OF MICROBIOLOGICAL PROCESSES IN THE RHIZOSPHERE (2014-2018)

Variant	Coefficients of		
	mineralization (SAA/MPA)	*immobilization (MPA/SAA)*	*transformation of organic matter (Пм)*
Control Rizoagrin n=5	0.89±0.13 0.94±0.12	1.12±0.27 1.06±0.19	46.4±7.7 51.0±10.3
Control Azorizin n=4	0.87±0.16 1.01±0.10	1.14±0.35 0.99±0.13	38.0±3.1 40.9±5.4
Control Mizorin n=3	1.05±0.28 0.76±0.09	0.95±0.06 1.31±0.17	65.3±11.3 107.8±13.7
Control Mival-agro n=5	0.95±0.24 0.99±0.09	1.06±0.29 1.01±0.16	45.8±7.2 92.6±32.4
Control Potassium humate n=5	0.89±0.13 0.92±0.10	1.12±0.27 1.09±0.12	46.4±7.7 61.3±4.7
Control Prestige n=3	0.99±0.41 0.79±0.05	1.01±0.50 1.26±0.09	50.9±10.5 71.6±17.1

Silicon-containing agent Mival-agro showed high efficiency in the prevention of late blight (table 2).

TABLE II. YIELD (T/HA) AND POTATO TUBERS INFESTATION DEPENDING ON SECONDARY TILLAGE WITH AGENTS OF COMPLEX ACTION, %

Variant	Yield, t/ha	Increase		Quantity of tubers affected by diseases, %		
		t/ha	*% to control*	*rhizoctonoise*	*scab*	*late blight*
Control, n=5-6	22.7±1.9	-	-	1.54 – 2.32	2.18 – 2.45	0.65 – 0.93
Rizoargin, n=5	26.2±1.3	3.5	15.4	0.92	1.83	0
Azorizin, n=4	23.0±2.3	0.3	1.3	0.98	1.45	0
Mizorin, n=3	30.6±2.2	7.9	34.8	0.65	0.65	0
Mival-agro, n=6	30.3±3.9	7.6	33.5	1.02	1.48	0.20
Potassium humate, n=4	29.4±2.1	6.7	29.5	1.42	1.68	0.72
Prestige, n=5	28.2±1.9	5.5	24.2	0.53	0.58	0.15

It should be noted that in the hyper humid 2018, the defeat of late blight on the treated variants was practically not manifested. The biostimulator potassium humate had the lowest efficiency to reduce the defeat of late blight.

The infestation with rhizoctonoise in the years of research fluctuated in the range of 1.5-2.3% to 1%. The most effective was the application of chemical agent Prestige – the incidence of rhizoctonoise was reduced to 0.53%, which is 3-4 times lower than the control. High efficiency was also observed from the application of biological preparations Mizorin, Rizoagrin, Azorizin.

In the application of microbial preparations on potatoes with the use of secondary tillage of tubers yield increases were obtained: from 0.3 t/ha to the maximum in the variant with Mizorin – 7.9 t/ha or 34.8% to the control Biostimulator potassium humate (gumi-20) increased the yield by 6.7 t/ha (29.5 %) relative to the control. Apparently, the value of the increase in yield was positively affected by the potassium contained in the preparation, since potatoes belong to crops with increased requirements for potassium content.

The increase in potato yield when treated with a chemical agent Prestige was 24%, which did not exceed the best option with the biological preparations Mizorin. Thus, inoculation of potato tubers with complex agents (Mival-agro, potassium humate, Prestige) allowed to increase potato yield by 5.5-7.6 t/ha or 24-33. 5% (table 2). At the same time, high yield increase was obtained in the variant with Mival-agro, silicon-containing plant growth stimulant, with the highest number of microorganisms in the rhizosphere.

Similar results on the reduction of potato tubers affected by rhizoctonoise, scab were obtained in the studies of Bulgarian scientists in the treatment of natural biological preparations with chitosan, grapefruit extract, citrus oil [18-20].

Under the conditions of 2014 and 2017, biological preparations contributed to the increase of potato resistance to drought, as evidenced by the obtained yield increases from 0.5 to 5.8 t/ha.

The agents used in our studies increased the marketability of potatoes by 4.4-6.6% to control, which is consistent with previous studies [8].

The average correlation (r = 0.40) between the total number of microorganisms in the variants of the experiment and potato yield was established. A closer relationship (r=0.57) was found between the number of bacteria decomposing organic nitrogen-containing compounds by MPA (including ammoniators) and potato yield, which is apparently related to the nitrogen nutrition regime of the crop. It is established that ammonium in chernozem soils of Western Siberia is rapidly nitrified. Plants also prefer the nitrate form of nitrogen, since the energy reserves for its consumption are lower than for the consumption of exchange ammonium [3].

In arid conditions, biological agents increased the stress resistance of potato plants, and in wet conditions reduced the spread of fungal diseases.

Judging by the ratio of the mass of tubers and tops, sufficient moisture promotes the growth of the mass of tops, and the use of biological stimulates this growth. The exception was the biological agent Mizorin contributing to the increase of the mass of tubers by 36.0 % compared to control. The correlation coefficient between the masses of tops and tubers is high (r = 0.71) under conditions of sufficient moisture (table 3).

In arid conditions, the mass indicators of tops and tubers were 20-35 % lower than in wet years. At the same time, the mass of tubers in most variants exceeded the mass of the tops. Biological preparation Mizorin 56% to control stimulated the growth of mass of potato tubers, as well as other preparations used for processing before planting. The correlation coefficient between the masses of tops and tubers in dry years was r = 0.98 (table 4).

IV. CONCLUSION

The greatest stimulating effect on the total quantity of microorganisms in the rhizosphere of potatoes had Mival-agro, Mizorin, Prestige agents.

TABLE III. ACCUMULATION OF MASS OF TOPS AND TUBERS IN CONDITIONS OF SUFFICIENT MOISTURE (70 DAYS AFTER PLANTING)

Variant	Mass from 1 plant, g		Ratio of tubers mass to tops mass
	tops	*tubers*	
Control	390	380	1: 1.03
Rizoargin	430	410	1:1.05
Azorin	450	390	1:1.15
Mizorin	480	520	1:0.92
Potassium humate	500	450	1:1.11
Mival-agro	460	420	1:1.09

TABLE IV. MASS ACCUMULATION OF TOPS AND TUBERS IN ARID CONDITIONS (70 DAYS AFTER PLANTING)

Variant	Mass from 1 plant, g		Ratio of tubers mass to tops mass
	tops	*tubers*	
Control	310	320	1:0.97
Rizoargin	300	290	1:1.03
Azorin	320	360	1:0.89
Mizorin	460	500	1:0.91
Potassium humate	400	450	1:0.89
Mival-agro	430	460	1:0.93

Between the total quantity of potatoes in the rhizosphere, as well as the bacteria count per MPA decomposing organic nitrogen-containing compounds, and the yield, the dependence of the average degree, r = 0.40 and 0.57, respectively, was established.

Judging by the ratio of groups of microorganisms on the media SAA/MPA, MPA/SAA and the coefficient of transformation of organic matter Ct in the rhizosphere of potatoes, immobilization processes prevailed, which intensified during the treatment of tubers with Mizorin, Mival-agro, Prestige agents.

The use of preparations of complex action, including microbial, for inoculation of potato tubers contributed to an increase in crop yield from 0.3 to 7.9 t/ha, reducing the defeat of rhizoctonoise from 2.3 to 0.53-1.0%, scab - 2 times, late blight - comparable to the action of the chemical preparation Prestige.

The agents used increased the marketability of potatoes from 90 to 96 %, by 4.4-6.6% relative to the control variant.

In arid conditions, biological agents increased the stress resistance of potato plants, and in wet conditions reduced the spread of fungal diseases.

In years with sufficient moisture, the correlation between the mass of tops and tubers of potatoes is lower than in dry years r = 0.71 and 0.98, respectively.

REFERENCES

[1] V. L. Omelyanskiy, Fixation of atmospheric nitrogen by soil microbes, Vol. 1. Moscow, 1953. (in russ.)

[2] V. N. Kudeyarov, V. N. Bashkin, A. Yu. Kudeyarova, and A. N. Bochkarev, Ecological problems of mineral fertilizers application. Moscow, 1984. (in russ.)

[3] G. P. Gamzikov, Agrochemistry of nitrogen in agrocoenosis. Novosibirsk, 2013. (in russ.)

[4] A.A. Alferov, L.S. Chernova, N.Ya. Shmyreva, A.A. Zavalin, "Assessing the efficiency of nitrogen fertilizer at the application of Rizoagrin to spring wheat," Plodorodie (Fertility), No. 6 (93), pp. 4-6, 2016. (in russ.)

[5] A. A. Belimov, Interaction of associative bacteria and plants depending on biotic and abiotic factors: Authors Abstract of Doctor of Science Dissertation. St. Petersburg, 2008. (in russ.)

[6] A. A. Zavalin, Biological preparations, fertilizers and yield. Moscow, 2005. (in russ.)

[7] V. K. Chebotar, A. N. Zaplatkin, A. V. Shcherbakov, N.V. Malfanova, A.A. Startsevf, and Ya.V. Kostin, "Microbial preparations on the basis of endophytic and rhizobacteria to increase the productivity in vegetable crops and spring barley (Hordeum vulgare L.), and the mineral fertilizer use efficiency," Sel'skokhozyajstvennnaya biologiya (Agricultural biology), No. 3, Vol. 51, pp. 335-342, 2016. (in russ.)

[8] O. F. Khamova, A. I. Cheremisin, and N. V. Dergacheva, "The Efficiency of Application of the Biological Preparations with Combined Effect at Potato Varieties under Conditions Southern Forest-Steppe of Western Siberia," Agrokhimiya (Agrochemistry), No. 9, pp. 33-38, 2016. (in russ.)

[9] Agrochemical methods of soil research. Moscow, 1975. (in russ.)

[10] J. Szegi, Talajmikrobiologial vizsgalati modszerek.Budapest, 1979. (in Hungarian)

[11] E. A. Simakov, N. P. Sklyarova, I. M. Yashina, Methodical instructions on the technology of potato breeding process. Moscow, 2006. (in russ.)

[12] E. Z. Tepper, V. K. Shilnikova, G. I. Pereverzeva, Microbiology workshop. Moscow, 1979. (in russ.)

[13] V. D. Mukha, "About indicators reflecting the intensity and direction of soil processes," in collection of scientific papers of Kharkov Agricultural Institute, Vol. 237. Kharkov, 1980, pp. 13-18. (in russ.)

[14] L. N. Korobova, A. V. Tanatova, S. A. Ferapontova, and A. A. Shindelov, Scientific-methodical recommendations on the use of microbiological indicators for assessing the status of agricultural soils in Siberia. Novosibirsk, 2013. (in russ.)

[15] B. M. Dospekhov, Technique of field experience. Moscow, 1985. (in russ.)

[16] N. N. Shuliko, O. F. Khamova, and E. V. Tukmacheva, "Phytotoxicity of leached chernozem soil under growing of spring barley," Vestnik Omskogo gosudarstvennogo agrarnogo universiteta (Bulletin of the Omsk State Agrarian University), No. 4 (24), pp. 52-57, 2016.

[17] A. Kh. Kulikova, A. V. Kozlov, and V. S. Smyvslov, "Influence of Silicon-Containing Materials on Soil Properties, Crop Condition and Yield of Grain Crops in the Conditions of Middle Volga Region," Agrokhimiya (Agrochemistry), No. 4, pp. 60-69, 2019. https://doi.org/10.1134/S0002188119040082

[18] H. Kurzawinska and S. Mazur, "Biological control of potato against Rhizoctonia solani (Kuhn)," Sodininkyste ir Darzininkyste, Vol. 27, No. 2, pp. 419-425, 2008.

[19] H. Kurzawinska and S. Mazur, "The effect of bio-preparations on the infestation of by Streptomyces spp.," Folia Horticulture, Vol. 20, No. 2, pp. 103-110, 2013. https://doi.org/10.2478/fhort-2013-0119

[20] S. Mazur, H. Kurzawinska, J. Nawrocki, and M. Nadziakiewicz, "Natural agents limiting diseases on potato tuber peel," Bulgarian Journal of Agricultural Science, Vol. 22, No. 3, pp. 458-464, 2016.