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ASEPTIC STUDIES ON SEED GERMINATION PATTERN IN *CORIANDRUM SATIVUM* LINN

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Abstract

This paper highlights the process of germination of seeds of *Coriandrum sativum* (CS), 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 % of seed germination, degree of contamination and seed color during the course of experiment was studied. The aim of this study is to establish best surface sterilization for *in-vitro* propagation of CS. The seeds of CS 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 0.15% for 5 minutes.

Keywords: *Coriandrum sativum* (CS) seeds, NaOCl, CH_2OH, $HgCl_2$ and sterilizing agent.

Introduction

The introduction of plant tissues/ explants to tissue culture involves a dynamic stage which aims at obtaining cultures that are devoid of microbial contamination. It often happens that, in spite of using proper method of surface sterilization of explants before their culture, the microbes are not completely eliminated and contamination occurs, this is especially the case where explants are taken directly from plants growing in open field and introduced to tissue culture. Many contaminants and microbes like endophytic bacteria are present in xylem vessel and are protected from the process of surface sterilization. Therefore, seeds are often assumed as the source of microbial contamination. So, in this paper we aim to study the effect of three sterilizing agents- mercuric chloride, sodium hypochlorite and ethanol on seed sterilization of *Coriandrum sativum* (CS) and physiological phenomenon involved in this process. Out of these three sterilizing agents, sodium 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]**. *Coriandrum sativum* (CS) commonly known as Dhania belonging to family Apiaceae, grows throughout the Indian with Rajasthan, Gujrat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh and Himachal Pradesh as the major coriander growing states in India. It is an important seed spice crop having prime position in flavoring food **[2]**. The seeds and leaves have been reported to show antimicrobial activity, Antioxidant activity, Anticonvulsant, Anxiolytic, sedative, anti-depressant and cognitive effects **[3]**. The essential oil of coriander is reported to show anti-fungal activity and insecticidal activity. Coriander seeds have also shown to exert hypoglycemic, hypolipidemic and antihypertensive effect **[4]**. The essential oil from coriander seeds have also been reported to show anti-biofilm activity **[5]**. Antibacterial activity has also been reported from essential oil of *Coriandrum sativum* Linn **[6]**. Coriander seed extracts have also reported to show *in-vitro and in-vivo*, anti-helminthic activities **[7]**.

Reports also showed that storage of dry seeds in freeze conditions led to delay in ageing and seed germinability was preserved for a long time. Hence, cryopreservation may be regarded as a convenient method for seed storage for studying comparative aspects of seed germination **[8]**. Various studies on some perennial weeds including *Taraxacum officinale* was done showing the effect of alternating temperature, light, nitrate and seed age as factor on seed germination and it was found that all factors affected seed germination to a considerable level **[9]**.

Materials and methods

Checking of seed viability

Firstly, the viability of CS seeds was checked by soaking them in water. Each seed was cut longitudinally in half using a razor blade. A 100 mm petridish was taken and filter paper containing the cut CS seeds was placed on it and further the petridish was covered with a 0.1% solution of triphenyltetrezolium chloride (TTC) at pH 7-8 and kept in a dark period for 12-24 hours. The seeds which were cut, were then examined for staining. The viable seeds changed the colorless TTC into an insoluble, red pigment which was seen in the endosperm and embryo. The unstained seeds remaining after staining were considered to be non-viable whereas, seeds exhibiting any stain were considered to be viable.

$$\text{Seed viability \%} = \frac{\text{No. of half cut seeds stained red}}{\text{Total No. of half cut seeds}} \times 100$$

Aseptic seed sterilization and germination

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

In another experiment seeds were rubbed with sand paper for 3-4 min and then washed 2-3

times with water, teepol solution and again washing was done 2-3 times with water and then finally washed 2-3 times with double distilled water and same procedure for seed germination was repeated as mentioned in previous experiment.

Results and discussions

It was found that 98% seeds were viable, after checking the viability.
The most critical step in germination of seed is breaking of seed dormancy. Several studies have shown that gibberellic acid, brassinosteroids and ethylene promote the germination of dormant seeds, but abscisic acid (ABA) is known to be primary mediator of seed dormancy. $HgCl_2$, used in different concentrations for different contact time periods resulted in total absence of contamination but seeds did not germinate even after keeping for so many days. Also it was noticed that due to use of $HgCl_2$, color of seed turned from light brown to dark brown and even black. The change in natural color of seeds shows that $HgCl_2$ is toxic for use in the process of seed sterilization. Hence, we can conclude that $HgCl_2$, is not suitable as a sterilizing agent for seed germination in *Coriandrum sativum*.

Alcohols are reported to be bactericidal rather than bacteriostatic against different vegetative forms of bacteria but do they not destroy bacterial spores **[10]**. Also the available literature suggest that ethanol is comparatively a better solvent than water in dissolving many phytoconstituents present in seeds therefore, its penetration power in seed is also good. So, it should be used for shorter time period for sterilization upto which ethanol does not penetrate and lead to dissolution of seed phytoconstituents further leading to contamination **[11]**. Using ethanol alone or in combination with NaOCl in different concentrations are depicted in table.1, led to contamination after 3-4 days. The seed color remained light brown throughout the process and hence there was no visible change in the color of seeds. The contamination level was highest in ethanol compared with the other two sterilizing agents. Henceforth, ethanol is cannot be considered as a sterlizing agent for seed germination in CS.

Using NaOCl, it was found that the degree of contamination was lesser at its different concentrations. After using ethanol, $HgCl_{2, \text{and}}$ NaOCl as sterlizing agents in various combinations it was found that NaOCl was found to be best sterilizing agent for aseptic germination of seeds in the concentration of 0.15% with contact time of 5 min (Fig. 1). Also there was no significant change observed in the seed colour, it means that sodium hypochlorite does not exhibit any detrimental effects on seed or seed coat. Almost 97% of CS 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 0.15% NaOCl for 5 min. Talking about the pattern of seed germinated it was noticed that seeds did not germinate at all in the months of June-November even after keeping for more than 25 days but in rest of the months the seeds showed rapid germination.

Endophytic bacteria 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 due to endophytic microbes as well as other factors **[12]**. The below tables show the various aseptic conditions for seed germination of CS.

Conclusion:

Although, significant challenges remain in the process of seed sterilization; we may conclude from above studies that rubbing of the seeds with sand paper reduced the seed germination period by 3-5 days using NaOCl as the chemical sterilant. Hence, aseptic germination of seeds of *Coriandrum sativum* is important from the view point of micro-propagation and *in vitro* cultures obtained may be devoid of any contaminant.

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Table 1: Optimisation of method for sterilization of seeds.

S.NO	Sterilant	Concentration (%)	Contact time (min)	Observations
1.	NaOCl	0.15% (25 ml in 100ml ddw)	5	No contamination was seen, seed color remained light brown & with seed germination.
2.	NaOCl	0.15% (25 ml in 100ml ddw)	6	No contamination was seen, seed color remained light brown & without seed germination.
3.	NaOCl	0.1% (25 ml in 100ml ddw)	6.5	No contamination was seen, seed color remained light brown & without seed germination.
4.	$HgCl_2$	0.1%	6	Seed color changed from light brown to dark brown & no contamination was seen.
5.	$HgCl_2$	0.1%	5	Seed color changed from light brown to dark brown, no contamination & no seed germination was observed .

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6.	$HgCl_2$	0.2%	7	Seed color changed from light brown to dark brown, no contamination and no seed germination was seen.
7.	NaOCl + CH_2OH	0.1% + 0.1% each	7	Seed color remained light brown & contamination was observed
8.	NaOCl + CH_2OH	0.15% + 0.1%	6.5	Seed color remained light brown, no contamination & No seed germination.
9.	NaOCl + CH_2OH	0.2% + 0.1%	7.5	Seed color remained light brown, no contamination & No seed germination.
10.	Ethanol	(97%)	8	Seed color remained light brown & contamination was observed.
11.	Ethanol	(97%)	9	Seed color remained light brown and minor contamination was seen.
12.	Ethanol	(97%)	10	Seed color remained light brown and contamination was observed.

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Table 2: Seed germination pattern throughout the year.

S.NO.	MONTH	TIME PERIOD OF GERMINATION (DAYS)
1.	December-May	All seeds germinated within 12 -13 days.
2.	June-November	Seeds did not show germination at all even after keeping for more than 30 days.

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