International Journal of Applied Research 2016; 2(8): 759-761

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(8): 759-761
www.allresearchjournal.com
Received: 12-06-2016
Accepted: 18-07-2016

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Effect of different sterilization methods on contamination and viability of nodal segment of *Vitex negundo* L

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Abstract
The present investigation was carried out to develop a protocol for surface sterilization of nodal segments of *Vitex negundo* for micropropagation that eradicate the higher per cent of the contamination along with higher per cent viability of explants. Different sterilants i.e. Plant preservative mixture (PPM), Mercuric Chloride ($HgCl_2$), Hydrogen peroxide (H_2O_2) and Ethyl Alcohol (EtOH) were tested for different concentrations or different time durations to test for the higher per cent of the establishment of microbes free culture. $HgCl_2$ was found to be a better sterilant.

Keywords: Sterilization methods, contamination, viability, nodal segment, *Vitex negundo* L

Introduction
Vitex negundo (Family Verbenaceae) commonly known as Nirgundi (Hindi, Sanskrit, Tamil), Shwari (Punjabi), Five leaved chaste tree or Monk's pepper (English), Nagod (Gujarati) etc (Venkateswarlu, 2012) [1]. All most all the parts of the plant are having the medicinal properties. The plant is tastes as pungent and bitter. It is useful to be used to cure inflammation, stomachic, cephalic, astringent, consumption, bacterial infections, cancer, bronchitis, asthma, biliousness, snake bite etc. (Kirtikar & Basu, 2008; Nadkarni, 2002; Alam & Gomes, 2003; Sharma *et al*., 2005; Venkateswarlu, 2012) [2, 3, 5, 4, 1].
The increased demand of the plant in pharma industry has resulted in the over-exploition leading towards its slow extinction. Its conventional methods of propagation are slow and less effective, as compared to its exploited rate. Sterilization is the basic and pre requirement of any *in vitro* culture to establish aseptic cultures. So, in the present investigation was carried out with an aim to develop the highly desirable and efficient protocol to effectively sterilize and establish viable cultures of the nodal explants in *in vitro* conditions on MS medium.

Material Method
Surface sterilization procedure is a primary and vital step in starting any *in vitro* propagation, as minute contamination in *in vitro* propagation can lead to loss of viability of the culture with wastage of time and labour. Nodal segments were excised into small pieces (1 - 1.5 cm) and washed with Tween -20 (3 drops per 100 ml distilled water) for 30 minutes. To solve the problem of contamination in the nodal segments, Plant Preservative Mixture (PPM), Mercuric Chloride ($HgCl_2$), Hydrogen peroxide (H_2O_2) and Ethyl Alcohol (EtOH) either of different concentrations or for different time durations were tested for the best surface sterilization, for *in vitro* culture establishment along with higher pre cent viability of cultures (Table -1, 2, 3 & 4). The explants were washed with double distilled water and trimmed by sterilized blade to remove any trace of the sterilized agent left adhering in the cut ends of the explants. Then, explants were inoculated on MS medium fortified 1 mg/l of BAP at 25±2 ^{0}C under 16 h photoperiod.

All aseptic manipulations were carried out under laminar airflow chamber for maintaining aseptic conditions. Thirty replicates per treatment were investigated in this experiment. The per cent aseptic cultures and per cent cultures surviving (out of total thirty per treatment) after 25 days of inoculation were recorded.

Result and Discussion

The results showed that among the four sterilizing agents investigated in this study, $HgCl_2$ (Mercuric Chloride) reported the highest per cent of aseptic cultures among the different sterilizing agents treatment tested at different concentrations. The PPM (Plant preservative mixture) treatments of different concentrations with increases of exposure duration reported an increase in the per cent aseptic cultures along with per cent viability. Similar results of per cent increase of aseptic cultures along with respective increase in per cent viability was reported in H_2O_2 (Hydrogen peroxide) and EtOH (Ethyl Alcohol) treatments as shown in Table 1, 3 & 4. However in $HgCl_2$ treatments, the best results were reported in $HgCl_2$ treatment of 0.1 mg/L for five minutes i.e. 100% observation of aseptic cultures with higher per cent (86.6%) viability (Table – 2). The lower concentration of $HgCl_2$ treatments (Table – 2) reported less per cent aseptic cultures and per cent viability. Though, higher concentration of $HgCl_2$ reported (Table – 2) 100 per cent aseptic cultures but per cent cultures surviving (viability) reported decline with increasing the concentration treatments of $HgCl_2$ (Table – 2).

$HgCl_2$ is a commonly used and a strong sterilizing agent (Gopal *et al*., 1998; Naika & Krishna, 2008; Anburaj *et al*., 2011; Das *et al*., 2012) [7, 10, 11, 6]. The higher exposure duration as well as higher concentration of sterilizing agents including $HgCl_2$ has side effects on the viability of the explants (Danso *et al*., 2011) [12]. This may be the reason for the decrease in the viability with increased exposure time. The sterilizing agents acts as disinfectant intents by damaging, denaturing, displacement, substitution, inactivation or blocking the important cell molecules or organelles (Patra *et al*., 2004; Patra & Sharma, 2000) [8, 9].

Table 1: Sterilizing agent (PPM) exposure in different Time Duration, Aseptic cultures in% and Culture Viability in%.

Sterilizing Agent	Concentration (ml/L)	Time Duration (Minute)	Aseptic cultures (%)	Viability (%)
PPM	10	10	13.3^{e}	10^{e}
PPM	10	20	20^{d}	16.6^{d}
PPM	10	30	33.3^{c}	26.6^{c}
PPM	10	40	63.3^{b}	56.6^{b}
PPM	10	50	73.3^{a}	66.6^{a}

Table 2: Sterilizing agent Mercuric chloride ($HgCl_2$) exposure in different Concentrations, Aseptic cultures in% and Culture Viability in%.

Sterilizing Agent	Concentration (mg/L)	Time Duration (Minute)	Aseptic cultures (%)	Viability (%)
$HgCl_2$	0.05	5	86.6^{b}	80^{b}
$HgCl_2$	0.1	5	100^{a}	86.6^{a}
$HgCl_2$	0.2	5	100^{a}	80^{b}
$HgCl_2$	0.4	5	100^{a}	76.6^{c}
$HgCl_2$	0.8	5	100^{a}	63.3^{d}

Table 3: Sterilizing agent Hydrogen peroxide (H_2O_2) exposure in different Time Duration, Aseptic cultures in% and Culture Viability in%.

Sterilizing Agent	Concentration (% w/v Solution)	Time Duration (Minute)	Aseptic cultures (%)	Viability (%)
H_2O_2	3	2	26.6^{e}	**16.6^{e}**
H_2O_2	3	4	43.3^{d}	36.6^{d}
H_2O_2	3	6	66.6^{c}	50^{c}
H_2O_2	3	8	73.3^{b}	56.6^{b}
H_2O_2	3	10	86.6^{a}	66.6^{a}

Table 4: Sterilizing agent Ethyl Alcohol (EtOH) exposure in different Time Duration, Aseptic cultures in% and Culture Viability in%.

Sterilizing Agent	Concentration (% v/v Solution)	Time Duration (Minute)	Aseptic cultures (%)	Viability (%)
EtOH	70%	5	6.6^{e}	**6.6^{e}**
EtOH	70%	10	13.3^{d}	10.0^{d}
EtOH	70%	15	23.3^{c}	20.0^{c}
EtOH	70%	20	26.6^{b}	23.3^{b}
EtOH	70%	25	36.6^{a}	26.6^{a}

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