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The Effects of Light and Some Presoaking Treatments on Germination Rate of St. John's Worth (*Hypericum perforatum* L.) Seeds

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Abstract: Light was found to be the most important factor affecting its seed germination. Under light conditions, soaking the seed for 30 min. in 50 ppm GA and 40°C hot water enhanced germination rate significantly. Under dark conditions, the seeds treated with KNO_3 were the only ones germinating effectively. The results suggest that *Hypericum perforatum* seeds exhibit both exogenous and endogenous dormancy. To over come former originating from a chemical inhibitor in exudate from capsule, washing with water, 40°C hot water and 50 ppm GA treatments; to over come later related with absence of light, 0.01 mol KNO_3 treatment were recommended.

Key words: *Hypericum perforatum,* germination, dormancy, light

INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilisation. The industrial revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. But, there has been an increasing tendency in using of plant-originated raw matters in medicinal treatment due to some reasons such as synthetic products have some adverse effects on human health and are very expensive or plant-originated matters have multi functional effects in contrast to synthetic products (Rates, 2001). Today, about 25% of the drugs prescribed worldwide and 121 active compounds being in current use come from plant. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant numbers are synthetic drugs obtained from natural precursors. It is estimated that 60% of anti-tumor and anti-infectious drugs already on the market or under clinical trial are of natural origin (Shu, 1998). The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plant (Hamburger and Hosstettmann, 1991).

One of plants used in drug production industry intensively is *Hypericum perforatum.* It, commonly known as St. John's worth, is a medicinally important plant native to relatively dry temperature zones of Europe and North America (Deltito and Bayer, 1998). The species belonging to *Hypericum* genus have been used as traditional medicinal plants due to their wound-healing (Yazaki and Okuda, 1990), bactericide (Ishiguro *et al*., 1998), anti-inflammatory (Dias *et al*., 1998). Diuretic and sedative properties (Holz and Ostrowiski, 1987) for last two hundred years. *Hypericum perforatum* contains, namely naphthodianthrons and phloroglucinols, biologically active secondary metabolites belonging to at least ten different classes (Greeson *et al*., 2001). Hypericin and pseudo hypericin, one of components this plant has, exhibit antiviral (Meruelo *et al*., 1988; Lavie *et al*., 1989) properties. That is why, it is suggested that *Hypericum perforatum* has a potential in using of Acquired Immuno Deficiency Syndrome (AIDS) treatment (Schinazi *et al*., 1990). In the clinical studies, anti-tumor (Diwu, 1995; Agostinis *et al*., 1995) and anti-cancer (Takahashi *et al*., 1989; Hudson *et al*., 1991 and Lavie *et al*., 1995) properties of *Hypericum perforatum* were determined. Especially using of this unique plant as an antidepressant is very popular today.

There are about 300 *Hypericum* living in mild temperature zone of the world. Turkey is an important center for *Hypericum.* There are 69 *Hypericum* species in Turkey, 24 of which are endemic (Tokur, 1988). *Hypericum perforatum* found in Turkey commonly is worldwide plant economically due to various secondary metabolites it has. This is evidenced by the fact that the market for *Hypericum perforatum* has exceeded $210 million in USA and $570 million worldwide annually (Sirvent *et al*., 2002).

Germination is a critical stage in the life cycle of weeds and crop plants and often controls population dynamics, with major practical implications (Radosevich *et al*., 1997). But, generally germination rate of *Hypericum perforatum* is very low due to seed dormancy (Macchia *et al*., 1983). The dormancy is caused by a chemical

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inhibitor exudate from the capsule (Campbell, 1985) and absence of light has an negative effect on germination (Thompson and Whatley, 1984). This plant prefers to be propagated vegetatively (Tokur, 1988). Both low germination rate and difficulty in cultivating this plant using vegetatif plant parts make *Hypericum perforatum* production difficult (Mustyatse, 1983). Over the past twenty years, dormancy has been widely studied but the regulatory principles behind changes in several types of dormancy remain unclear (Rehman and Park, 2000). Nevertheless, plant growth regulators such as GA and IAA (Hilhorst and Karssen, 1992; Iglesias and Babiano, 1997); chemical matters like KNO_3 (Kevseroğlu, 1993; Hartmann *et al*., 1997) and hot water treatments (Hermansen *et al*., 1999) has been recommended in breaking dormancy and to enhance germination. The objectives of this study were to determine the effect of light and exogenously applied GA, KNO_3 and hot water on germination and to find an effective method for breaking seed dormancy of *Hypericum perforatum* L.

MATERIALS AND METHODS

In the study, the 6 month-old seeds obtained from *Hypericum perforatum* plants collected from the mountain pasture named Sisdağı in Trabzon province of Turkey were used. Before treating of different factors, seeds were bulked and washed with tap water to remove chemical inhibitor in exudate from capsule from seed surfaces (Campbell, 1985). Germination rates of the seeds exposed to different treatments were determined with enumerations made 20 days later.

Presoaking treatments used in the study were Different GA doses, hot water and 0.01 mol KNO_3. Before placing in petri dishes, seeds were soaked in 50 and 100 ppm GA solution and 40 and 60°C hot water for 30 minutes. For KNO_3 treatment, petri dishes were wetted with 0.01 mol KNO_3 solution. To evaluate the effect of light on germination, the study was performed under both continuous illumination (1200 lux white light (Macchia *et al*., 1983)) and darkness in growth chambers. Heat was fixed with 18°C (Campbell, 1985).

For each application 3 x 100 = 300 seeds were placed in petri dishes and germinating seeds were counted for 20 days after treatments. Obtained numbers were analysed according to design of randomised plots using MSTAT packet program. Differences among treatments were assessed according to least significant differences (LSD) test (Yurtsever, 1984).

RESULTS AND DISCUSSION

The germination rates of *Hypericum perforatum* seeds treated with different factors were shown in Table 1 as a group. All treatments applied in this study had a significant effect on germination rate ($p<0.01$).

According to the results of variance analysis, effect of light on germination rate was significant. As untreated control seeds gave second highest germination rate under light conditions with 60.67%, the lowest germination rates was obtained from control seeds under darkness (1.00%). These results are similar to that of Macchia *et al*. (1983), Thompson and Whatley (1984), Campbell (1985) and Tokur (1987) reporting that germination was very low in absence of light in *Hypericum perforatum* seeds. Also, germinating seeds under darkness formed etiolated seedling (Fig. 1a) but, that of germinating under light conditions were green and looks alive (Fig. 1b).

The effect of light on seed germination is various. Light has no effect on germination of many crops seeds economically important but, some of them need light to germinate at the highest level (Kacar, 1996). Likewise in a study conducted out to determine the effect of light on germination of seven different cacti species, Arechiga *et al*. (1997) obtained similar results.

As mean of light and dark conditions, among presoaking treatments, 40°C hot water and 50 ppm GA treatments gave the highest germination rates with 41.33 and 39.67%, respectively. The percentages were followed by 100 ppm GA and 0.01 mol KNO_3 treatments found in the same statistical group (31.83 and 30.67%, respectively). The lowest germination rate was obtained from seeds treated with 60°C hot water. Hot water applications have enhanced germination of hard coated seeds by elevating water and O_2 permeability of testa (Aydın and Uzun, 2001). Hermansen *et al*. (1999) reported that germination was enhanced by presoaking in 44, 49 and 54°C hot water for 5-40 minutes in carrot seeds. Hot water treatments also can have a detrimental influence on germination, which is special concern in high value seeds such as hybrid cabbage (Williams, 1980). In this study germination rate was very low in seeds presoaked in 60°C hot water. But, adverse effect of hot water on germination can be minimised by reducing the length of exposure to hot water (Smit and Davies, 1989).

Endogenus GAs are widely studied in relation to the breaking of dormancy in seeds of various plant species (Seiller, 1998). Imbibition stimulates GA secretion from embryo. Secreted GA increases synthesis of hydrolytic enzymes located under aleuron layer. Synthesised enzymes are transported to endosperm via scutulum and are used for decomposing of stored food to supply the energy required for germination (Cetin and Onay, 1994). In this study, GA increased germination rate significantly depending on used doses. Similar results were obtained from the studies carried on other species such as *Haplopappus gracilis* (Galli *et al*., 1975), *Sesamum*

Table 1: The effects of light and some presoaking treatments on germination rates of *Hypericum perforatum* seeds

	Germination rates (%)						
	Different presoaking treatments						
	Control	Hot water (40°C)	Hot water (60°C)	GA (50pmm)	GA (100ppm)	KNO_3 (0.01mol.)	Mean
Light	60.67b	72.67a	16.00d	75.33a	59.33b	30.00c	52.33a
Darkness	1.00e	10.00de	3.00e	4.00e	3.33e	31.33c	8.78b
Mean	31.83b	41.33a	8.50c	39.67a	31.33b	30.67b	

LSD presoaking treatments: 6.497; LSD light x presoaking treatments: 9.188. CV:%13.07**There is no difference between means marked with the same letter in%1 probability level

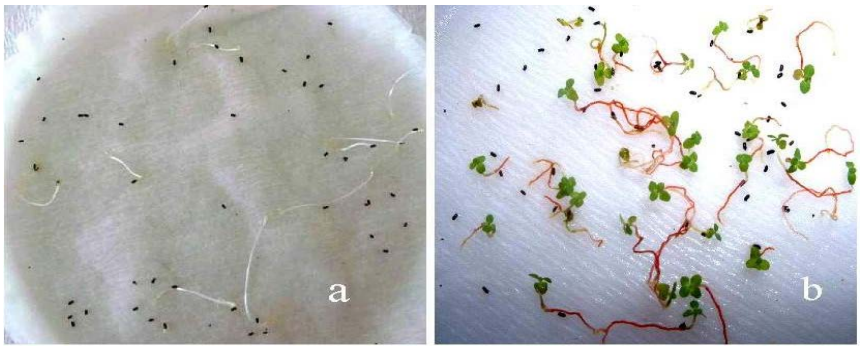

Fig. 1: Germination of *Hypericum perforatum* seeds under light and dark conditions

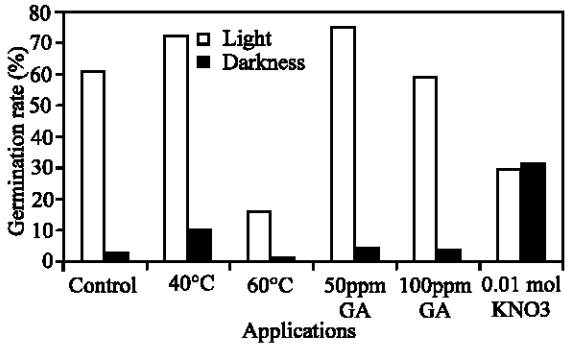

Fig. 2: Light x different presoaking treatments interaction

indicum (Kyauk *et al*., 1995), *Rumex dentatus* (Ali and Helal, 1996), *Zea mays* and *Glycine max* (Wang *et al*., 1996) and *Opuntia tomentosa* (Carrillo *et al*., 2003).

Nitrates have been commonly used for breaking of dormancy in seeds requiring light for germination. Nitrates have increased seed germination by offsetting the light requirement on a large scale (Kacar, 1996). In a study whose main objective was to evaluate seed treatments for reducing or eliminating the light requirement of *Lesquerella fendleri* seeds, KNO_3 was reported as an effective agent for reducing light requirement and enhancing germination (Puppala and Fowler, 2003). In this study, similarly, germination enhancing effect of KNO_3 was more evident under darkness (Table 1).

Besides each treatment, an interaction ($P<0.01$) between treatments was determined as statistically significant (Fig. 2). In this respect, the highest germination rate was obtained from 50 ppm GA and 40°C hot water treatments (75. 33 and 72.67%, respectively) under light conditions. The numbers were followed by untreated control (60.67%) and 100 ppm GA treatment (59.33%) under light conditions. All treatments decreased germination rate under darkness statistically except for 0.01 mol KNO_3 treatment when compared to that of light conditions.

According to the results of this study, *H. perforatum* seeds exhibit both exogenous and endogenous dormancy. Former originating from a chemical inhibitor in exudate from capsule could be eliminated 40°C hot water and 50 ppm GA treatments effectively. All treatments performed under light conditions increased germination rate when compared to that of darkness. But, 0.01 mol KNO_3 was the only treatment causing significant increase for germination under darkness. The effect of KNO_3 probably was related to its light requirement reducing property. The result suggest that there is an endogenous dormancy related to absence of light in *Hypericum perforatum* seeds except for exogenous dormancy.

In this study, effects of light and some presoaking treatments on germination rate of *Hypericum perforatum* were investigated. According to the results, light was the most significant factor affecting germination. Under light

conditions, 50 ppm GA and 40°C hot water treatments increased germination significantly. Under darkness, the seeds treated with 0.01 mol KNO_3 were the only ones germinating effectively. The results suggest that *Hypericum perforatum* seeds exhibit both exogenous and endogenous dormancy. To over come former originating from a chemical inhibitor in exudate from capsule, washing with water, 40°C hot water and 50 ppm GA treatments; to over come later related with absence of light, 0.01 mol KNO_3 treatment were recommended. But, more studies will be needed about endogenous dormancy.

REFERENCES

Agostinis, P., A. Vandenbogaerd, A. Donella-Dean, L.A. Pinna, K.T. Lee, J. Goris, W. Merlevede, J.R., Vandenheede and D. De Witte, 1995. Photosentetized inhibition of growth factor-regulated protein kinases by hypericin. Biochem. Pharmacol., 49: 1615-1622.

Ali, A. and A. Helal, 1996. Studies on germination of *Rumex dentatus* L. seeds. J. Arid Envir., 33: 39-47.

Arechiga, M.R., O.S. Alam and V.Y. Carlos, 1997. Effect of light on germination of seven species of cacti from the Zapotitlan Valley in Puebla, Mexico J. Arid Envir., 36: 571-578.

Aydın, I. and F. Uzun, 2001. The effects of some applications on germination rate of Gelemen Clover seeds gathered from natural vegetation in Samsun. Pak. J. Biol. Sci., 4: 181-183.

Campbell, M.H., 1985. Germination, emergence and seedling growth of *Hypericum perforatum*. Weed-Research, UK., 25: 259-266.

Carrillo, Y.O., J.M., B. Guzman, S.C. Victor, O. Esther and S. Alma, 2003. Germination of the hard seed coated *Opuntia tomentosa* S.D., a cacti from the Mexico valley. J. Arid Envir., (In press).

Cetin, H. and A. Onay, 1994. Buyume ve Gelisme Fizyolojisi. In: Bitki Fizyolojisi, Cetin, H. and A. Onay (Eds)., Dicle Universitesi Basimevi, Diyarbakir.

Deltito, J. and D. Bayer, 1998. The scientific, quasi-scientific and populer literature on the use of St. John's wort in the treatment of depression. J. Affect. Disort., 51: 345-351.

Dias, A.C. P., A. Francisco, T. Barberan, F. M. Ferreria and F. Ferreres, 1998. Unusual flavanoids produced by callus of *Hypericum perforatum*. Phytochemistry, 48: 1165-1168.

Diwu, Z., 1995. Novel therapeutic and diagnostic applications of hypoccrellins and hypericins. Photochem. and Photobiol., 61: 529-532.

Galli, M.G., S. Elio and M. Caroi, 1975. Comparative effects of fusicoccin and gibberellic acid on the promotion of germination and DNA synthesis initiation in *Haplopappus gracilis*. Plant Science Letters, 5: 351-357.

Greeson, J., B. Sanford and D.A. Monti, 2001. St. John's worth (*Hypericum perforatum*): a review of the current pharmacological, toxicological and clinical literature. Psychopharmacol., 153: 402-414.

Hamburger, M. and K. Hosstettmann, 1991. Bioactivity in plants: the link between pyhtochemistry and medicine. Pyhtochemistry, 30: 3864-3874.

Hartmann, K., C. Krobb and A. Mollwo, 1997. Phytochrome-mediated photocontrol of the germination of the Scentless Mayweed, *Matricaria inodora* L. and its sensitization by nitrate and temperature. J. Photochem. and Photobiol. B: Biology, 40: 240-252.

Hermansen, A., G. Brodal and G. Balvoll, 1999. Hot water treatments of carrot seeds: Effects on seed-borne fungi, germination, emergence and yield. Seed Sci. Technol., 27: 599-613.

Hilhorst, H.W.M. and C.M. Karssen, 1992. Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. Plant Growth Regulation, 11: 225-238.

Holz, J. and E. Ostrowiski, 1987. St. John's worth HPLC analysis of main components and their variability in the populations. Deuts. Apothekertz, 127: 1227-1230.

Hudson, J.B., I. Lopez-Bazzocchi and G.H.N. Towers, 1991. Antiviral activities of hypericin. Antiviral Res., 15: 101-112.

Iglesias, R.G. and M.J. Babiano, 1997. Endogenous abscisic acid during the germination of chickpea seed. Physiol. Plant, 100: 500-504.

Ishiguro, K., N. Nagareya and H. Fukomoto, 1998. A phloroglucinol derivative from cell suspension cultures of *Hypericum perforatum*. Phytochemistry, 47: 347-369.

Kacar, B., 1996. Bitki fizyolojisi. Ankara Univ., Ziraat Fak. Yayinlari, Toprak Bolumu, No. 1447. Ankara, Turkey.

Kevseroğlu, K., 1993. Doğal floradan toplanan datura (*Datura stramonium* L.) tohumlarının çimlenmesine bazı fiziksel ve kimyasal iþlemlerin etkisi. Doğa-Tr. J. Of Agricultural and Foresty, 17: 727-735.

Kyauk, H., N.W. Hopper and R.D. Brigham, 1995. Effects of temperature and presoaking on germination, root length and shoot length of sesame (*Sesamum indicum* L.) Envir. and Exper. Botany, 35: 345-351.

Lavie, G., Y. Mazur, D. Lavie, B. Levin, Y. Ittah and D. Meruelo, 1995. Hypericin as an antiretroviral agent. Reprinted from: Aids: Anti-HIV agents. Therapies and Vaccines of the Annals of the New York Academy of Sciences, 616: 556-562.

Lavie, G., F. Valentine, B. Levin, Y. Mazur, G. Gsllo, G. Lavie, D. Weiner, D. Macchia, N. Benvenuti and A. Angelini, 1989. Germination characteristics of some seeds of medicinal plants. Notiziario-di-Ortoflorofrutticoltura, 9: 241-247.

Macchia, N., A. Benvenuti and L. Angelini, 1983. Germination characteristics of some seeds of medicinal plants. Rastitel'nye-Resursy, 21: 461-463.

Meruelo, D., D. Lavie and G. Lavie, 1988. Therapeutic agents with dramatic retroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. natl. Acad. Sci. USA., 85: 5230-5234.

Mustyatse, G.I., 1983. On the establishment of industrial plantations of *Hypericum perforatum*.Tezisy Dokladov 7 Delegatnogo S"ezda Vsesoyuznogo Botanicheskogo Obshchestva, Donetsk, 11-14 Mai, 1983. 1983, 201. Leningrad, USSR.

Puppala, N. and J.L. Fowler, 2003. *Lesquerella* seed pretreatment to improve germination, Industrial Crops and Products, 17: 61-69.

Radosevich, S., J. Holt and C. Ghersa, 1997. Weed Ecology Implications for Management, Wiley, New York.

Rates, S.M.K., 2001. Plants as source of drugs. Toxication, 39: 603-613.

Rehman, S. and H. Park, 2000. Effect of scarification, GA and chilling on the germination of golden-tree (*Koelreuteria paniculata* Laxm.) seeds. Scientia Horticulturae, 85: 319-324.

Schinazi, R.F., C.K. Chu, J.R. Babu, B. Oswald, V. Saalman, D.L. Cannon and B.F. Erickson,1990. Anthraquinones as a new class of antiviral agents against AIDS. Antiviral Res., 13: 265-272.

Seiller, G.J., 1998. Seed maturity, storage time and temperature and treatment effects on germination of two wild sunflowers. Agron. J., 90: 221-226.

Sirvent, T., L. Walker, N. Vance and G. Donna, 2002. Variation in hypericins from wild populations of *Hypericum perforatum* L. in the Pasific Northwest of the U.S.A. Economic Botany, 56: 41-49.

Smit, W.A. and P.S. Davies, 1989. Elimination of D*iaporthe phaseolorum* and *Neocosmospora vasinfecta* from rooibos tea seeds by hot-water treatment and acid scarification. Phytophylactica, 21: 297-299.

Shu, Y.Z., 1998. Recent natural products based drug development: A pharmaceutical industry perspective. J. Nat. Prod., 61: 1053-1071.

Takahashi, I., S. Nakanishi, E. Kobayashi, H. Nakano, K. Suzuki and T. Tamaoki, 1989. Hypericin and pseudohypericin specifially inhibit protein kinase C: possible relation to their antiretroviral activity. Biochemistry Biophys. Res. Comm., 3: 1207-1212.

Thompson, K. and J.C. Whatley, 1984. A thermogradient bar apparatus for the study of germination requirement of buried seed in situ. New-Phytologist., 96: 459-471.

Tokur, S., 1988. Bazı *Hypericum* türlerinin ekolojisi üzerine araþtırmalar. Doğa TU Botanik D., 6: 323-331.

Wang, Q., Z. Feng and D. Smith, 1996. Application of GA_3 and kinetin to improve corn and soybean seedling emergence at low temperature. Envir. and Exper. Botany, 36: 377-383.

Williams, P.H., 1980. Black rot: a continuing threat to world crucifers. Plant Diseases, 64: 736-742.

Yazaki, K. and T. Okuda, 1990. Procyanins in callus and multiple shoots of *Hypericum erectum*. Planta Med., 56: 490-491.

Yurtsever, N., 1984. Deneysel istatistik metotlari. TC Tarim orman ve Koyisleri Bakanligi Koy Hizmetleri Genel Mudurlugu Yayinlari, Genel Yayin No. 121, Technic Yayin No. 56.