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Research on the Effect of Pre-sowing Treatment on Seed Germination of *Hypericum perforatum* L.

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Abstract

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Trials were carried out for improvement of seed germination of St. John's Wort (*Hypericum perforatum* L.) based on studies of main sowing properties after treatments as follows: soaking, soaking and heating and combined seed stratification. Recommendations were made for the best pre-sowing seed treatment, 24 h soaking in cool water bath at room temperature and 7-day stratification at 0.5 °C.

Key words: St. John's Wort (*Hypericum perforatum* L.), seeds, germination, stratification

Introduction

In the recent years, *Hypericum perforatum* L. proved a valuable medicinal plant due to the content of hypericin in the flowers - a compound of cancer drugs with antibiotic, adaptogenic and stimulating effect on human organism. The need for raw material has increased multiple times. The demand is for raw material with high hypericin content. This triggered extensive investigations of natural habitats in search of chemoraces and forms characterized with synthesis of higher hypericin concentrations.

Literature data on this plant with rich content of biological substances are far from ample. The reason is that for the time being the resources of natural habitats are sufficient and the random seed collection for creating new plantations is irrelevant.

The first and foremost problem of cultivation is the identification of biological peculiarities of seeds as well as seed pretreatment for fast and successful germination and cultivation.

The marketable parts of St. John's Wort are the aboveground ones (Herba Hiperici), collected prior to or at the time of full blossom by picking the leafy stems with flowers and buds at 20-30 cm from the top (Staneva et al., 1982).

The plant contains different biologically active substances. All parts, except the root, contain about 2% photoactive condensate anthracene derivatives, the main

ones being hypericin and pseudohypericin (up to 0.3 - 0.4%). They are dark red and are characteristic of the genus *Hypericum*. The drug contains up to 1.8% flavonoids and flavone glycosides - hyperoside (0.7% in *herba* and 1.1% in flowers); essential oil (up to 0.10%) colored green and consisting mainly of terpenes and sesquiterpenes; common oil; tannins (3.8 - 10%); nicotinic and ascorbic acid, choline, pectin, coumarins and the antibiotic substance hyperforin. Flavonoids - hyperisid, rutin, quercetin and isoquercetin are important for the healing effect of drugs. Essential oil has the following compounds: α-pinene, β-pinene, cineole, mircene and cadinene (Isaev et al., 1977; Stoyanov, 1966; Mutaze, 1981; Gamermann et al., 1980; Bomme, 1968).

The various chemical composition accounts for the different pharmacological effects of the plant (Bomme, 1968).

The presence of hypericin, psuedohypericin and essential oil in the drug is a main prerequisite for its anti-inflammatory, wound-healing and astringent effect in treatment of burns, infected suppurating and slowly healing skin and mucosa injuries. The effect on inflammatory diseases of digestion organs as well as digestion disorders such as stomach and duodenum ulcer and chronic gastritis, etc., is remarkable (Jordanov and Kozuharov, 1970; Staneva et al., 1982; Isaev et al., 1977; Stoyanov, 1966; Gamermann et al., 1980).

The seeds of *Hypericum perforatum* L. are very small. Maximum length is 0.8 - 1.1 mm and width - 0.5 mm. The absolute weight of 1000 seeds is 0.10 - 0.12 g or 8000 - 10000 seeds in 1 g (Jordanov and Kozuharov, 1970; Royal Horticultural Society, 1996).

Seed material is often embryo dormant depending on origin and age. The studied batches showed germination rate of 1 to 90%. Freshly harvested seeds are mostly embryo dormant for a period of 3-11 months. The author explains dormancy with the effect of suppressing substances (possibly, essential oil) in seeds, especially seed testa.

The factors accounting for post-harvest seed dormancy and increase of endogenous gibberellinic complex are as follows: seed storage, washing, high temperature and light during germination, preliminary moisturizing and stratification. This is a way to reduce the period of dormancy and germination. It has been experimentally proven that 24-hour seed treatment with gibberellinic acid (500 - 1000 ppm GA3) considerably increases germination rate.

The following has been found efficient for germination rate in greenhouses: soil warming, covering with glass or foil or ensuring additional light as well as substrate and soil temperature of 20 - 25 °C. Light and variable temperatures of ~30 °C in daytime and ~20 °C at night are especially suitable (Jankulov, 1999).

Regular germination of *Hypericum perforatum* L. seeds requires low temperature treatment - 0-5 °C that is achieved by pre-winter sowing and natural stratification or preliminary stratification in spring sowing. Some findings show that certain species do not require low temperature treatment (Royal Horticultural Society, 1996).

The objective of the present work is to make an updated overview of research on seed biology and growing of *Hypericum perforatum* targeting successful seed germination and plant cultivation.

• For this purpose, the issues related to the effect of different factors on seed germination were studied, namely:

• Duration of seed soaking at a temperature of 18 °C;

• Combination of soaking and heating at a temperature of 28 °C to 35 °C;

• Combination of low temperature stratification (2 °C to 1 °C) and duration of soaking;

• Recommending the best pre-sowing treatment of *Hypericum perforatum* L. seeds.

Material and Methods

The study took place in the period 1998-1999 in field conditions with seed material from a Bulgarian population of *Hypericum perforatum* L., propagated on the area of the Institute of Rose and Essential Oil Cultures in Kazanlak.

The project involved 10 variants with 3 controls among them:

Variant 1: Seeds soaking for 48 hours and stratified in sand for 14 days

Variant 2: Seeds soaking for 48 hours and stratified in sand for 7 days

Variant 3: Seeds soaking for 48 hours and heated for 48 hours

Variant 4: Seeds soaking for 48 hours and heated for 24 hours

Variant 5: Seeds soaking for 24 hours and heated for 24 hours

Variant 6: Seeds soaking for 48 hours

Variant 7: Seeds soaking for 24 hours

Control 1. Dry seeds stratified in sand for 14 days

Control 2. Dry seeds stratified in sand for 7 days

Control 3. Dry seeds

Seeds of all variants were placed for testing on one and the same day under the same conditions:

➢ In Petri dishes in 2 replications of 100 seeds

➢ On germination bed of medium moistened filter paper

➢ In light conditions and air temperature of 17 °C

The research topics included the following:

➢ Study of morphological peculiarities of seeds

➢ Absolute weight identification

➢ Water absorption rate

➢ Evaluation of germination rate

➢ Germination energy

Absolute seed weight was estimated by counting 1000 seeds in 2 replications and weighing them on electronic scales with an accuracy of 0.01.

Water absorption rate was identified by means of seed soaking and weighing at certain intervals of time.

Germination rate was reported on the 21st day of seed placement.

Germination energy was monitored for a period of 70 days with air temperature recorded on a daily basis.

Results and Discussion

The seeds of Bulgarian population of *Hypericum perforatum* L. are cylindrical and kidney shaped with longitudinal meshy wrinkling (Figure 1), dark brown. Their average dimensions are: length ~0.9 mm and width ~0.5 mm. Therefore, sowing should be done on hard seedbed on preliminary rolled soil. Germination starts with the appearance of the hypocotyl that is ~ 0.4 mm thick. About 2 days later the radicle protrudes that is very thin and could be easily torn. Primarily it's bare and gets covered with small radicle hairs later. The testa detaches soon after germination with seed leaf growth. Retarded growth has been observed in cases of hard seed coats with low rupture capacity.

Seed leaves are of oblong to elliptic shape with entire margin (Figure 1b). Their average length is ~1.7 mm and width - ~1.2 mm.

Germination of St. John's Wort seeds is epigeal, i.e. above-ground, because cotyledons open above the ground. They acquire photosynthetic functions.

Due to the extremely small size of seeds, the results reported from 2 replications of 1000 seeds each are the same, i.e. the absolute weight of 1000 seeds is 0.12 g.

In order to establish water absorption dynamics for a period of 48 hours at room temperature of 18 - 20 °C, 1000 seeds were soaked in 2 replications.

The results of this investigation showed that the dynamics of water absorption after the 1st hour of soaking gradually increased to reach the highest values on the 24th hour followed by a complete stop (Table 1 and Drawing 1). This lead to the conclusion that the maximum duration of soaking, required for pre-sowing seed treatment (if this is the procedure applied) should be 24 hours.

Germination capacity is an important biological property of seeds to be used for sowing. High germination rate is a prerequisite for the fast and uniform seed germination that, combined with the optimal agrotechnical events, guarantees good growth.

Table 1
Absolute weight of 1000 seeds after soaking

Soaking duration, h	Weight of 1000 seeds, g
1	0.17
6	0.18
12	0.21
18	0.23
24	0.24
36	0.24
42	0.24
48	0.24

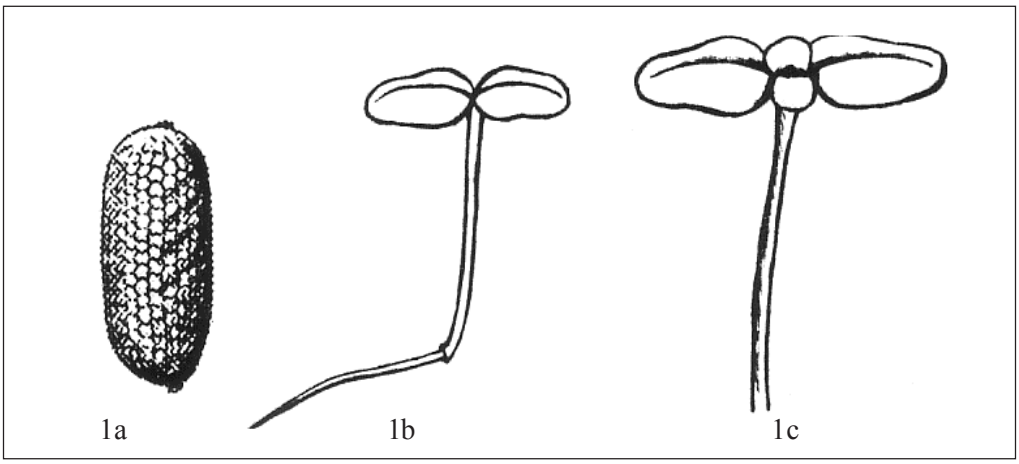


Fig. 1. Seed and germination phases
1a – seed, 1b – cotyledon phase, 1c – beginning of 1st true leaf

The results showed that on the 21st day, dry seeds having germinated without pretreatment, showed the highest germination rate (19%) and those soaked for 24 hours showed a germination rate as low as 1% (Table 2).

Figure 3 and 4 show germination dynamics (in numbers of seeds from 2 replications). The table shows also seed germination rate on the last day of observations, i.e. on the 70th day of beginning of trial.

Different treatments of seeds of separate controls of the tested population showed low and long germination rate - within 29.5 - 40%.

Dry seeds were reported with comparatively good results but 7- and 14-day stratification had a suppressing effect and reduced germination to 8 - 10.5%.

Comparing the results of variants I and II that were soaked for 48 hours with different stratification duration we observed 26.5% germination intensification at 7-day stratification.

Reference data show that the time of seed stratification is within 2 weeks and 2 months and that longer stratification results in considerable germination intensification. Our data showed that it would be more precise to say that different samples have different stratification optimums. The duration observed in the investigations of this population was 7 days.

Comparing the data of variant III and IV identified the factor of seed forcing duration by means of heating for 48 and 24 hours. It was not only that heating did not increase germination but had a strong depression effect - 23 and 9.5 %, respectively, at 40% for the control.

Table 2

Seed germination rate, %

Variants	Germination rate, %
I	11
II	3.5
III	11
IV	3.5
V	2.5
VI	2
VII	1
control 1	9
control 2	12
control 3	19

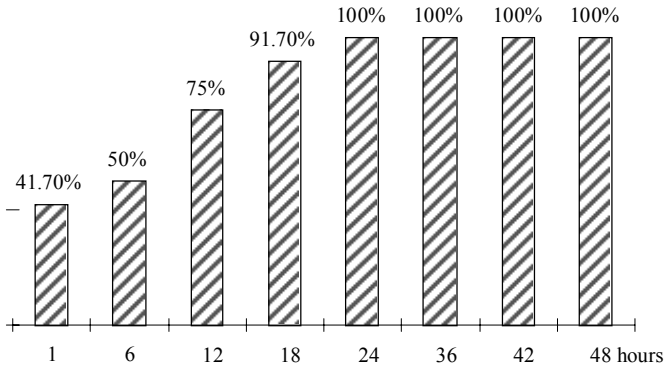
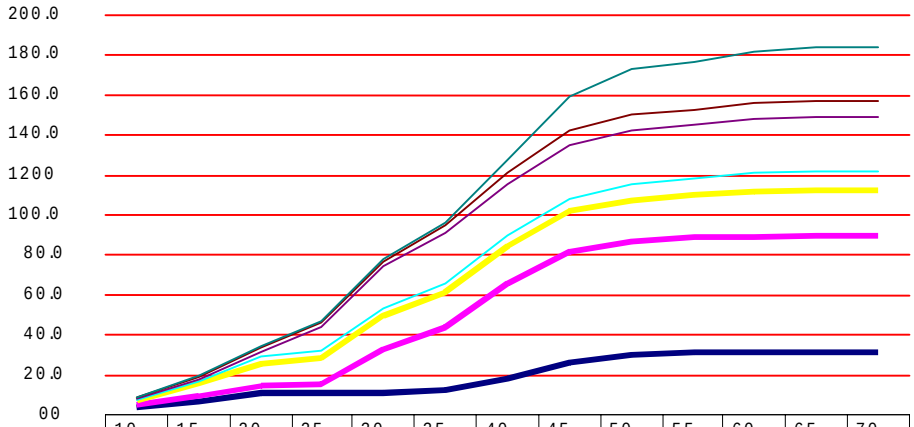
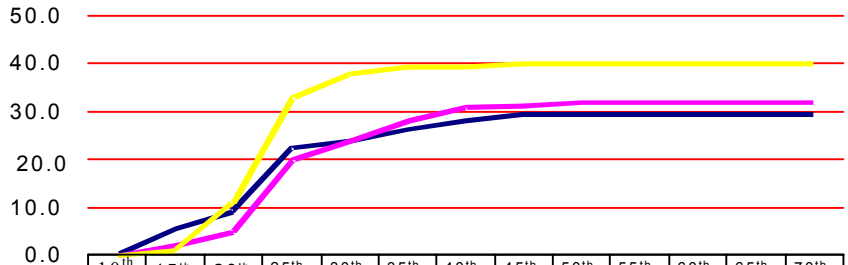


Fig. 2. Quantity of absorbed water, % of seed weight



	10-th Day	15-th Day	20-th Day	25-th Day	30-th Day	35-th Day	40-th Day	45-th • Day	50-th Day	55-th Day	60-th Day	65-th Day	70-th Day
Variant 7	0.5	0.5	1.0	1.0	1.0	1.0	7.0	16.5	23.0	24.5	26.0	27.0	27.0
Variant 6	0.5	1.5	2.0	2.0	2.5	4.0	5.5	7.5	7.5	7.5	8.0	8.0	8.0
Variant 5	0.5	1.0	2.5	12.0	21.5	25.5	26.0	27.0	27.0	27.0	27.0	27.0	27.0
Variant 4	0.0	0.5	3.5	3.5	3.5	4.0	4.5	5.5	8.0	8.0	9.5	9.5	9.5
Variant 3	2.5	6.5	11.0	13.5	16.5	17.5	19.5	20.5	21.0	21.0	22.5	23.0	23.0
Variant 2	1.5	3.0	3.5	4.0	22.0	31.5	47.5	56.0	56.5	57.5	57.5	58.0	58.0
Variant 1	3.5	6.5	11.0	11.0	11.0	12.5	18.0	26.0	30.0	31.5	31.5	31.5	31.5

Fig. 3. Germination dynamics, number



	10th day	15th day	20th day	25th day	30th day	35th day	40th day	45th day	50th day	55th day	60th day	65th day	70th day
control 1	0.5	5.5	9.0	22.5	24.0	26.5	28.0	29.5	29.5	29.5	29.5	29.5	29.5
control 2	0.0	2.0	5.0	20.0	24.0	28.0	31.0	31.5	32.0	32.0	32.0	32.0	32.0
control 3	0.0	1.0	11.0	33.0	38.0	39.5	39.5	40.0	40.0	40.0	40.0	40.0	40.0

Fig. 4. Germination dynamics, number

Seed soaking as an independent method for seed germination intensification was reflected in the results of variant VI and VII. They showed that 48-hour soaking

reduced germination and brought it down to 8%, i.e. 5 times lower that the dry control. Almost the same results were obtained in comparing variants IV and V, where seed soaking for 24 hours and heating for the same duration resulted in 3 times higher germination percentage compared to 48-hour soaking.

Conclusions

Seeds of the studied population germinate slowly. They have a low germination percentage. The weight of 1000 seeds is 0.12 g. Maximum water absorption is 100% and takes place in 24 hours.

The optimal combination of factors influencing germination is 24-hour soaking in aerated and cool water bath at room temperature and 7-day stratification in sand at a temperature of ~ 0?Ñ that result in 58% germination vs. 40% for control.

Continuous soaking, heating and stratification considerably reduce germination percentage - up to 4 times.

Data show that it would be wrong to recommend methods for seed germination enhancement for the species *Hypericum perforatum* L. - seed biology is origin or variety dependent, i.e. genetically controlled. It is obvious that each seed origin - variety, form, line and cultivar has its own optimum for demonstrating its maximum germination capacity.

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