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Studies on aseptic seed germination pattern in *Taraxacum officinale* Weber

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ABSTRACT

This paper signifies the process of aseptic germination of seeds of *Taraxacum officinale*, using various sterilizing agents and their influence on the physiology of seed. The three sterilizing agents, mercuric chloride ($HgCl_2$), sodium hypochlorite (NaOCl) and ethanol (CH_2OH) were used in the experiment alone and also in combination with each other in various concentrations. The degree of contamination, seed colour and % of seed germination during the course of experiment was studied. The aim of this study is to establish best surface sterilization for *in-vitro* propagation of Dandelion. The seeds of *Taraxacum officinale* were tried to germinate aseptically under various conditions and finally it was observed that seeds showed good germination using sodium hypochlorite (NaOCl) at concentration of 0.1% for 7 minutes.

Keywords: *Taraxacum officinale*, seeds, sterilizing agent, NaOCl, CH_2OH, $HgCl_2$.

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INTRODUCTION

The introduction of plants to tissue culture involves a critical stage which aims at obtaining cultures that are free of microbial contamination. Sometimes inspite of following proper method of surface sterilization of explants prior to their culture, the microbes can not be eliminated, this is especially the case where explants are taken directly from field growing plants and introduced to tissue culture. Many contaminants like endophytic bacteria are present in xylem vessel, and are protected from the process of surface sterilization. Hence, seeds are often assumed as the source of microbial contamination. So, in this paper we aim to study the effect of three sterilizing agents sodium hypochlorite, mercuric chloride and ethanol on seed sterilization of *Taraxacum officinale* and physiological phenomenon involved in this process. Out of these three sterilizing agents, hypochlorite is generally effective against microorganisms even in low concentrations, whereas ethanol is phytotoxic so the plant material should be exposed to it for a shorter period of time and mercuric chloride is toxic for humans as well as plants, so it must be handled with care.[1] *Taraxacum officinale*, commonly known as Dandelion belonging to family Asteraceae is a stemless perennial herb, found abundantly in Kashmir and Northern parts of India and distributed in almost every temperate and subtropical regions of the world. Whole plants possess anti-hyperglycemic, anticoagulatory, analgesic and anti-allergic properties and flowers are used for making wine [2]. Also it is known to benefit in cancer as it induces apoptosis,[3] Alzheimer's and parkinsonism,[4] COX inhibitor[5], Acetyl cholinesterase inhibitor[6]. The high seed production and germination rates are perhaps responsible for strong competitive and dispersal abilities of dandelion. The reported germination capacity of dandelion seeds varies[7] but normally the germination is between 80-90% [8]. Studies indicated that germination in seeds lacking a pappus is 31% lower than the seeds with an intact pappus and that the seeds devoid of a pappus required more time to germinate. Also it was found that there was no effect of relative humidity on germination [9]. Reports also showed that freezing storage of dry seeds led to delay in ageing and seed germinability was preserved for a long time. Hence, cryopreservation is a convenient method for seed storage for studying comparative aspects of seed germination [10]. Studies on five perennial weeds including *Taraxacum officinale* was done showing the effect of light, nitrate, alternating temperature and seed age as factor on seed germination and it was found that all factors affected seed germination to a considerable level [11].

MATERIALS AND METHODS

Collection and authentification of seeds

Seeds of *Taraxacum officinale*, were collected from natural plant growing in herbal garden of Jamia Hamdard, New Delhi, in the months of April. The authentification of plant and seeds were done at Pusa institute, NISCAIR (Raw Materials Herbarium and Museum, its voucher specimen number is NISCAIR/RHMD/Consult/2013/2273/53.

Checking of seed viability

Firstly, viability of these seeds was checked by soaking them in water. Using a razor blade each seed was cut longitudinally in half. A 100 mm petridish was taken and filter paper containing the cut seeds was placed in it and further the petridish was covered with a 0.1% solution of triphenyl tetrezolium chloride (TTC) at pH 7-8 and kept in dark for 12-24 hours. The cut seeds were then examined for staining. The seeds that were viable changed the colorless. TTC into an insoluble, red pigment which was observed in the embryo and endosperm. The seeds which remained unstained were considered to be non-viable. Seeds showing any stain were considered to be viable.

Seed viability % = No. of half cut seeds stained red/ Total No. of half cut seeds × 100

Aseptic seed sterilization and germination

After confirming their viability, these were then washed with water 2-3 times, teepol solution, then again washed with water 2-3 times, then washed with double distilled water 2-3 times and then sterilized with 0.1% sodium hypochlorite for 6 min. After that seeds were washed repeatedly 2-3 times with autoclaved double distilled water. About 7-8 seeds were then transferred aseptically into 5 autoclaved petriplates containing absorbent cotton and filter paper in laminar air flow. After that petriplates were covered with aluminum foil and kept in BOD at a temperature of 24 ± 2ºC and alternate dark and light period for germination.

In other experiment seeds were rubbed with sand paper for 2-3 min and then washed with water 2-3 times, teepol solution and again washed with water 2-3 times and then washed with double distilled water 2-3 times and same procedure mentioned above for seed germination was repeated.

RESULTS AND DISCUSSION

The seeds were checked for viability and it was found that 98% seeds were found to be viable. The most critical step in seed germination is breaking of seed dormancy. Several studies have shown that ethylene, gibberellic acid and brassino steroids promote the germination of dormant seeds, but now it's a well known fact that abscisic acid (ABA) is primary mediator of seed dormancy. $HgCl_2$, used in various concentrations for different contact time resulted in total absence of contamination but seeds did not show germination even after keeping for so many days. Also it was noticed that colour of seed turned from light brown to dark brown and black. The change in colour of seeds

shows that $HgCl_2$ is toxic for use in the seed sterilization process. Hence, we can say that $HgCl_2$, is not appropriate as a sterilizing agent for seed germination in *Taraxacum officinale.*

It has been reported that alcohols are bactericidal rather than bacteriostatic against vegetative forms of bacteria but do not destroy bacterial spores [12]. Also the reports suggest that ethanol is a better solvent than water in dissolving many phytoconstituents present in seeds and hence its penetration power in seed is also comparatively good. So, due to this it is important to use it for shorter period of time for sterilization upto which ethanol does not penetrate and dissolve the seed phytoconstituents further leading to contamination [13]. Use of ethanol alone or in combination with NaOCl in various concentrations, shown in table.1. resulted in contamination after 3-4 days. The colour of seed remained light brown throughout and hence there was no change in the colour of seeds. The degree of contamination was highest in ethanol compared with the other two sterilizing agents. Henceforth, ethanol is not successful as a sterilizing agent for seed germination in *Taraxacum officinale*.

Using NaOCl, it was found that the degree of contamination was lesser with NaOCl using its different concentrations. After using $HgCl_2$, NaOCl and ethanol as sterilizing agents in various combinations it was found that NaOCl was found to be best for aseptic germination of seeds in the concentration of 0.1% with contact time of 7 min (Figure. 1). Also there was no significant change noticed in the colour of seeds, it means that sodium hypochlorite does not exhibit any detrimental effects on seed coat or seed. Almost 98% of seeds germinated within a time span of 10-12 days. Also the seeds which were rubbed with sand paper germinated within a time span of one week using 0.1% NaOCl for 7 min. Talking about the pattern of seed germinated it was noticed that seeds did not germinate at all in the months of may-october even after keeping for more than 25 days but in rest of the months the seeds showed rapid germination.

Endophytic bacteria are the micro-organisms that colonize the internal tissues of the plant without causing any external sign of infection or negative effect on their host. Hence, effective seed sterilization is very important to eliminate the contamination arising out of endophytic microbes as well as other factors. Endophytic organisms can be derived from any part of the plant including seeds. Various endophytic microorganisms are present in the plant out of which two endophytic fungi has been isolated from dandelion leaf which shows inhibitory activity against staphylococcus aureus. Reports also suggest the isolation of actinomycetes from *Taraxacum officinale* leaves [14]. Also there is a possibility that *Taraxacum officinale* growing in different geographical regions due to difference in flora and fauna may show some variations in seed sterilization process.

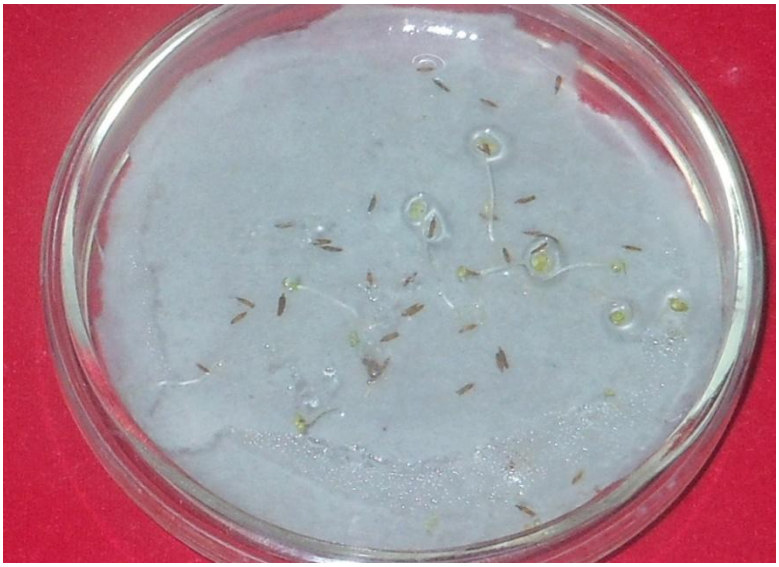

Figure. 1: Aseptically germinated seedlings of Taraxacum officinale by using 0.1% NaOCl as sterilizing agent for 7 min as contact time

Table 1: Optimization of method for sterilization of seeds.

S. No	Sterilant	Concentration(%)	Contact time (min)	Observations
1.	NaOCl	0.1%(25 ml in 100ml ddw)	6	No contamination, seed colour remained light brown, without seed germination.
2.	NaOCl	0.1%(25 ml in 100ml ddw)	7	No contamination, seed colour remained light brown, with seed germination.
3.	NaOCl	0.1%(25 ml in 100ml ddw)	7.5	No contamination, seed colour remained light brown, with seed germination.
4.	Ethanol	(97%)	7	Seed colour remained light brown, contamination was seen.
5.	Ethanol	(97%)	8	Seed colour remained light brown, contamination was seen.
6.	Ethanol	(97%)	10	Seed colour remained light brown, contamination was seen.
7.	$HgCl_2$	0.1%	5	Seed colour changed from light brown to dark brown, no contamination was seen.
8.	$HgCl_2$	0.1%	7	Seed colour changed from light brown to dark brown no contamination, without seed germination .
9.	$HgCl_2$	0.2%	5	Seed colour changed from light brown to dark brown, no contamination, without seed germination.
10.	NaOCl + CH_2OH	0.1% + 0.1%	6	Seed colour remained light brown , contamination was seen.
11.	NaOCl + CH_2OH	0.15% + 0.1%	7	Seed colour remained light brown, no contamination, without seed germination.
12.	NaOCl + CH_2OH	0.2% + 0.1%	7.5	Seed colour remained light brown, no contamination, without seed germination.

Table 2: Seed germination pattern throughout the year.

S.No.	Month	Time period of germination
1.	November-April	All seeds germinated within 10 -12 days.
2.	May-October	Seeds did not show germination at all even after keeping for more than 30 days.

CONCLUSION:

Nevertheless, significant challenges remain in seed sterilization, we may conclude from above studies that rubbing of the seeds with sand paper reduced the seed germination period by 2-4 days using NaOCl as the chemical sterilant. Hence, aseptic germination of seeds of *Taraxacum officinale* is important from the micropropagation point of view and *in vitro* cultures may be obtained devoid of any contaminant.

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