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Effects of Mixed Fermentation Agents of Biocontrol Fungi on the Growth of Peanut Seedlings

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Abstract

In this laboratory, two kinds of fungi were fermented and cultured to obtain a biocontrol preparation that could promote plant growth and defend against Aspergillus flavus pollution. In the growth process of peanut seedlings, the effects of different concentrations of biocontrol preparation on the growth of peanut seedlings were studied. The experiment was divided into 1 control group and 5 experimental groups. The control group used sterile water for seed invasion and leaf spraying, and the experimental group soaked seeds with 10-fold dilution of preparation, and used sterile water and 0.5%, 1%, 2% and 4% of preparation for spraying during seedling period, respectively. The seedlings were sprayed once every two days and sampled for 10 consecutive times to measure the growth index, chlorophyll content and enzyme activity. The results showed that the spraying agent could effectively promote the growth of peanut seedlings. When the concentration was 2%, the promoting effect was the most obvious. The root length and fresh weight of peanut seedlings could be significantly increased, which were 49% and 42% higher than that of the control, respectively. Chlorophyll content increased slightly after spraying. The results of enzyme activity determination showed that the spraying of preparation could increase the enzyme activity of seedlings. When the spraying concentration was 2%, the activities of PAL, LOX, Rubisco, PEPC and GAPD in carbon metabolizing enzymes and GS and NR in nitrogen metabolizing enzymes all reached the highest values. Therefore, leaf spraying can promote seedling growth and increase metabolism-related enzyme activities, and the best effect is when the spraying concentration is 2%.

Keywords

Biocontrol Preparation; Peanut Seedling; Leaf Spraying; PAL; RuBisCo.

1. Introduction

Trichoderma harzii, a species of Trichoderma harzii, is a fungus with important biocontrol value, which can be parasitic to 29 plant pathogens in 18 genera [1]. Studies have shown that fungal parasitism is one of the main antagonistic mechanisms of Trichoderma, and a series of cell wall degrading enzymes secreted by Trichoderma, such as β-1, 3-glucanase, chitinase, cellulase, protease, etc. play an important role in the fungal parasitic process including growth, recognition, contact, entangling and penetration [2]. These enzymes can degrade fungal cell walls and release oligosaccharides, which can be used as immune activators to induce plant innate immunity, synthesize antimicrobial substances, stimulate plant gene defense [3-6], and enhance plant disease resistance [7]. Nge et al. Found that the addition of chitooligosaccharides in culture medium can promote the growth of orchid tissue [8]. Studies on the growth parameters showed that chitosan and its oligomers could

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obviously promote the growth and yield of crops, vegetables, fruits and flowers. In the field experiment, the number of panicles and the number of grains per panicle of wheat were significantly increased by soaking seeds or spraying chito-oligosaccharide on wheat at different stages, thus increasing the wheat yield [9]. On the other hand, physiological studies have shown that chito-oligosaccharide treatment can also increase the chlorophyll content of plants and expand the volume of chloroplasts, thus promoting the process of photosynthesis [10-11]. In addition, chitosan and its oligosaccharides enhance photosynthesis by amplifying the activities of various enzymes involved in carbon and nitrogen metabolism and enhancing photocooperation [12]. As a result, our laboratory using heavy parasitic characteristics of trichoderma viride, co-culture with aspergillus flavus, get a kind of fermented with low poly oligosaccharide preparation, take the object of peanut seedling, by spraying different concentrations of the preparations, research on the growth of seedlings and the influence of metabolism related enzyme activity, understand the biological effect of jiujixingjun powder.

2. Methods

2.1 Experimental materials

The peanut seeds were Yueyu 7, provided by Guangdong Academy of Agricultural Sciences. Biological control preparation is provided by Jinan University.

2.2 Test method

2.2.1 Preparation of mixed formulations

The preparation was prepared by Laboratory 219, Department of Biomedicine, Second Science and Technology Building, Jinan University.

2.2.2 Seed treatment

Pick peanut seeds with the same degree of fullness, disinfect the surface with 0.1% mercury liters, rinse with sterile water for 4 to 5 times, put the seeds in a 10-fold preparation dilution, soak them at room temperature for 8 hours, and take 11 seeds respectively The seeds were placed in a petri dish covered with moist filter paper, and cultured in a 28°C light incubator for 72 hours. The sterile water was used as a blank control group**.**

2.2.3 Seed planting

After 72 hours, the germinated peanut seeds were loaded into sterilized black soil flower pots, with 3 peanut seeds in each pot, with 3 repetitions for each treatment; cultivated under natural conditions with regular hydration. After the peanuts emerge, spray the leaves on the leaf surface and the back of the leaf. The dosage is appropriate for the drop. The spray concentration is 0.5%, 1%, 2%, 4% of the infestation concentration, and regularly every two days. Sprayed, sprayed 10 times continuously, and the control group was sprayed with distilled water.

2.2.4 Determination of growth indicators of seedlings

After planting, remove the seedlings, remove the black soil on the roots, measure the plant height, root length, and lateral branch length of the peanut seedlings, and count the fresh weight and the number of lateral branches.

2.2.5 Determination of chlorophyll content

Take 100 mg of seedling leaves and determine the chlorophyll content. The chlorophyll content determination method refers to the method of Qiu et al. [31].

2.2.6 Seedling enzyme activity determination

Take 500 mg of seedling leaves and place them in a pre-cooled mortar. Add 2 mL of phosphate buffer (pH 7.4). After being fully ground in an ice bath, add 8 mL of phosphate buffer to mix well, and put it in 15 mL for centrifugation. The tube was centrifuged at 4000 r/min at 4°C for 10 min, and the supernatant was collected. The supernatant was centrifuged at 8000 g at 4°C for 10 min. The supernatant was the crude enzyme extract. Measure Phenylalanine Ammonia Lyase (PAL), Lipoxygenase (LOX), Ribulose-1,5 diphosphate carboxylase (oxygenase) (RuBisCO), and

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Phosphorene according to the instructions of the Elisa enzyme kit. Alcohol Pyruvate Carboxylase (PEPC), Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH), Sucrose Phosphate Synthase (SPS), Glutamine Synthetase (GS), Nitrate Reductase (NR) enzyme activities.

2.3 Data processing

Use Excel 2010 and SPSS (version 22.0) to process and analyze the data, and use Graphpad software for graphing.

3. Results and analysis

3.1 Effects of different concentration of preparation solution on peanut seedling traits

After spraying the preparation on the leaf surface, the growth indexes of peanut increased in different degrees except for the number of lateral branches (Table 1). Under different concentration preparations spraying processing, with the increase of concentration, the growth index also increased, to reach the highest when the concentration of 2%, the plant height, root length, lateral branch length and fresh weight than the control group increased by 18%, 49%, 11% and 42% respectively, and with spraying concentration increased to 4%, the growth index has a little decrease, so the spraying on the leaf concentration was 2% had better effect on the growth of peanut seedling, and spraying on the leaf of root length and fresh weight of seedlings to promote the most obvious effect.

Table 1. Effects of different spraying concentration groups on growth indexes of peanut seedlings

Treatment	stem length/cm	Root length/cm	Lateral branch number	Lateral branch length/cm	raw weight/g
CK	6.96	10.67	4	4.07	2.3
0%	7.55	13.67	4	4.2	2.63
0.5%	7.56	13.85*	4	4.23	2.8
1%	7.92	14*	4	4.4	2.88
2%	8.82 **	15.94**	4	4.51	3.27*
4%	8.57 *	13.8	4	4.3	3.04

Note: * and ** indicate significant differences between the treatment group and the control group at the levels of 0.05 (P<0.05) and 0.01 (P<0.01) in the same column of data, respectively.

CK: Sterile water was used during the seed invasion stage and seedling stage; 0%, 0.5%, 1%, 2%, 4% : 10 times diluted preparation was used in the seed invasion stage, and sterile water and 0.5%, 1%, 2%, 4% preparation of invasion concentration were sprayed respectively in the seedling stage (the same below).

Table 2 Effects of different spraying concentration groups on the chlorophyll content of peanut seedlings

Treatment	Chlorophyll a (mg/g)	change	Chlorophyll b (mg/g)	change	Chlorophyll (mg/g)	change
CK	1.76		0.74		2.5	
0%	1.8	2.27%	0.74	0.00%	2.54	1.60%
0.5%	1.77	0.57%	0.75	1.35%	2.5	0.00%
1%	1.84	4.55%	0.78	5.41%	2.59	3.60%
2%	1.83	3.98%	0.79	6.76%	2.63	5.20%
4%	1.82	1.11%	0.79	6.76%	2.57	1.18%

3.2 Effect of different concentration of preparation solution on the chlorophyll content of peanut seedlings

The influence of different spraying concentration of peanut seedling chlorophyll conditions (table 2), 2% of the highest concentrations of chlorophyll, and contrast ratio increased by 5.2%, the rest of the group the chlorophyll and has no obvious change, but as the spraying concentration increases, the chlorophyll b increase, to 2% when the highest, increased by 6.76%, to 4% when the chlorophyll b

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has no obvious increase. In the same group, the increase of chlorophyll b was greater than that of chlorophyll a, so the foliar spraying of preparation was beneficial to the increase of chlorophyll b of peanut seedlings and the absorption of blue violet light. Therefore, the optimal spraying concentration is 2%

3.3 Effects of different concentrations of preparation solution on phenylalanine ammonia-lyase and lipoxygenase in peanut seedlings

There was no significant change in PAL activity at the four spraying concentrations except for A significant increase at 2% (Fig. 1 (A)). However, with the increase of spraying concentration, the activity of LOX increased at first and then decreased (Figure 1 (B)). The activities of PAL and LOX were increased by 14.54% and 19.63%, respectively, when the activity of PAL and LOX increased by 2%.

For LOX, when the foliar spraying concentration was greater than 1%, the effect was significantly improved. At 1% and 4%, the enzyme activity was increased by 12.53% and 18.38%, respectively. In conclusion, the activities of phenylalanine ammonia-lyase and lipoxygenase were increased by leaf spraying, and the activities of the two enzymes were the highest when the spraying concentration was 2%.

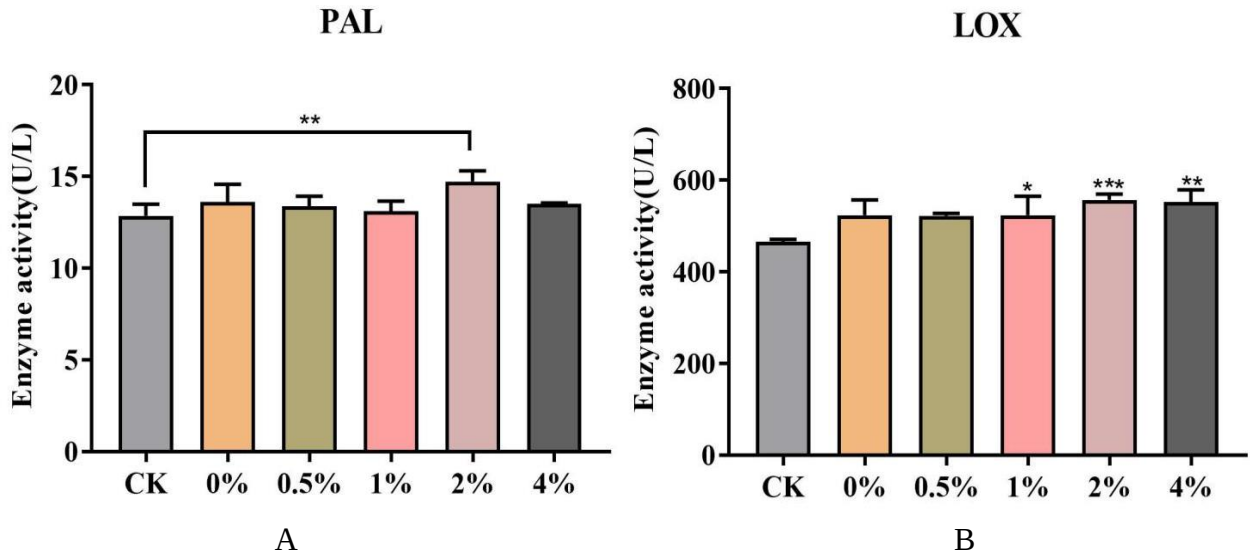

Figure 1. Changes of PAL and LOX activities in peanut seedling leaves under different spraying concentrations

Note: *, ** and *** indicate significant differences between the treatment group and the control group at 0.05 (P<0.05), 0.01 (P<0.01) and 0.001 (P<0.001) levels, respectively.

3.4 Effects of different concentrations of preparation solution on carbon metabolism enzyme activities of peanut seedlings

Fig. 2 shows the changes in activities of carbon metabolism-related enzymes such as ribulose-1, 5-diphosphate carboxylase (RuBisCo), phosphoenolpyruvate carboxylase (PEPC), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and sucrose phosphate synthase (SPS) in peanut seedling leaves under different spraying concentrations. It can be seen that the enzyme activities of Rubisco, PEPC and GAPDH in nitrogen metabolism enzymes increased first and then decreased with the increase of spraying concentration, and the enzyme activities were all higher than those in the control group. It can be seen that spraying leaves with preparation can improve the activities of Rubisco, PEPC and GAPDH in carbon metabolism, increase the carbon metabolism level of peanut seedling leaves, and promote the growth of peanut.

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Compared with the control group, the enzyme activity in 0% group was significantly increased by 9.14% (P<0.05). Therefore, the activity of Rubisco at seedling stage could be improved by soaking peanut seeds with preparation. The activities of Rubisco were increased by 10.84%, 11.25%, 28.24% and 27.98% in 0.5%, 1%, 2% and 4% foliage spraying groups, respectively. The activities of Rubisco in 2% group increased the most, and the enzyme activities of this concentration were also the highest in PEPC and GAPDH, which increased by 11.94% and 17.34%, respectively. Therefore, the optimal spraying concentration was 2%, when the activities of RuBisCo, PEPC and GAPDH could reach the highest. In addition, SPS enzyme activity did not change significantly under the four spraying concentrations, indicating that SPS activity of peanut leaves was not significantly affected by the spraying of the preparation under the concentration range.

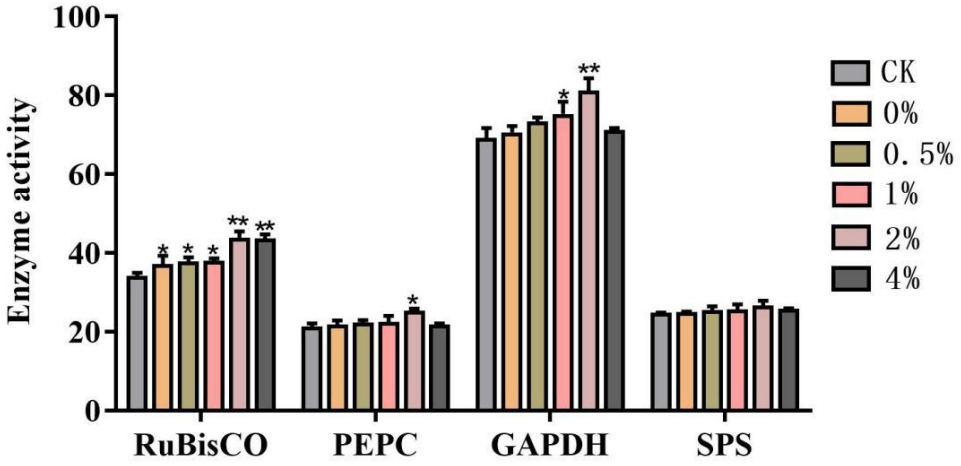

Figure 2. Changes of carbon metabolism enzyme activities in peanut seedling leaves under different spraying concentrations

Note: *, **respectively indicate significant differences at 0.05 (P<0.05) and 0.01 (P<0.01) levels between the treatment group and the control group in the same column of data. Rubisco, SPS, GAPDH :(U/ L); PEPC :(U/ L)

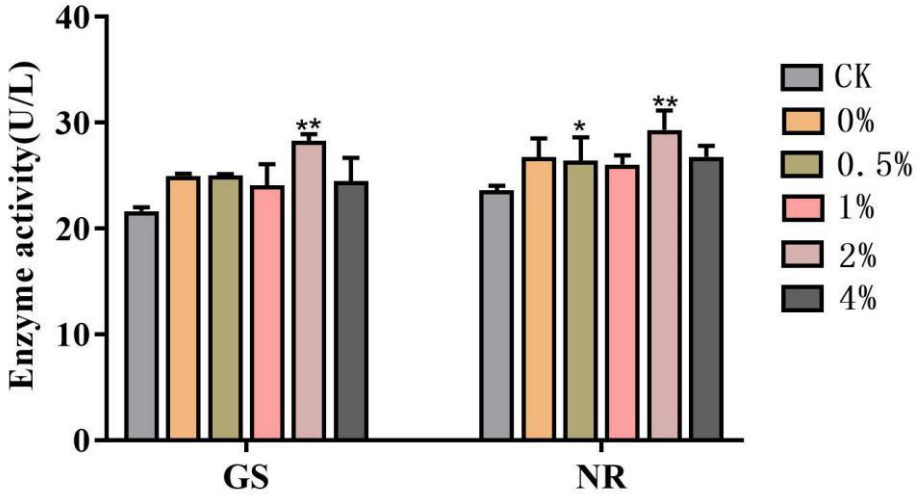

Figure 3. Changes of nitrogen metabolism enzyme activity in peanut seedling leaves under different spraying concentrations

Note: *, **respectively indicate significant differences at 0.05 (P<0.05) and 0.01 (P<0.01) levels between the treatment group and the control group in the same column of data

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3.5 Effects of different concentrations of preparation solutions on the activities of nitrogen metabolism enzymes in peanut seedlings

Figure 3 shows the changes in the activities of plant nitrogen metabolism enzymes such as glutamine synthetase (GS) and nitrate reductase (NR) in peanut seedling leaves under different spraying concentrations. It can be seen from the figure that as the spraying concentration increases, the activities of the two enzymes both increase first and then decrease. At 0%, the activities of the two enzymes were increased by 15.44% and 13.25% respectively compared with the control. Therefore, soaking the seeds with the formulation can increase the activity of nitrogen metabolism enzymes in the seedling stage. The activities of GS and NR both reached the highest value at a concentration of 2%, at which time the enzyme activity increased by 30.79% and 24.08%, respectively. Enzyme activity begins to decrease when the concentration is 4%. It shows that when the spraying concentration is 2%, the activities of glutamine synthetase (GS) and nitrate reductase (NR) in seedling leaves can reach the maximum, and this concentration is the best spraying concentration.

4. Discussion

Studies have found that the application of chitosan can promote the growth and development of plants in different growth stages, and the use of chitosan in the seedling period can improve the germination and seedling vigor of millet [13]. In this experiment, after spraying peanut seedlings with the formulation, each concentration promoted the growth of seedlings to different degrees, and the root length and fresh weight of the seedlings increased significantly. At a concentration of 2%, the root length and fresh weight of the seedlings were increased by 49%. And 42%, which is consistent with the experimental results of Ahmad and Jaleel, they used COS to increase the root length of peppermint and lemongrass [14-15]; in addition, the application of chitosan and its oligosaccharides improved various plants The content of photosynthetic pigment [16-17], among the various concentrations sprayed in this experiment, except for 2%, the chlorophyll increased by 5.20%, and the other concentrations did not change significantly, but the chlorophyll b was at 1%, 2%, and 4%. The concentration increased by 5.41%, 6.76%, and 6.76% respectively. Therefore, spraying of this formulation can increase the content of chlorophyll b in the seedlings and increase the absorption of blue-violet light by the seedlings. Both PAL and LOX play an important role in the response of plants to stress resistance, and their activity is closely related to the strength of plant disease resistance [18]. PAL is the first rate-limiting enzyme of plant phenylpropane metabolism, and is considered to be a plant One of the main defensive enzymes [19], which can convert the amino acid phenylalanine to cinnamic acid and provide substrates for other phenolic compounds such as phenolic acid, lignin and flavonoids [20-21]; LOX can catalyze the conversion of polyunsaturated fatty acids into hydroperoxides, and ultimately generate jasmonic acid, an important plant resistance response signal molecule. In this paper, foliar spraying increased the activities of PAL and LOX, and when the spraying concentration was 2%, the two enzymes had the highest activity, increasing by 14.54% and 19.63% respectively. Therefore, spraying the seedlings can improve the defense against pathogen infection. Ability.

In plant carbon metabolism, the C-3 cycle is the main mechanism of carbon fixation. Rubisco catalyzes the first step of the cycle and promotes the synthesis of 3-phosphoglyceric acid (3-PGA) [22]. Zhang's research shows that wheat After applying COS to seedlings, the activity of Rubisco was significantly increased, and its activity increased by 66.4%, and 3-PGA also increased, which indicated that COS enhanced the fixation of CO2 in seedlings. A carboxylation reaction is catalyzed by PEPC. PEPC uses bicarbonate ions to form oxaloacetate to promote the carboxylation of PEP [23]. Zhang and Jaleel proved that COS treatment significantly increased the activity of PEPC in wheat and lemongrass [24,15]; GAPDH mediates the conversion of glyceraldehyde-3-phosphate into 1,3-diphosphate glyceride, produces NADH, or combines with phosphoglycerate kinase (PGK) to produce ATP[25], Chamnanmanoontham research shows that rice The expression levels of GADPH, enolase and phosphoglucase increased in seedlings after chitooligosaccharide treatment [26]. In this experiment, the activities of three plant carbon metabolizing enzymes, RuBisCO, PEPC and GAPDH, of peanut seedlings were measured. After the preparation was sprayed, the activities of the three

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enzymes all increased to varying degrees, and reached the highest value at a spraying concentration of 2%. , An increase of 28.24%, 11.94% and 17.34% respectively. Therefore, the use of the preparation can increase the level of carbon metabolism of seedlings, thereby promoting plant growth. In addition, in the synthesis of plant secondary metabolites, sucrose is the most important source of carbon metabolism. The synergistic effect of fructose-1,6-bisphosphatase (FBPase) and sucrose phosphate synthase (SPS) is considered to be a key regulatory step in sucrose biosynthesis. The accumulation of sucrose is directly affected by the regulation of the activities of these two enzymes, and the overexpression of FBPase and SPS will lead to the accumulation of sucrose in plants [27]. Zhang reported that the sucrose level of wheat seedlings increased by 1.1 times after using COS heptamer, because the activities of FBPase and SPS increased by 17.5% and 29.4%, respectively [26]. However, in this experiment, there was no significant difference in the SPS activity of the four concentrations of formulations sprayed, which may be because spraying in this concentration range had no significant effect on the SPS activity of peanut leaves.

Nitrogen is one of the most important essential nutrient elements required for plant growth. It is the main component of chlorophyll and protein, which are closely related to the color, growth state and yield of crop leaves [28]. The main enzymes of N metabolism are nitrate reductase (NR), glutamine synthase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH). The activities of these enzymes have been used as The main biochemical indicators for evaluating N status in plants [29]. Mondal research showed that the application of chitosan and chitosan at 75ppm and 100ppm concentrations significantly improved the NR activity of tomato and spinach [30]. After using chitosan heptamer in wheat, the activities of NR, GS, GOGAT and GDH all increased, and the level of glutamate also increased by 80.0% [23]. Therefore, the use of chitosan oligosaccharides can increase the activities of enzymes related to nitrogen metabolism in plants, resulting in an increase in amino acid content in the body. In this article, the enzyme activities of GS and NR increased after the four concentration preparations were used, and reached the highest value at 2%, which increased by 30.79% and 24.08%, respectively. It can be seen that the use of the preparation can improve the nitrogen metabolism of peanut seedlings. Level, increase the activity of related enzymes.

In summary, the formulation developed by our laboratory has the ability to promote seedling growth and improve defense against pathogen infection. Spraying the formulation during peanut seedling stage can increase defense enzymes such as PAL and LOX in the body, as well as RuBisCO, PEPC, GAPDH, GS and NR and other metabolic enzyme activities can promote the growth of seedlings, and the growth promotion effect is best when the spray concentration is 2%.

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References

[1] Harman GE, Howell CR, Viterbo A, et al. Trichoderma species--opportunistic, avirulent plant symbionts. [J]. Nature Reviews Microbiology, 2004, 2(1):43-56.

[2] Xu Tong, Liu lianghao. Chitinase from Trichoderma spp. and its antagonism against plant pathogenic fungi [J]. Acta phytopathologica Sinica, 2002,32 (002): 97-102. (In Chinese)

[3] Das SN, Madhuprakash J, Sarma PVSRN, et al. Biotechnological approaches for field applications of chitooligosaccharides (COS) to induce innate immunity in plants[J]. Critical Reviews in Biotechnology, 2015, 35(1):29-43.

[4] Zhang Fuyun, Zhao Xiaoming, Bai Xuefang, et al. Research progress of chitooligosaccharide induced plant disease resistance [J]. Chinese Journal of biological control, 2008, 24 (002): 174-178. (In Chinese)

ISSN: 2414-1895 DOI: 10.6919/ICJE.202105_7(5).0035

[5] Hu Ju Wei, Liu Hui, Ma Xiu Ming, et al. Review on antibacterial, inducing and promoting effects of chitosan and its application in agriculture [J]. Jiangsu Agricultural Sciences, 2018,046(015):1-5.(In Chinese)

[6] Hamel LP, Beaudoin N. Chitooligosaccharide sensing and downstream signaling: contrasted outcomes in pathogenic and beneficial plant–microbe interactions[J]. Planta, 2010, 232(4):787-806.

[7] Lei Fei, Zhang Dongming, Tan Hao, et al. Effect of foliar spraying chitooligosaccharide on Yield and quality of cherry tomato and control effect of late blight [J]. Guizhou Agricultural Sciences, 2019, 047 (009): 74-77. (In Chinese)

[8] Nge KL, Nwe N, Chandrkrachang S, Stevens WF. Chitosan as a growth stimulator in orchid tissue culture. Plant Science, 2006, 170: 1185-1190.

[9] Yin, Heng, Zhao, et al. Effects of chitosan oligosaccharides on the yield components and production quality of different wheat cultivars (Triticum aestivum L.) in Northwest China[J]. Field Crops Research, 2015, 172: 11-20.

[10] Limpanavech P, Chaiyasuta S, Vongpromek R, Pichyangkura R, Khunwasi C, et al. Chitosan effects on floral production, gene expression, and anatomical changes in the Dendrobium orchid. Scientia Horticulturae, 2008, 116: 65-72.

[11] Zhang XQ, Li KC, Liu S, Xing RG, Yu HH, et al. Size effects of chitooligomers on the growth and photosynthetic characteristics of wheat seedlings. Carbohydrate Polymers, 2016, 138: 27-33.

[12] Ahmed KBM, Khan MMA , Siddiqui H , et al. Chitosan and its oligosaccharides, a promising option for sustainable crop production- a review[J]. Carbohydrate Polymers, 227.

[13] Manjunatha, G., Roopa, K. S, Prashanth, G. N., & Shekar Shetty, H. (2008). Chitosan enhances disease resistance in pearl millet against downy mildew caused by Sclerospora graminicola and defence-related enzyme activation. Pest Management Science: formerly Pesticide Science, 64(12), 1250–1257.

[14] Ahmad, B, Khan, MMA, Jaleel, H, Sadiq, Y, Shabbir, A, & Uddin, M. (2017). Exogenously sourced γ-irradiated chitosan-mediated regulation of growth, physiology, quality attributes and yield in Mentha piperita L. Turkish Journal of Biology, 41(2), 388–401.

[15] Jaleel, H., Khan, M. M. A., Ahmad, B., Shabbir, A., Sadiq, Y, Uddin, M, & Varshney, L. (2017). Essential oil and citral production in Field-Grown Lemongrass in response to gamma-irradiated chitosan. Journal of Herbs, Spices & Medicinal Plants, 23(4), 378–392.

[16] Ali, A. (2014). Response of Eucalyptus citriodora hook to the application of gamma irradiated polysaccharides. Doctoral dissertationAligarh Muslim University.

[17] Brash A R.Lipoxygenases:occurrence, functions, catal-ysis, and acquisition of substrate[J]. J. Biological Chemis-try, 2006, 163 (3) :348-57

[18] activities and reduced boron concentration in Cucumis sativus L. Acta Physiologiae Plantarum, 39(1), 31.

[19] Zikang, Cai, Rong, et al. Effect of preharvest application of Hanseniaspora uvarum on postharvest diseases in strawberries - ScienceDirect[J]. Postharvest Biology and Technology, 2015, 100(100):52-58.

[20] Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. The Plant Cell, 7(7), 1085.

[21] Ververidis, F, Trantas, E, Douglas, C, Vollmer, G, Kretzschmar, G, & Panopoulos, N. (2007). Biotechnology of flflavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. Biotechnology Journal: Healthcare Nutrition Technology, 2(10), 1214–1234.

[22] Tamoi, M., Nagaoka, M, Yabuta, Y, & Shigeoka, S. (2005). Carbon metabolism in the Calvin cycle. Plant Biotechnology, 22(5), 355–360.

[23] Zhang, X, Li, K, Liu, S, Zou, P, Xing, R., Yu, H., & Li, P. (2017). Relationship between the degree of polymerization of chitooligomers and their activity affffecting the growth of wheat seedlings under salt stress. Journal of Agricultural and Food Chemistry, 65(2), 501–509.

[24] Zhang, X, Li, K, Xing, R, Liu, S, Chen, X., Yang, H, & Li, P. (2018). miRNA and mRNA expression profifiles reveal insight into chitosan-mediated regulation of plant growth. Journal of Agricultural and Food Chemistry, 66(15), 3810–3822.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202105_7(5).0035

[25] Plaxton, W.C. (1996). The organization and regulation of plant glycolysis. Annual Review of Plant Biology, 47(1), 185–214.

[26] Chamnanmanoontham, N, Pichayangura, R, Comai, L, Chadchawan, S, & Chadcahwan, S (2014)A genetic screen of chitosan-resistant mutant in Arabidopsis thaliana. Genomics and Genetics, 7(1), 51–60.

[27] Tamoi, M., Nagaoka, M., Yabuta, Y., & Shigeoka, S. (2005). Carbon metabolism in the Calvin cycle. Plant Biotechnology, 22(5), 355–360.

[28] Fageria, N. K, Moreira, A, & Coelho, A.M. (2011). Yield and yield components of upland rice as inflfluenced by nitrogen sources. Journal of Plant Nutrition, 34(3), 361–370.

[29] Ren, B., Dong, S., Zhao, B., Liu, P., & Zhang, J. (2017). Responses of nitrogen metabolism, uptake and translocation of maize to waterlogging at diffffferent growth stages. Frontiers in Plant Science, 8, 1216.

[30] Mondal, M. M. A., Rana, M. I. K., Dafader, N. C., & Haque, M. E. (2011). Effffect of foliar application of chitosan on growth and yield in Indian spinach. J. Agrofor. Environ, 5(1), 99–102.

[31] Qiu NianWei, Wang xiushun, Yang Fabin, et al. Rapid extraction and precise determination of chlorophyll [J]. Acta botany Sinica, 2016, 51 (05): 667-678. (In Chinese)