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Ultrasound seed treatment for organic farming

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Abstract

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Seed treatment is usually associated with the application of chemical, biological or physical agents to control pest and diseases, transmitted by the seeds. The conventional crop technology relies mostly on the chemical fungicides and insecticides that are unacceptable in organic farming. Common winter wheat is a major cereal culture all over the world. The main objective of the present study is to investigate the influence of ultrasonic treatment on the improvement of germination rate and the initial development of seedlings of common winter wheat and the destruction of pathogens transported by the seeds. Treating common wheat seeds with ultrasound is an alternative to conventional agriculture, meeting the requirements for minimal chemistry. Treatment with 42 kHz in an ultrasonic device having various duration: 3-9-21 min, at constant germination temperature of 22°C, has been tested on healthy and inoculated seeds. Greatest differences between the control and treated variants of healthy seeds were observed after sonification for 3 and 9 min. Sonication of contaminated seeds is particularly valuable as the shortest duration -3 min, gives significant improvement of their initial development – germination energy increases by 46%, germination rate by 35% and growth of seedling by 33%. Shorter irradiation for 3 and 9 min leads to enlarged leaves of the young plantlet on the 5^{th} and 9^{th} day from the germination. Longer treatments are unnecessary as in this case serious negative effect is shown – decrease with more than 20 % of the length of the leaves. The development of the contaminated seeds after different duration of ultrasound treatment is stronger and quicker, with no symptoms of any plant disease, but the results from analyses of coleoptile length, even statistically significant, are contradictory. Shorter exposition to ultrasound for 3 minutes enhances the coleoptile length on the 9^{th} day. Quicker development of the leave on the 3^{rd} day is observed at longer sonication for 9 min. Biggest accumulation of total biomass is achieved at the longest treatment of the seeds. The method of sonication with 42 kHz in water bath has been shown to be a good alternative for destroying fungi in seeds and accelerating the growth rate of young shoots which improves their competitiveness against weeds and avoids the need to use a herbicide as an alternative to organic farming.

Keywords: ultrasound; seed treatment; wheat; germination; organic farming

Introduction

The quality seed is the first step in the agricultural production. Well established and developed crops have better phytosanitary conditions, nutrition and yield capacity. Pathogens and pests transmitted by seeds are controlled mainly by chemical substances unappropriated for use in the organic farming. This treatment is often accompanied by chemical additives for promotion of seed germination and early development of the shoots. Seed invigoration by various methods has many advantages and is still challenging the seed industry (De Araújo et al., 2016).

The use of physical methods to stimulate plant growth is increasingly attracting farmers as an alternative to chemical methods by offering ways to improve food quality without compromising their safety. Physical methods used in agronomic science are varied and include the treatment of seeds and plants with electrical or magnetic field, sound, ultrasound, radiation with laser radiation of different wavelengths.

Decontamination of seed from different cultures by ultrasound treatment is one of the less studied strands. It is clear from the literature that ultrasonic irradiation is mainly used to stimulate germination of seeds (Suslick, 1994; Yaldagard et al., 2008b,c).

In biotechnological processes, the ultrasonic method is widely used on a laboratory scale and does not require complicated equipment and great technical expertise. It has been found that ultrasound treatment can alter the state of the substances and even speed up the reactions (Suslick, 1994). The chemical effects of ultrasound are varied and involve significant improvements in chemical reactions. The structure and function of biological molecules can be changed by ultrasonic irradiation. The most common mechanisms of interaction involved in this case are either thermal or chemical effects and acoustically induced cavitation activity. In addition, changing the function of biomolecules by ultrasound can also be caused by a mechanical effect, which is the shear stress resulting from eddy currents (Eddie) resulting from shock waves of ultrasound.

Ultrasound exerts its effects mainly through the phenomenon called cavitation. Cavitation is the formation, growth and sometimes implosion of microbubbles created in a fluid in which ultrasound waves propagate. Bubble collapse leads to accumulation of energy at hot spots where the temperature reaches 1000 K and the pressures are approximately 500 (Suslick, 1990). It is known that ultrasound waves have the potential to affect living cells. Ultrasound acts as an alternative stress on cells or tissues and can therefore be used to kill pathogenic organisms.

Research on the benefits of ultrasound technology in agrobiology dates back more than half a century (Gordon, 1963), but is still very attractive (Goussous et al., 2010; Kwiatkowska et al., 2011). The application of ultrasonic stimulation to increase seed germination in the early stages of plant growth has been investigated for different crops: chickpeas, wheat, pepper and watermelon (Kwiatkowska et al., 2011), corn (Goussous et al., 2010; Hebling et al., 1995), rice (*Oryza sativa* Nipponbare) (Liu et al., 2003), pepper, tomatoes and cucumbers (Markov et al., 1987), fodder (Rubtsova, 1967), radishes (Shimomura, 1990), carrots (Aladjadjiyan, 2002), decorative trees (Aladjadjiyan, 2003a,b). Ultrasonic seed treatment is also used for industrial purposes such as oil extraction and malt preparation (Aladjadjiyan, 2011; Kobus, 2008; Yaldagard et al., 2008b,c), as the destruction of cells by shock from the high intensity ultrasound waves facilitates the extraction of substances.

The effect of ultrasonic treatment at 22 kHz and a power of 150 W on the germination energy and germination of carrot seed (*Daucus carota* L.) were studied by Yaldagard et al. (2008). Maximum effect was found in 5-minute treatment.

Toma et al. (2001) investigates the effect of ultrasound treatment with a higher incidence on lens and wheat development. Short exposure times have been selected. Low-intensity ultrasound stimulates the germination of seeds from several plant species: barley (Aladjadjiyan, 2011; Kobus, 2008), carrot (Markov et al., 1987), corn (Hebling et al., 1995), feed fodder (Rubstova, 1967), spruce from Norway (Kwiatkowska et al., 2011). However, the comparison of these results is difficult because they are obtained at different frequencies, different ultrasound intensities and different treatment duration. Studies have shown that stimulation of seed germination by ultrasound is due to increased enzyme activity, such as alpha-amylase, involved in the maltose and maltodextrin metabolism (Yaldagard et al., 2008a).

Studies on the impact of ultrasound on white yarrow are known (Davoud Salar Bashi, 2012). In these studies, the yarrow seeds were treated with ultrasound, laser beam, magnetic field, gamma and beta radiation respectively for 210 and 300 s. It has been established that the pretreatment of the yarrow seeds with ultrasound and magnetic field results in improved yields and yields (Davoud Salar Bashi, 2012).

Experiment

The main objective of the present investigation is to study the influence of ultrasonic treatment on the improvement of germination rate and the initial development of seedlings of common winter wheat and the destruction of pathogenic fungi transmitted by the seeds.

To achieve this goal, the following tasks are addressed:

- Investigation of the influence of ultrasonic treatment on the increase of germination rate of wheat;
- Investigation of the possibilities of ultrasonic treatment for the destruction of pathogens in wheat seeds.

The experimental work was carried out in the Laboratory of Physics of the Agricultural University of Plovdiv, in June and July 2017. Seeds of common winter wheat, variety Zebrets have been studied: healthy and infected with phytopathogens. The healthy and diseased seeds were pre-soaked in water in separate dishes for 2 days, and then irradiated with a 42 kHz ultrasound. The irradiation duration was 3 min, 9 min and 21 min. A CARRERA – SINUS ultrasonic bath was used Model: 2501 (Figure 1) with the following technical data:

- Supply voltage: 230 V; 50 Hz; 50 W;
- Ultrasonic frequency: 42 kHz

Fig. 1. CARRERA – SINUS ultrasonic bath used to irradiate wheat seeds

All experimental variants were set in two replicates of 30 seeds per variation. Germination rate measurements were performed on days 3, 5 and 9 as follows:

- On the 2nd day, the number of germinated seeds was counted;
- On the 5th day, the height of the seedlings was recorded;
- On the 9th day the height of the seedlings was recorded; length of 2nd leaf and the total plant biomass.

Results and Discussion

Treatment with 42 kHz in a water bath ultrasonic CARRERA – SINUS for 3 – 9 and 21 min has statistically significant impact on the development of the seeds and seedlings of common winter wheat. Calculated germination energy and seed germination in percent as a ratio of seed germination to total seed number on days 2 and 5 respectively, and the percentage of normal developed seedlings on day 9 are present on Table 1. Greatest differences between the control and treated variants of healthy seeds are observed after sonification for 3 and 9 min. Shorter irradiation can be offered as practical priming method for enhancement the generation speed, as found by Goussous et al. (2010), and early establishment of the crop on the field.

Sonication of contaminated seeds is particularly valuable as the shortest duration -3 minutes, gives significant improvement of their initial development – germination energy increases by 46%, germination rate by 35% and growth of seedling by 33% (Table 2). No symptoms of any disease have been observed on the irradiated seedlings. Further analyses for the explanation of the mechanism for overcoming the infestation by the young plantlets are needed. This early emergence and wheat establishment can be useful in cases of late sowings in the autumn. For organic farming the production of healthy foods, clear of pesticides, can be stimulated by such improvement of the germination energy, germination rate and quicker establishment of crop with healthy seedlings without use of any chemical fungicide or plant stimulator.

The Germination energy (%) of Zebrec seeds on 2nd, 5th and 9th day as function of time for irradiation with ultrasound with frequency 42 kHz, for 3, 9 and 21 min are presented on Figures 2, 3 and 4.

The positive effect of the ultrasound treatment with 42 kHz in water bath CARRERA – SINUS of common winter wheat seeds is obvious and on the growth of the seedlings (Table 3). Shorter irradiation for 3 and 9 min leads to enlarged leaves of the young plantlet on the 5th and 9th day from the germination. Longer treatments are unnecessary as in this case serious negative effect is shown – decrease with more than 20 % of the length of the leaves is shown on Table 3.

The development of the contaminated seeds after different duration of ultrasound treatment is stronger and quicker, with no symptoms of any plant disease as shown

Table 1. Germination rate of heathy Zebrec seed treated with 42 kHz ultrasound for different time of exposition

ET	GE	Δ GE	GR	Δ GR	GS	Δ GS
0 – healthy control	63		70		73	
3 min	77	122	77	110	80	110
9 min	70	111	80	104	90	113
21 min	53	84	60	75	67	74

Legend: ET- Exposition time, GE, germination energy (germinated/undeveloped seeds) in %; GR, germination rate (germinated/undeveloped seeds) in %; GS – growth of seedlings, in %; Δ difference in percent

The statistical significance of the differences was proved by the test of Student:

$t = 0.189 < t_{tabl.} = 2.02$ (irradiation for 3 min);

$t = 0.01 < t_{tabl.} = 2.02$ (irradiation for 9 min);

$t = 1.45 < t_{tabl.} = 2.02$ (irradiation for 21 min).

Table 2. Germination rate of contaminated Zebrec seed treated with 42 kHz ultrasound for different time of exposition

ET	GE	Δ GE	GR	Δ GR	GS	Δ GS
0 – contaminated control	50		57		60	
3 min	73	146	77	135	80	133
9 min	67	92	77	100	77	96
21 min	63	94	67	87	70	91

Legend: ET- Exposition time, GE, germination energy (germinated/undeveloped seeds) in %; GR, germination rate (germinated/undeveloped seeds) in %; GS – growth of seedlings, in %; Δ difference in percent

The statistical significance of the differences was proved by the test of Student:

$t = 0.189 < t_{tabl.} = 2.02$ (irradiation for 3 min);

$t = 0.01 < t_{tabl.} = 2.02$ (irradiation for 9 min);

$t = 1.45 < t_{tabl.} = 2.02$ (irradiation for 21 min).

The Germination energy (%) of Zebrec seeds on 2nd, 5th and 9th day as function of time for irradiation with ultrasound with frequency 42 kHz, for 3, 9 and 21 min are presented on Figures 2, 3 and 4.

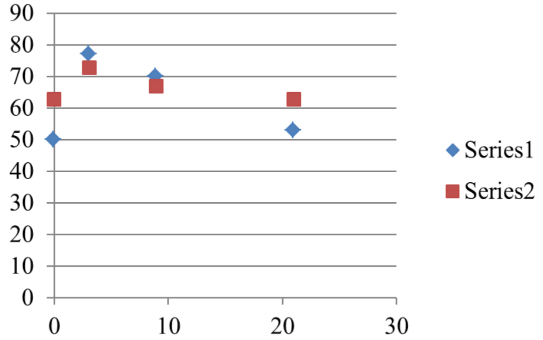

Fig. 2. Germination energy (%) of Zebrec seeds on 2nd day as function of time for irradiation with ultrasound with frequency 42 kHz, for 3, 9 and 21 min. (Series 1 – healthy; 2 – contaminated)

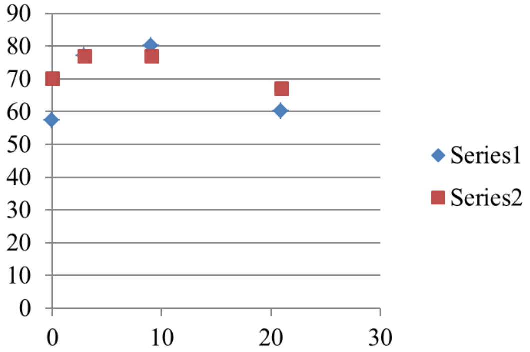

Fig. 3. Germination (%) of Zebrec seeds on 5th day as function of time for irradiation with ultrasound with frequency 42 kHz, for 3, 9 and 21 min. (Series 1 – healthy; 2 – contaminated)

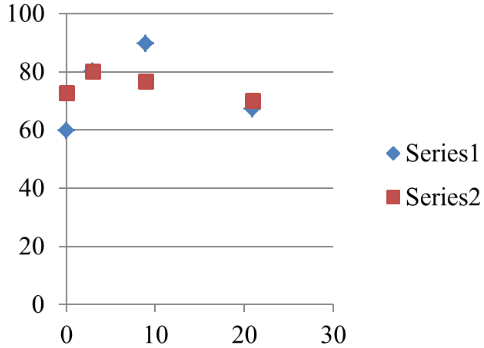

Fig. 4. Normally developed seedlings (%) of Zebrec seeds on 9th day as function of time for irradiation with ultrasound with frequency 42 kHz, for 3, 9 and 21 min. (Series 1 – healthy; 2 – contaminated)

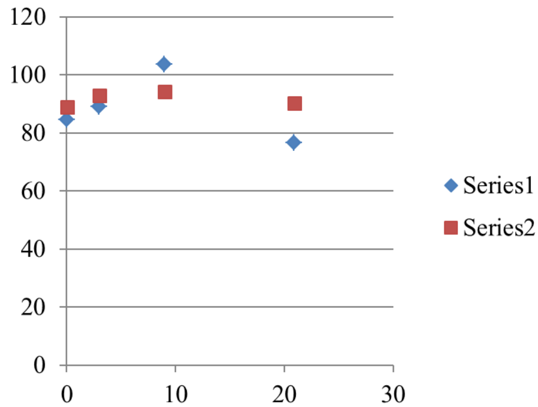

Fig. 5. Coleoptile length (mm) of Zebrec, measured on the 5th day after irradiation of the seeds with ultrasound with frequency 42 kHz, for 3, 9 and 21 min, as function of time. (Series 1 – healthy; 2 – contaminated)

Table 3. Growth parameters for healthy Zebrec seedlings, irradiated with ultrasound with frequency of 42 kHz for 3, 9 and 21 min

Time for irradiation with US, minutes	Coleoptile length on 5th day, mm	Δ	Coleoptile length on 9th day, mm	Δ	Total plant weight on 9th day, mg	Δ
0 – healthy control	85		194.06		158.82	
3 min	89.09	105	221.62	114	195.42	123
9 min	103.29	116	245.32	111	220.32	113
21 min	76.39	74	194.3	79	173	79

Table 4. Growth parameters for contaminated Zebrec seedlings, irradiated with ultrasound with frequency of 42 kHz for 3, 9 and 21 min

Time for irradiation with US, min	Coleoptile length on 5th day, mm	Δ	Coleoptile length on 9th day, mm	Δ	Total plant weight on 9th day, mg	Δ
0 – contaminated control	88.63		185.36		178.57	
3 min	69.17	78	214.96	116	174.58	98
9 min	94.25	136	205.78	96	173.48	99
21 min	89.91	95	198.38	96	182	105

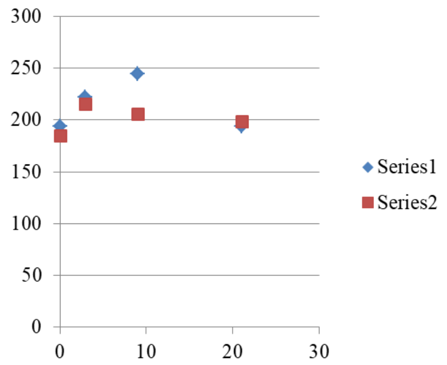

Fig. 6. Coleoptile length (mm) of Zebrec, measured on the 9th day after irradiation of the seeds with ultrasound with frequency 42 kHz, for 3, 9 and 21 min, as function of time. (Series 1 – healthy; 2 – contaminated)

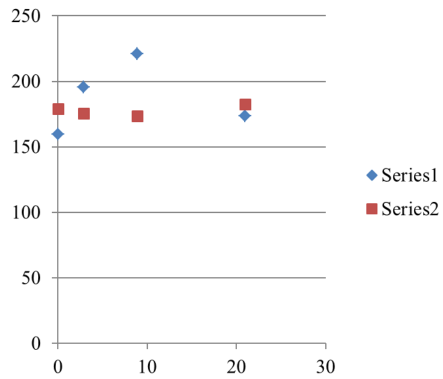

Fig. 7. Total plant weight (mg) of Zebrec, measured on the 9th day after irradiation of the seeds with ultrasound with frequency 42 kHz, for 3, 9 and 21 min, as function of time. (Series 1 – healthy; 2 – contaminated)

before, but the results from analyses of coleoptile length, even statistically significant, are contradictory (Table 4). Shorter exposition to ultrasound for 3 min enhances the coleoptile length on the 9th day. Quicker development of the leave on the 3rd day is observed at longer sonication for 9 min. The biggest accumulation of total biomass is achieved at the longest treatment of the seeds.

The coleoptile length (mm) of Zebrec, measured on the 5th and 9th day after irradiation of the seeds with ultrasound with frequency 42 kHz, for 3, 9 and 21 min, as function of time are shown on Figures 5 and 6.

Prolonged treatment (21 min) of wheat seeds with ultrasound at a frequency of 42 kHz leads to single cases of strain deformation. Observation only, without additional research does not allow conclusions to be drawn about their nature. Physical methods for inducing mutations (hereditary changes of DNA molecules) are known and used in plant selection. More in-depth studies on the importance and practical use of ultrasound in wheat selection improvement will follow.

The total plant weight (mg) of Zebrec, measured on the 9th day after irradiation of the seeds with ultrasound with frequency 42 kHz, for 3, 9 and 21 min, as function of time is present of Figure 7.

Although attempts were made with seeds infected with phytopathogenic fungi (visible black germ), no diseased roots or co-optides were detected in any variant (Figure 8).

Fig. 8. Coleoptiles of Zebrec, pictured on the 9th day after treatment of contaminated seeds with ultrasound with frequency 42 kHz.

No pathogens were observed in laboratory vessels. It can be assumed that any ultrasound treatment of the applied resulted in complete destruction of the phytopathogen. Seeds are not treated with chemical preparations. The soil mixture was autoclaved at 131°C with over 2 atmospheres for 2 h.

Conclusion

Ultrasonic irradiation at a frequency of 42 kHz with duration of 3 min and 9 min has been found to increase the germinating energy of seeds of zebra seed, the effect being more pronounced in three-minute exposure.

Ultrasonic irradiation at a frequency of 42 kHz with duration of 3 min and 9 min has been found to increase the germination of seeds of the Zebrets seed variety, the effect being more pronounced in the nine-minute irradiation.

It has been established that pathogenic fungi developed on Zebrets *s*oft wheat seeds can be destroyed by a harmless physical method, namely by ultrasound irradiation at a frequency of 42 kHz with a duration of more than 3 min.

The pretreatment of 42-kHz wheat seeds of the Zebrets seed with a frequency of 42 kHz for 21 min has a negative effect on all the measured biometric parameters, namely: germinating energy, germination, coleoptilia length.

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