IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-1, 2019, pp330-335
http://www.bipublication.com

Research Article

Evaluation of the Activity of Spring Wheat Extracts (*TRITICUM AESTIVUM* L.)

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[Received: 19/12/2018; Accepted: 23/01/2019; Published: 24/01/2019]

ABSTRACT.

Background. Application of growth stimulators based on plant-derived substances is a highly potential approach to the increase of agricultural crops production. This approach is especially relevant in organic farming. Seeds are one of the most valuable sources of substances that exert stimulating effect. During germination of seeds, a wide range of phytohormones (auxins, gibberellins) are expressed that stimulate plant growth. Seed extracts can be obtained by different methods.

The aim of the present study was to evaluate the influence of seed extracts (water, ethanol, enzyme), obtained from germinated spring wheat seeds (*Triticum aestivum* L.), on the growth and development of plants, and to assess their fungicide activity towards *Fusarium oxysporum*.

Scope. The main method of the study was the evaluation of the influence of the extracts, obtained from germinated wheat seeds, on the growth of plant models and of the fungicide activity in laboratory conditions.

The analysis of different wheat extracts (water, ethanol, enzyme) was performed. It was established that the enzyme extracts exerted expressed growth stimulating effect at the early stages of wheat development and contributed to the enhancement of seed germination. The activity of the wheat extracts without vernalization had higher activity than the ones with vernalization. Only wheat water extracts exerted fungicide activity towards *Fusarium oxysporum*.

Conclusion. Materials of the study showed that wheat enzyme extracts can be used for the development of organic growth stimulators suitable for the application in organic farming.

Key words: extracts, growth stimulators, phytohormones, fungicide activity, *Fusarium oxysporum*, spring wheat

INTRODUCTION:

Regulation of plant growth and development remains one of the main tasks in crop management (Sharma et. al., 2013). Application of plant growth regulators (PGRs) allows the producers to optimize the plants growth, root:shoot ratio, stimulate photosynthesis and enhance plants stress resistance (Rajala and Peltonen-Sainio, 2001; Cromey et al., 2004; Bingham and McCabe, 2006; Beasley et al. 2012; Akram et al. 2012; Barányiová and Klem, 2016).

For this purpose, natural and synthetic phytohormones are used, as well as the substances that influence on the expression and activity of phytohormones (Yan et al., 2011).

Application of biologically active substances expressed by plants is becoming more widespread in the development of growth regulators and biofungicides (Wilson et. al., 1998; Andresen, &Cedergreen, 2010). Application of plant extracts as PRGs is explained by the fact that they contain different substances, including growth-promoting hormones (Ulfa et al., 2013). Amino acids and proteins contained in plant extracts exert expressed growth stimulating effect when applied to different farm crops (Calvo et. al., 2014).

Many plant extracts have an anti-stress effect and can be used as biological products for pathogens control and abiotic stress protection (Helmy, 1972; Davidson, 1998; Barkai-Golan, 2001; Arya and Perelló, 2010). High inhibiting activity of some plant extracts towards numerous diseases was observed after wheat and other grain crop seeds treatment (Antoniazzi et al., 2008; Yassin et al. 2012; Perelló et al., 2013). Some garlic and onion extracts enhanced wheat seeds germination and inhibited the growth of *Ustilago tritici* (Rajendra et al., 2014). At the same time, the studies on the evaluation of the effectiveness of different extracts were conducted primarily on wild or medicinal plants. There were no studies performed on the evaluation of extracts activity towards wheat seeds germination and their adaptogenic activity.

MATERIALS AND METHODS

The extracts were obtained from spring wheat seeds (*Triticum aestivum* L.) of Joldyz variety. Before the germination, the seeds were sterilized in 70% ethanol for 5 minutes. After drying out in filter paper, the seeds (100 kernels in three replications) were placed in moist germination chambers (germination units with sterile filter paper and sterile water). Germination was performed by two methods: 1) at +24°C (without vernalization) for 7 days, 2) at +24°C for 3 days and then at + 8°C (with vernalization) for 4 days in cool chamber. After 7 days, the seeds were dried out at 14% of humidity and fined in a lab mill. As a result, fine mass for extraction was obtained. Air-dry, fine, dark gray forest soil (humus content 5.2%) was used as a standard.

Extraction. 1 g of the studied material and 1 g of soil was used for extracts preparation.

Water extraction. 10 ml of sterile distilled water (85°C) was added to 1 g of the studied material. Extraction time was 12 hours. After the extraction, the supernatant fluid was filtered via a paper filter. Sterile water was added to 5 ml of the extract to the volume of 10 ml. All the procedures were performed in three replications.

Ethanol extraction. 10 ml of 95% ethanol (25°C) was added to 1 g of the studied material. Extraction time was 12 hours. After the extraction, the supernatant fluid was filtered via a paper filter. Ethanol was evaporated and the residue was diluted in 10 ml of sterile water.

Enzyme extraction. 1 mg of pancreatin (digestive enzyme, 10 000 UA) was added to 1 g of the material and 10 ml of sterile water (35°C). Extraction time was 12 hours. After the extraction, the supernatant fluid was filtered via a paper filter. Sterile water was added to 5 ml of the extract to the volume of 10 ml. All the procedures were performed in three replications.

Tests variants. 1. Wheat water extract without vernalization - WWEWV; 2. Wheat ethanol extract without vernalization - WEEWV; 3. Wheat enzyme extract without vernalization – WEnEWV; 4. Wheat water extract with vernalization - WWEV; 5. Wheat ethanol extract with vernalization – WEEV; 6. Wheat enzyme

extract with vernalization – WEnEV; 7. Soil water extract – SWE; 8. Soil ethanol extract – SEE; 9. Soil Enzyme extract – SEnE.

Evaluation of the extracts activity towards spring wheat germination. Joldyz variety wheat seeds were used as the test object. The seeds were kept in the extracts for 4 hours at +25°C. The seeds were dried out and placed into humid chambers in Petri dishes (10 pcs in three replications). Humid chambers were placed into a thermostat at +23°C for 7 days. After that, germination (number of seeds that gave normal sprouts and roots) was evaluated, maximum length of primary roots and sprouts length were measured.

Evaluation of fungicide activity. Pathogenic micromycete *Fusarium oxysporum,* that causes root rot in wheat crop, was used as the object of the test. The tests were performed in Potato-Dextrose Agar (PDA) medium. A disc with mycelial culture 5 mm in diameter was taken by a sterile probe from the peripheral area of the ten-day culture and was placed into the center of a Petri dish with medium at 28 ± 2°C. Sterile paper discs 1 cm in diameter were treated with the extracts and placed in the medium. The measurement of the area of fungus growth inhibition was performed on the 5^{th} day of the incubation at +28°C. Two series of tests were performed in four replications.

RESULTS

The test results of the extracts influence on seeds germination are presented in Table 1.

Table 1. Germination of spring wheat seeds after the treatment with extracts, %

Test variant	Laboratory germination, %	Significance of the difference as compared with the control
Water - control	76.7	
WWEWV	93.3*	+
WEEWV	70.0	–
WEnEWV	93.3*	+
WWEV	86.7*	–
WEEV	90.0*	+
WEnEV	76.5	–
SWE	86.3*	+
SEE	70.0	–
SEnE	90.0*	+

Note: * – significant difference as compared to the control P=0.05

The most significant stimulating effect on the germination of wheat seeds was exerted by wheat water and enzyme extracts without vernalization (WWEWV and WEnEWV). Positive effect on the seeds germination in these test variants was more significant than in the tests with soil extracts.

The influence of extracts on maximum length of the primary root and sprouts is presented in Table 2. As it can be seen in the table, maximum sprout length and development of the primary roots were observed after the seeds treatment with wheat enzyme extract without vernalization. This test variant results exceeded the control and all the soil extracts parameters values.

All the wheat extracts without vernalization were characterized by the expressed stimulation of the root system growth, significantly exceeding the values observed in the tests variants with the seeds vernalization.

Growth stimulation of sprouts was observed after the treatment with wheat enzyme extract with vernalization.

Table 2. Length of sprouts and maximum length of the primary root, mm

Test variant	Length of wheat sprout, mm	Maximum length of the root, mm
Water – control	20.78	29.44
WWEWV	29.39*	**63.33***
WEEWV	33.76*	**64.91***
WEnEWV	**45.12***	**71.29***
WWEV	33.04*	44.77*
WEEV	34.19*	39.52*
WEnEV	**42.79***	58.21*
SWE	19.12	31.77
SEE	22.47	40.14*
SEnE	24.63	37.07*

Note: * – significant difference as compared to the control P=0.05

The test results on the influence of extracts on pathogen fungus *Fusarium oxysporum* are presented in Table 3.

Table 3. Influence of extracts on the growth of *Fusarium oxysporum* in Potato Dextrose Agar Medium (PDAM)

Test variant	Area of fungus colony growth inhibition, mm	Fungicide effect
Water – control	0	-
WWEWV	4.2	+
WEEWV	0	-
WEnEWV	0	-
WWEV	2.1	+
WEEV	0	-
WEnEV	0	-
SWE	0	-
SEE	0	-
SEnE	0	-

Phytopathogen fungus growth inhibition was observed after the seeds treatment with wheat water extracts without vernalization and with vernalization (WWEWV and WWEV). In all the other cases, the inhibition of fungus colony growth was not registered. Probably, the substances that exerted fungicide activity remained only in wheat water extracts.

DISCUSSION.

The spring wheat extracts exert stimulating effect on the development of plants at early stages of ontogenesis. The most intensive activity was observed after the application of wheat enzyme extracts without vernalization. Seed treatment with the extracts significantly enhanced the seeds germination and increased the length of the roots and sprouts. Probably, during enzymatic hydrolysis, the substances that stimulate plants development are synthesized, like tryptophan that takes part in the synthesis of a phytohormone auxin (Zhang et al. 2008). Seeds vernalization does not enhance the extracts activity, which can be associated with the slowdown of phytohormone synthesis during germination. Fungicide activity towards *Fusarium oxysporum* was observed only after the application of wheat water extracts, primarily, without vernalization. Since water extracts model the processes that are observed in soil under natural conditions during wheat germination, this effect can be one of the mechanisms of crop protection against fusaria root rot.

CONCLUSION.

Spring wheat enzyme extracts enhance seeds germination and exert a growth stimulation effect, so they can be used for the development of plant-derived growth stimulators.

Recommendations.

The obtained results can be used as the basis for the development of growth stimulators for the treatment of spring wheat seeds.

ACKNOWLEDGEMENTS.

The present study was conducted with the financial support of the Ministry of Education and Science of the Russian Federation. The number of the agreement for the subsidy is №14.610.21.0017. The unique identification number of the project is RFMEFI61017X0017.

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