Research Article

IN VITRO EFFECT OF STERILIZING AGENTS ON CONTAMINATION AND REGENERABILITY OF *JATROPHA CURCAS* SEED: A BIOFUEL PLANT

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ABSTRACT

The seeds of *J.curcas* were categorized into three groups on the basis of their size and naked embryo weight viz. small (1.0-1.75g), medium (2.0-2.75g), large (above3.0g).They were further sub divided into four types i.e. intact seed (with seed coat), halved seed, naked embryo (without seed coat) and halved naked embryo. The naked embryos of small, medium and large seeds expressed highest contamination (22.8%, 20.5% and 12.8%) when sterilized with $HgCl_2$ (0.1%, 12 minutes). Contamination was lowest (11.4%, 7.5%, and 2.7 %) when treated with streptocycline (0.1%, 8minutes) + $HgCl_2$ (0.1%, 21 minutes). The small, medium and large category seeds of both the types (intact seeds and naked embryo) exhibited maximum viability (4.9%, 5.6%, 6.5%) and (25.9%, 49.7%, 95.7%) respectively when treated with streptocycline (0.1%,8 minutes)+$HgCl_2$(0.1%, 12 minutes). The naked embryo of large sized seeds sterilized with streptocycline (0.1%, 8 minutes) + $HgCl_2$ (0.1%, 12minutes) exhibited maximum viability and lower contamination.

Keywords: Sterilizing agents, Contamination, Regeneration, Viability, Seed.

INTRODUCTION

The oil yielding plant *J.curcas* (L.) or physic nut is a multipurpose and drought resistant large shrub or small tree. It is a native of tropical Central America; it has now been domesticated in a widespread manner in Africa and Asia mainly due to its ability to grow in a number of climatic zones in tropical and subtropical regions of the world particularly in marginal lands. *J.curcas* is easy to establish, grows relatively quickly and is hardy. *J.curcas* has immense economic potential and ecological and environmental significance. The uses of this crop range from traditional medicine for common human and animal ailments, protection against land erosion, as a boundary fence to newly found potential for fossil fuel replacement. In the recent years, energy conservation and its production has acquired significant importance in the wake of the world energy crisis. A number of options for production of liquid fuel as an alternative source have been considered in many countries. The *J. curcas* oil has been identified as an efficient substitute to be used as fuel for diesel engines[1]. The engine performance and fuel consumption with *J. curcas* oil has been compared favorably with normal diesel oil[2]. Hence utilization of *J. curcas* oil as a new source of oil for diesel engine has tremendous scope in contributing to growing needs of country for energy resources. Since, *J. curcas* does not compete with conventional crops for cultivation, the dilemma of food verses fuel does not arise. Seeds and cuttings are widely used for propagation of *J. curcas.* Plant propagated through seeds lead to significant variations in seed yield and oil content[3]. Furthermore, seeds of *J.curcas* have a limited viability and can only be stored for 15 months after which its viability is reduced by 50%[4]. *In vitro* cultured seeds expressed higher percent germination in a short time period in comparison to *ex vitro* grown seed but it depends upon size, weight, sterilizing agents and their durations used. Contamination with microorganism is one of the most serious problems in plant tissue culture[5]. Leifert *et al*. 1991[6] reported wide range of microorganisms like filamentous fungi, yeast, bacteria, viruses and viroids *i.e.* mites and thrips as contaminants in plant tissue culture. Contaminant microorganisms may overrun the cultures killing the explants[7]. Successful increasing attention is being paid to ascertain sources of contamination, to develop procedures for their elimination by avoidance, rigorous manipulation of the nutritional and environmental conditions and by specific antibiotic treatments[8]. The use of anti-microbial agents (anti-bacterial as well as anti-fungal) to control contamination is the preferred method[9]. However, their indiscriminate use may lead to phytotoxicity problems[10]. Taking into account the present investigation was planned to control the microflora contamination of *in vitro* raised seedlings of *J.curcas*.

MATERIALS AND METHODS

Seeds of *J. curcas* were collected from the Experimental Orchard of the Department of Horticulture and Home Sciences Garden of CCS Haryana Agricultural University, Hisar for *in vitro* seed germination. The seeds of *J.curcas* were categorized into three groups on the basis of their size and naked embryo weight viz. small (1.0-1.75g), medium (2.0-2.75g), large (above 3.0g). The seeds were washed with a mild detergent solution, soaked in Bavistin (0.2%) for 30 minutes and rinsed with distilled water after washing under running tap water. The seeds were gently cracked to expose the zygotic embryos surrounded by kernel. The seeds of each of the above categories were

further sub divided into four types i.e. intact seed (with seed coat), halved seed, naked embryo (without seed coat) and halved naked embryo. Seeds were surface sterilized with $HgCl_2$ (0.1%) alone and in different combination with streptocycline (0.1%) followed by successive washing with sterile distilled water three times in a laminar flow. The seeds were cultured on MS medium supplemented with GA_3 (2.0mgl^{-1}) + citric acid (0.0025%) and ascorbic acid (0.005 %). The pH of the medium was adjusted to 5.8 using 1N KOH or HCl, prior to autoclaving at 1.05 kgcm^{-2} pressure at 121◦C for 20 min. The cultures were maintained at 25±2°C under a 16-h photoperiod with light intensity of 35–40µmol m^{-2} s^{-1} (cool white fluorescent tubes). The effect of different durations of $HgCl_2$ (0.1%) alone and in combination with streptocycine (0.1%) on surface sterilization and viability of above categories and types of *J.curcas* seeds was studied. Data was analyzed at the p = 0.05 level using the Newman-Keul's multiple range test.

RESULTS AND DISCUSSION

Data on the effect of sterilizing agents on sterilization of *J.curcas* seeds is presented in table-1 which indicates that the intact seeds and naked embryos of small, medium and large seeds when sterilized with $HgCl_2$ (0.1 %, 12 minutes) exhibited maximum contamination (48.6%, 45.4%, 42.5%) while it was minimum (21.8 %, 19.5 %, 17.2%) when treated with streptocycline (0.1 %, 8 min) + $HgCl_2$ (0.1 %, 21 minutes).

The naked embryos of small, medium and large seeds expressed highest contamination (22.8%, 20.5%, and 12.8%) when sterilized with $HgCl_2$ (0.1%, 12 minutes). Contamination was lowest (11.4%, 7.5%, and 2.7 %) when treated with streptocycline (0.1 %, 8minutes) + $HgCl_2$ (0.1 %, 21 minutes). It is also evident from the data in table-1 that the intact seeds of all the three categories exhibited higher contamination in comparison to the naked embryos irrespective of the seed size and duration of treatments and contamination was higher in small size seeds in comparison to large size seeds irrespective of the sterilization treatments and the type of seeds i.e. intact seed or naked embryo.

Data on the effect of sterilizing agents on percent viability of *J.curcas* seeds is tabulated in table-2.

The small, medium and large category seeds of both the types (intact seeds and naked embryo) exhibited maximum viability (4.9 %, 5.6 %, 6.5 %) and (25.9 %, 49.7 %, 95.7 %) respectively when treated with streptocycline (0.1%, 8 minutes) + $HgCl_2$ (0.1 %, 12 minutes). Minimum viability (1.8 %, 2.5 %, 2.9 %) and (7.4 %, 12.5 %, 43.7 %) was observed in intact seed and naked embryos when treated with streptocycline (0.1 %,8 minutes) + $HgCl_2$ (0.1 %, 21 minutes) (Figure 1).

Naked embryos of large sized seeds showed highest viability percentage may be because of the removal of the hard seed coat. Hard seed coat prevents seed germination by interference with water uptake, gaseous exchange. Also seed coat supplies inhibitors to the embryo or prevent the exhibit of the inhibitors[11]. This also may be the reason behind less germination percent of intact seed. It is observed that when halved seeds are used, germination percent was increased over the intact ones. It appears that physical action of cutting the seeds in half did not impede development rather improves it by removing a part of hard seed coat. Some germination inhibiting substances are present in the cotyledons whose affect is reduced when the seeds are halved[11]. This may be because, while dissecting, damage may be caused in the meristematic region of the embryos. Similar pattern of growth of halved embryos was also observed in *Rubus* seed germination[12].

Table 1: Effect of sterilizing agents on contamination of *J.curcas* seeds

Treatments and durations	Percentage Contamination					
	Category of seed					
	Small seed		Medium seed		Large seed	
	Intact seed	Naked embryo	Intact seed	Naked embryo	Intact seed	Naked embryo
$HgCl_2$ (0.1%,12 min.)	48.6±0.28^{a}	22.8±0.14^{a}	45.4±0.24^{b}	20.5±0.14^{a}	42.5±0.26^{a}	12.8±0.10^{a}
$HgCl_2$(0.1%) (15 min.)	42.7±0.24^{c}	20.4±0.15^{b}	47.0±0.22^{a}	18.4±0.10^{b}	38.9±0.17^{b}	10.5±0.09^{b}
$HgCl_2$(0.1%) (18 min.)	34.9±0.24^{d}	18.3±0.12^{c}	33.2±0.18^{e}	15.3±0.16^{c}	30.6±0.14^{c}	9.4±0.09^{c}
$HgCl_2$(0.1%) (21 min.)	28.5±0.15^{e}	16.5±0.09^{d}	25.4±0.14^{g}	14.5±0.10^{c}	22.8±0.12^{d}	7.5±0.06^{d}
Streptocycline (0.1%, 8min. +$HgCl_2$ (0.1%) (12 min.)	45.9±0.34^{b}	18.9±0.07^{c}	40.2±0.24^{c}	12.7±0.07^{d}	34.6±0.21^{c}	5.6±0.07^{e}
Streptocycline (0.1%) (8min +$HgCl_2$ (0.1%) (15min.)	39.4±0.24^{c}	15.2±0.11^{d}	36.8±0.23^{d}	10.4±0.10^{e}	32.4±0.23^{c}	4.6±0.10^{f}
Streptocycline (0.1%) (8min +$HgCl_2$ (0.1%) (18min.)	34.9±0.22^{d}	13.1±0.11^{e}	31.4±0.14^{f}	9.9±0.11^{e}	28.4±0.12^{e}	3.3±0.07^{g}
Streptocycline (0.1%)(8min +$HgCl_2$(0.1%)(21min.)	21.8±0.23^{f}	11.4±0.13^{f}	19.5±0.15^{h}	7.5±0.14^{f}	17.2±0.14^{f}	2.7±0.08^{h}

Mean followed by the same letter are not significantly different according to Newman-Keul's multiple range test (p=0.05).

Table 2: Effect of sterilizing agents on viability of *J.curcas* seeds

Treatments and durations	Percentage Viability					
	Category of seed					
	Small seed		Medium seed		Large seed	
	Intact seed	Naked embryo	Intact seed	Naked embryo	Intact seed	Naked embryo
$HgCl_2$(0.1%,12min.)	4.6±0.28[a]	20.8±0.14[b]	5.0±0.24[a]	40.5±0.14[b]	5.5±0.26[b]	88.8±0.10[b]
$HgCl_2$(0.1%) (15 min.)	3.5±0.24[b]	14.4±0.15[c]	4.0±0.22[b]	35.4±0.10[c]	4.9±0.17[c]	70.5±0.09[c]
$HgCl_2$(0.1%) (18 min.)	2.0±0.24[c]	10.3±0.12[d]	3.2±0.18[c]	18.3±0.16[d]	3.6±0.14[d]	50.7±0.09[d]
$HgCl_2$(0.1%) (21 min.)	1.5±0.15[d]	5.5±0.09[e]	2.1±0.14[d]	11.5±0.10[e]	2.6±0.12[e]	42.6±0.06[e]
Streptocycline(0.1%,8min +$HgCl_2$(0.1%)(12min.)	4.9±0.34[a]	25.9±0.07[a]	5.6±0.24[a]	49.7±0.07[a]	6.5±0.21[a]	95.7±0.07[a]
Streptocycline(0.1%,8min +$HgCl_2$(0.1%) (15min.)	3.8±0.24[b]	15.2±0.11[c]	4.2±0.23[b]	36.4±0.10[c]	3.4±0.23[d]	72.6±0.10[c]
Streptocycline(0.1%,8min +$HgCl_2$(0.1%)(18min.)	2.3±0.22[c]	13.1±0.11[c]	3.4±0.14[c]	19.5±0.11[d]	3.8±0.12[d]	52.0±0.07[d]
Streptocycline(0.1%,8min +$HgCl_2$(0.1%)(21min.)	1.8±0.23[d]	7.4±0.13[e]	2.5±0.15[d]	12.5±0.14[e]	2.9±0.14[e]	43.7±0.08[e]

Mean followed by the same letter are not significantly different according to Newman-Keul's multiple range test (p=0.05).

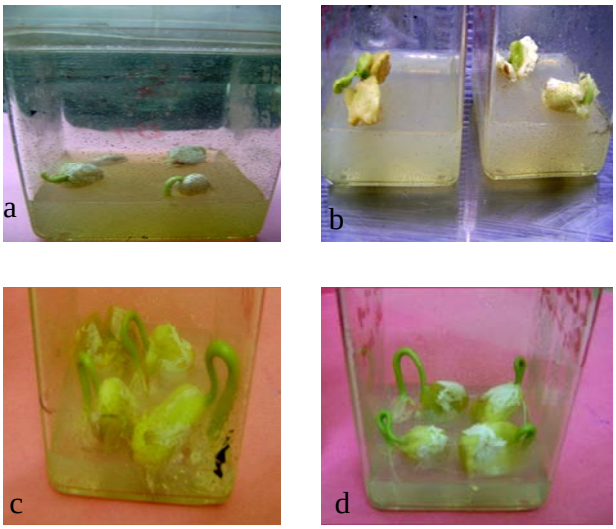

Figure1. a, b. Naked embryo of small *J.curcas* seed expressed low percent viability; **c.** Naked embryo of medium size *J.curcas* seed expressed lesser viability in comparison to larger seed; **d**. Naked embryo of large *J.curcas* seed expressed 100% viability

Naked embryo of larger seeds having average weight showed more viability and germination percentage. This may be because seedling shoot-length and dry matter yield significantly affected by seed weight[13]. Seedlings grown from the heaviest seeds were 51% taller and 91% heavier than those from the lightest ones. The improved seed and seedling quality, as associated with greater seed weight, is attributed to better membrane integrity and increased availability of energy in the endosperm[14].

On the overall basis of data presented in table 1 and 2 ,it is revealed that naked embryo of large sized seeds sterilized with streptocyclin (0.1%, 8 minutes) + $HgCl_2$ (0.1 %, 12) exhibited maximum viability and lower contamination. Hence, streptocycline (0.1 %, 8 minutes) + $HgCl_2$ (0.1 %, 12) proves to be the best seed sterilization treatment for *in vitro* viability of *J.curcas* seeds.

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