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Effect of Atonik preparation on germination ability of milk thistle seeds (*Silybum marianum* (L.) Gaertn.)

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Abstract Treatment of milk thistle seeds with Atonik for 6 hours increased the germination percentage, root length, and shoot length, and reduced germination time as compared to the control. When the milk thistle seeds were soaked for 6 hours in 0.3% Atonik solution, seed germination was 94.5%, germination time was 1.6 days, and root length reached 25.6 mm, the shoot length reached 14.2 mm. It is suggested that Atonik 0.3% can be used to treat milk thistle seeds.

Keywords: Germination percentage, Mean germination time, *Silybum marianum* (L.) Gaernt

Introduction

Germination is an essential process in the morphogenetic cycle of plants (Kibinza *et al*., 2011). The seeds could germinate quickly, and short germination time is an essential factor; it had a significant influence on the growth and ability of seedlings to withstand adverse conditions (Zhu *et al*., 2004; Gorian *et al*., 2007; Arellano and Peco, 2012). Seed germination was not only necessary for survival and dispersal but also required for plant establishment and depends on seed dormancy (Zutic and Dudai, 2008; Carrera *et al*., 2020); it was regulated by internal factors such as abscisic acid and external conditions (Buijs, 2020; Yan and Chen, 2020). Milk thistle (*Silybum marianum* (L.) Gaertn.) is a species of the Asteraceae family, grown for its fruits (achenes), in fact called seeds, used as medicinal herbs. The seeds of milk thistle contained about 20-35% fatty acids and polyphenolic compounds, and taxifolin was used medicinally to treat liver diseases such as jaundice, gallstones, hepatitis, and fatty liver (Morazzoni and Bombardelli, 1995; Ramasamy and Agarwal, 2008). Milk thistle seeds do not have a profound physiological dormancy. However, milk thistle seeds have a smooth seed coat, so their ability to absorb water is poor. Mohammadi (2009)

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and Thiam *et al*. (2013) suggested that salt and osmotic pressure were essential factors limiting and delaying seed germination and seedling formation. In addition, milk thistle seeds had a high percentage of fatty oil, accounting for 22.1% - 25.1%, so the seeds had a short shelf life and low durability (Long *et al*., 2015), which led to a decrease in the percentage of milk thistle germination rate over time (Liava *et al*., 2022).

In addition, harvesting has been one of the complex problems in milk thistle cultivation. For this plant, the ripening of inflorescences is uneven. The flower clusters at the top of the stem develop and ripen earlier, followed by the flower clusters on the side branches. The riper the main flower head is, the higher the silymarin content in the seeds (achenes) is. The highest levels of flavonolignans were found in dark-colored achenes (Carrier *et al*., 2003; Dyduch and Najda, 2007). Therefore, increasing the germination rate and shortening the average germination time was the basis for the milk thistle population to grow and develop more evenly, increasing the percentage of flower heads reaching maturity on harvesting. It was a meaningful issue in production practice in the field. There have been several authors who studied milk thistle seed treatment (Abdollahi *et al*., 2011; Nasiri *et al*., 2014; Siadat *et al*., 2015; Dorri *et al*., 2019; Mosavikia *et al*., 2020); however, the highest germination percentage according to publications was 84.9%, with an average germination time of 4.5 days when seeds were treated with 150 mM sodium chloride (Solouki *et al*., 2015); and achieved the lowest mean germination time of 2.26 days when using 3% KNO_3, but this rate was as low as 65.0% (Parmoon *et al*., 2013).

Atonik is a commercial product containing nitrophenolate as the main ingredient, a natural plant-derived compound that stimulates plant growth. Djanaguiraman *et al*. (2004) suggested that the growth regulator Atonik was an organic compound with small amounts that could support and promote germination. According to Purba *et al*. (2019), nitrophenolate was a growth regulator that increased seed germination and promoted cell division. It stimulated root growth and activated the ability to absorb nutrients. In growth, regulators could improve seed germination and seedling growth. The application of Atonik on seed treatment to improve seed germination has been reported in some studies. El-Shabrawy (2009) recommended seed treatment with Atonik for 8 hours at a concentration of 0.05% combined with foliar spraying at 0.05% for the highest vegetative value and yield. Djanaguiraman *et al*. (2005) reported that the treatment of cottonseed and tomato with Atonik 3mg/l gave the best results regarding germination, formation, and enzyme activity. The research finding was aimed to evaluate the effects of Atonik using for seedling germination and development to contribute to improving milk thistle production efficiency.

Materials and methods

Materials

Milk thistle seed sources were selected from imported varieties grown from September 2022 to April 2023 at the Nursery, Institute of Applied Research and Development, Hung Vuong University, Nong Trang ward, Viet Tri city, Phu Tho province, Vietnam has coordinates 21°20'21.3"N 105°22'45.2"E. Atonik 1.8 SL has the composition of Sodium-5-Nitroguaiacolate 3g/l; Sodium-O-Nitrophenolate 6g/l; Sodium-P-Nitro phenolate 9g/l.

Morphological analysis of milk thistle seeds

Milk thistle seeds were harvested when the flowers dried in early April 2023. Fruit samples were chosen with typical morphological characteristics, such as plump appearance and intact state with no signs of pests or diseases. After collection, the samples were stored in plastic boxes in well-ventilated conditions at room temperature until being dehydrated before the experiments.

Analysis of the morphological characteristics of the seeds

30 seeds of milk thistle were randomly selected. The shape and color of the seed shell were observed. Seed length (mm) and seed width (mm) were measured using a Mitutoyo 500-181-30 electronic palm caliper. Weight of 1,000 seeds were measured. Each seed lot was randomly taken to be one sample of 1,000 seeds and repeated thrice. The mass of 1,000 seeds was determined using an Ohaus PA214 electronic balance. Root and shoot length (mm) were determined by randomly selecting five germinated seeds, marking and measuring the length of the roots, and shooting on the day 7^{th}. Root length was measured from the root collar to the root tip. Shoot length was measured from the root collar to the tip of the longest leaf.

Atonik treatment for Milk thistle seeds

The experiment was arranged in a completely randomized design with four treatments and three replications under laboratory conditions at the Center for Medicinal Materials, Institute of Applied Research and Development, Hung Vuong University, Phu Tho Province, Vietnam. Room temperature was 25 °C ± 1 °C. The implementation period was from May 2023 to October 2023.

Treatments were as follows: control formula (soaked the seeds in pure water at room temperature for 6 hours), Atonik formula 0.1% (soaked the seeds in a 0.1% Atonik preparation solution for 6 hours), Atonik formula 0.3% (soaked the seeds in a 0.3% Atonik preparation solution for 6 hours), and Atonik formula 0.5% (soaked the seeds in a 0.5% Atonik preparation solution for 6 hours).

Before starting the experiment, the Petri dishes and filter papers were sterilized in an autoclave at 120 °C for 2 hours. Milk thistle seeds (total 1,600 seeds, with 100 seeds recipe^{-1} repeat^{-1}) were surface disinfected with 2.5% sodium hypochlorite solution for ten minutes, and then rinsed with distilled water. Seeds treated according to the treatments were then put in a Petri dish with a diameter of 9 cm (20 seeds dish^{-1}) on six layers of blotting paper. It was moistened with distilled water. Seeds with more than 2 mm sprout length were counted as germinated seeds. Germination was recorded daily until day 10th.

Tracking indicators

Germination percentage (GP) (%) was calculated by the formula (Scott *et al*., 1984): GP (%) = [(Total germinated seeds) × 100] / (Total seeds tested). Mean germination time (day) (MGT) was calculated by the formula (Orchard, 1977): MGT = $\sum f_d / \sum f$. Where: f was the number of seeds germinated on day *d*; *d*: day.The first day of germination (day) was the day on which the first sprout appeared (Al-Mudaris, 1998).The last day of germination (day) was the day the last sprout appeared (Al-Mudaris, 1998).The coefficient of velocity germination (CVG) was calculated by the formula (Jones and Sanders, 1987): CVG (%) = $\Sigma N_i \times 100 / \Sigma N_i T_i$. Where: N_i was the number of seeds germinated each day; T_i was the number of days corresponding to N_i.Speed germination (SG) was calculated by the formula (Jones and Sanders, 1987):SG = $\sum[n_1/d_1 + (n_2 - n_1)/d_2 + \ldots + (n_n - n_{n-1})/d_n]$. Where n was the number of seeds germinated in a day; *d* was the number of days. Seedling vigor index (SVI) was calculated by (Abdul-Baki and Anderson, 1973): SVI = Germination percentage (%) × seedling length (root length + shoot length).The germination index (GI) was calculated by (Arnold and Fenner, 1991): GI = $(10 \times N_1) + (9 \times N_2) + \ldots + (1 \times N_{10})$. Where: N_1, N_2,... N_{10} was the number of seeds germinated on days 1st, 2nd,..., 10th. The germination rate index (% day^{-1}) (GRI) was calculated by the formula (Esechie, 1994):GRI = $G_1/1 + G_2/2 + \ldots + G_x/d$. Where: G_1 was the GP × 100 on the first day after the germination test; G_x was the GP × 100 on day *d* after the germination test.

Time spread of germination (day) was the time between the first and last day of sprout appearance (Al-Mudaris, 1998). Root and shoot length (mm) were determined by randomly selected five germinated seeds in each formula in all

three replicates, marked and measured the length of the roots and shoots on the day 7th.

Data processing methods

The data were analyzed for variance using IRRISTAT 5.0 software. The least significant difference (LSD) of the parameters between experiments was calculated at a confidence level of 95% ($p < 0.05$).

Results

Some morphological and biological characteristics of milk thistle seeds

Milk thistle was a nut oval which characterized by a long white papilla, which was shedding seed-dispersing hairs. Depending on the level of growth, the seed shell was beige and brown. Mature seeds were brown; the shell was thick, shiny, and had poor water absorption. Immature seeds were usually lighter in color. Seed size was an indicator that affected the quality and germination ability of the seed. Seeds of the same species still had variations in size due to environmental influences on the seed development stage and genetic variations. Milk thistle seeds had an average seed length of 6.5 mm and an average seed width of 3.2 mm. The weight of 1,000 seeds was 25.4 g. The MGT under normal conditions was 6.8 days, and the GP was 62.6%. After seven days of seed germination, the root length was 10.6 mm, and the shoot length was 12.4 mm (Table 1).

Effect of Atonik on germination and development of seedlings from milk thistle seeds

Seed treatments were external influences on seeds to break dormancy or stimulate germination to save the seeds, and nursery areas, to help the seedlings grew quickly, less susceptible to pests and diseases. Evaluating the effects of treating seeds with Atonik solution with different concentrations on the germination process of milk thistle seeds was summarized in Table 2. The results showed in control that the starting time of germination was the day fourth, the end of germination was the day ninth, and the germination period was six days. In formulas with Atonik treatment showed the seeds germinated early after 24 hours. However, the concentration of Atonik treatment depened on germination time ranges from two to four days.

Table 1. Some morphological and biological characteristics of milk thistle seeds

Evaluation criteria	$\bar{X}$ ± SD	CV (%)
Seed length (mm)	6.5 ± 0.18	2.7
Seed width (mm)	3.2 ± 0.10	3.2
Length/width index of seed	2.03 ± 0.08	4.0
Weight of 1,000 seeds (g)	25.4 ± 0.70	2.8
Mean germination time under normal conditions (day)	6.8 ± 0.31	4.6
Root length (mm)	10.6 ± 0.42	4.1
Shoot length (mm)	12.4 ± 0.55	4.5
Germination percentage (%) (GP)	62.6 ± 0.62	1.0

$\bar{X}$: Mean; SD: Standard Deviation; CV: Coefficient of Variation; GP: Germination percentage.

The MGT is accurately measured the time, it took for many seeds to germinate, which was the most seeds germinated. It indicated seed vitality and resistance to unfavorable conditions. The longest MGT in the control was 6.8 days. The Atonik treatment formulas were started to germinate on the same first day, they had different MTG values, in which the Atonik 0.3% and Atonik 0.5% treatment formulas had the shortest MTG of 1.6 days (Table 2).

Table 2. Effect of Atonik concentration on milk thistle seed germination time

Experimental formula	First day of germination (day)	Last day of germination (day)	Germination time (day)	Mean germination time (day)
Control	4	9	6	6.8[a]
Atonik 0.1%	1	4	4	2.1[b]
Atonik 0.3%	1	2	2	1.6[c]
Atonik 0.5%	1	2	2	1.6[c]
$LSD_{0.5}$				0.62E-01
CV (%)				1.3

Different letters (a, b, c) in the same column represent significant differences $p < 0.05$,
MGT: Mean germination time.

The result was measured by GP and speed germination, which was an indicator reflecting the vitality of seeds and the influence of stimulants in the seed treatment process. It showed that the GP in the control was only 64.8%, while the Atonik-treatment formulas were high from 82.5% to 94.5% (Table 3).

Seed treatment with 0.3% Atonik solution for 6 h reached the highest rate was 94.5%. The analysis of variance showed that the concentration of Atonik significantly affected the GP at the 95% confidence level.

The GI is the most comprehensive measurement, combining GP and speed germination. All treatments treated with Atonik had higher GI, in which milk thistle seed treatment at 0.3% Atonik concentration had the highest GI of 886.0 while the control only achieved 272.0 (Table 3).

Seed treatment with Atonik had affected to increase the GRI. However, depending on the treatment concentration, this value was differently achieved. Seed treatment with 0.3% Atonik solution for 6 h had the highest GI of 65.0% day^{-1}, while the control treatment was only achieved at 10.0 % day^{-1} (Table 3).

Table 3. Effect of Atonik concentration on some parameters of milk thistle seed germination

Experimental formula	Germination percentage (%)	Germination index	Germination rate index (% day^{-1})
Control	64.8[a]	272.0[a]	10.0[a]
Atonik 0.1%	82.5[b]	734.3[b]	48.1[b]
Atonik 0.3%	94.5[d]	886.0[d]	65.0[d]
Atonik 0.5%	88.5[c]	832.0[c]	62.0[c]
$LSD_{0.5}$	*1.65*	*12.3*	*0.96*
CV (%)	*1.3*	*1.2*	*1.3*

Different letters (a, b, c, d) in the same column represent significant differences $p < 0.05$,
GI: Germination index, GRI: Germination rate index.

SG is the percentage of germination in a certain period, also called germination energy. This indicator shows the health of the seed and the seedlings produced from that seed. The control had SG of only 10.0, while the 0.3% Atonik formula achieved this value up to 65.0. This result showed that treating milk thistle seeds with Atonik significantly increased germination (Table 4).

Result showed that CVG values differed between treatments, in which the control treatment had the lowest CVG of 14.7, while seeds were treated with Atonik 0.5% leading to 62.5. Thus, the formulas with high GI and CVG showed that the seeds had high vigor. The SVI is one factor that evaluates the seed's vigor. The SVI in control was the lowest at only 880.6. The seeds were treated with Atonik had the SVI ranging from 2,326.5 - 3,761.1, of which seeds were treated with 0.3% Atonik reached the highest value of 3,761.1 (Table 4).

Table 4. Effect of Atonik concentration on milk thistle seed vigor

Experimental formula	Speed germination	Coefficient of velocity of germination	Seedling vigor index
Control	10.0[a]	14.7[a]	880.6[a]
Atonik 0.1%	48.1[b]	47.6[b]	2,326.5[b]
Atonik 0.3%	65.0[d]	61.6[c]	3,761.1[d]
Atonik 0.5%	62.0[c]	62.5[d]	3,097.5[c]
$LSD_{0.5}$	0.96	0.64	72.6
CV (%)	1.3	0.9	1.9

Different letters (a, b, c, d) in the same column represent significant differences $p < 0.05$,
SG: Speed germination, CVG: Coefficient of velocity of germination, SVI: Seedling vigor index.

The results showed that seedling root and shoot lengths were significantly affected when treated seeds with Atonik solution (LSD test, $p < 0.05$) as seen in Table 5. In the control formula, the root length reached 8.2 mm, and the shoot length was 5.4 mm. Meanwhile, in formulas with Atonik treatments were significantly higher than the control. When increasing the Atonik concentration from 0.1% to 0.3%, the size of milk thistle sprouts increased. Atonik 0.3% showed the most significant size, the root length reached 25.6 mm, the shoot length reached 14.2 mm, and the total length of the sprout was 39.8 mm. However, when the Atonik concentration was increased to 0.5%, the root length and shoot length tended to decrease.

Table 5. Effect of Atonik concentration on milk thistle sprout size

Experimental formula	Root length (mm)	Shoot length (mm)	Total length of the sprout (mm)
Control	8.2[a]	5.4[a]	13.6[a]
Atonik 0.1%	18.4[b]	9.8[b]	28.2[b]
Atonik 0.3%	25.6[d]	14.2[d]	39.8[d]
Atonik 0.5%	22.4[c]	12.6[c]	35.0[c]
$LSD_{0.5}$	*0.69*	*0.40*	*0.79*
CV (%)	*2.4*	*2.5*	*1.7*

Different letters (a, b, c, d) in the same column represent significant differences $p < 0.05$.

Discussion

Morphological characteristics and germination ability of milk thistle seeds

The characterizing the morphology of milk thistle seeds grown in Phu Tho province, Vietnam resulted to similarities with reports by Abd-El-hady and Arafa (2019); Marceddu *et al*. (2022); Andrzejewska *et al*. (2011). However, Ghavami and Ramin (2008) noted that the mass of 1,000 seeds was 23.9 g with smaller

than the mass of 1,000 seeds in this study. Evaluation of the germination ability of milk thistle seeds under common seed treatment conditions in this study is found to be consistent with the research of Andrzejewska *et al*. (2011); Zahra *et al*. (2021) stated that the GP of milk thistle seeds were only 65% to 75% in all ecological phenotypes. Thus, the GP of milk thistle seeds was only averaged under laboratory conditions. In production, limiting factors such as external conditions and seed aging during storage reduced the GP of seeds. Therefore, increasing the GP is confirmed the vital in production to save seeds and nursery areas, and to help the seedlings grown quickly.

Evaluate the effects of the Atonik treatment regimen on the germination and development of seedlings from milk thistle seeds

Some authors had studied the milk thistle seeds to stimulate germination and reduce the MGT of milk thistle seeds, but showing the highest GP which according to published reports was 84.9 (Solouki *et al.,* 2015) and the lowest MGT was 2.26 days (Parmoon *et al*., 2013). Solouki *et al.* (2015) reported that the most suitable factor for treating milk thistle seeds was a magnetic field at 100 mT for a GP of 62.7% with a MGT of 5.3 days; hydro-priming gave a GP of 61.0%, MGT which was 4.4 days, sodium chloride was 150 mM GP reached 84.9%, MGT was 4.5 days. Nasiri *et al*. (2014) selected NaCl and salicylic acid at the most suitable concentration when treating milk thistle seeds showed the highest GP, NaCl at a concentration 40mM, for a GP of 66.5%, MGT was 5.19 days and 0.5 mM salicylic acid showed a GP of 67.07%. Siadat *et al*. (2015) showed that using cytokines at a concentration of 500 ppm to treat milk thistle seeds for 24 hours gave the highest GP of 56%, and daily germination of 8 days. Abdollahi *et al*. (2011) reported the smoke solution at a concentration of 1:250 showed a GP of 55%. Parmoon *et al*. (2013) recommended using 3% KNO_3 to treat milk thistle seeds, giving the GP of 65% and MTG of 2.26 days. Dorri *et al*. (2019) showed that using NaCl at a concentration of 50 mM when treating milk thistle seeds resulted in a GP of 83.3% and the lowest MGT of 2.93 days. Mosavikia *et al*. (2020) recommended using NaCl at a concentration of 50 mM when treating milk thistle seeds, giving a GP of 69.6% with the lowest MGT of 3.89 days, while using chitosan nanoparticle 0.25% gave a GP of 64.0%, the shortest MGT was 3.6 days.

Atonik is a biostimulant synthesized from phenolic compounds: sodium-para-nitrophenolate (0.3%), sodium-ortho-nitrophenolate 0.2%, and sodium-5-nitroguaiacolate 0.1%. Up to date, the mechanism of action of nitrophenolate-based biostimulants has not been fully explained, but using nitrophenolate as

seed treatment increased endogenous auxin concentrations, which increased biomass accumulation and seedling growth (Djanaguiraman *et al*., 2005).

In this study, milk thistle seeds' vitality and growth were determined through the GP, root length, and shoot length when seeds were treated with 0.3% Atonik showing the best results. The increase in the above technical parameters might be due to increase in endogenous auxin content and phenolic compounds contained in Atonik. The interaction and cross-talk of endogenous auxin with gibberellin and ethylene can influence germination and seedling establishment (Fu and Harberd, 2003; Chiwocha *et al*., 2005). Endogenous auxin could influence seed germination by influencing the activity of enzymes, as in the case of germinating pea seeds, the activity of glyoxalase was regulated by endogenous auxin, leading to higher rates of cell growth and development (Thornalley, 1990; Hentrich *et al*., 2013).

In addition, in mature seeds, some chemical compounds, such as polyphenols and lignin, could accumulate on the seed coat and make mature seeds hydrophobic, causing low seed GP. Atonik contains nitro-organic compounds that stimulate physiological and metabolic processes, causing plant nutrients to be optimally metabolized and absorbed, increasing the seed germination rate (Siregar *et al*., 2005). It could stimulate all tissues to develop biochemically and directly permeate plant tissues to accelerate seed metabolism. Nitrogen-active ingredients could enhance seed germination by adjusting the K^+/Na^+ ratio and increasing ATP production and seed respiration (Zheng *et al*., 2009). Water is one of the necessary factors in the germination process of seeds. Water was a factor to note in handling hard-shelled seeds; this was very useful in germination. Therefore, soaking seeds in Atonik could make for absorbing water easier so they germinated faster (Penelitian and Surabaya, 2017).

Treating milk thistle seeds with Atonik increased the germination percentage, reduced the mean germination time, increased germination speed, and increased the seedling root length and shoot length. When treating milk thistle seeds by soaking for 6 hours in 0.3% Atonik solution, the seeds germinated after 24 hours of treatment, the last day of germination was on the second day, and the MGT was 1.6 days. With this time, the germination as the above had shown that the milk thistle seeds germinated very concentratedly, had good vitality, and created uniformity of seedlings while shortening the seed incubation time, saving time, money, and costs in production activities. From this result, using Atonik 0.3% to treat milk thistle seeds improved seed germination and vitality, thereby contributing to improve the production efficiency for this species.

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